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Absorption Curve and Germination of Jucá Seeds under Different Salts

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Abstract

The plants are subjected to conditions of multiple stresses that limit their growth and development, which affects their chances of survival wherever they grow. To evaluate the plant tolerance to these conditions, one of the most used methods is the observation of seed germination under salt stress. The action of salts on the seeds varies widely among species and can exert toxic effects, causing damage before and/or after the onset of germination. Therefore, the objective of this work was to evaluate the influence of different salts in water absorption and germination in jucá seeds (*Libidibia ferrea*). For absorption and germination curve, were used non-scarified and scarified seeds, and subjected to the treatments in distilled water (control) and NaCl solutions, KCl, $CaCl_2$ and K_2HPO_4, in an electric conductivity of 5 dS m^{-1}. The saline solution composed by K_2HPO_4 influence on water absorption of *L. ferrea* in intact seeds. The scarified and non-scarified seeds of *L. ferrea* have different times for the physiological phases of water absorption. The action of salts in the germination and vigor in non-scarified and scarified seeds, does not significantly reduce these values due to electrical conductivity of 5 dS m^{-1} not be enough to affect the seed germination of *L. ferrea*.

Keywords: forest seeds, *Libidibia ferrea*, mathematical modeling, salinity, vigor

1. Introduction

The plants are subjected to conditions of multiple stresses that limit their growth and development, which affects their chances of survival wherever they grow. Larcher (2000), states that one of the most widespread methods for determining the tolerance of plants to water and salt stress is observing the germination of seeds in these conditions. Germination is an early stage of plant development in which the seed must have internal and external conditions favorable for both. Among the external conditions, temperature, salinity, light, water, oxygen concentration and alkalinity are one of the main environmental factors that may influence the germination of seeds.

Saline soils have various types of soluble salts, each of which has different effects on initial plant growth (Tobe et al., 2003), and its composition in soils will differ widely between locations (Tobe et al., 2004). In fertilized soils, the presence of salts can reach high levels, and significantly affect germination (Sangoi et al., 2009). Excessive soluble salts in soils, such as sulphates, bicarbonates, borates and especially sodium chloride, will interfere in the water potential of the soil, reducing the potential gradient between the soil and the seed surface, which causes a restriction in the water inlet embryo (Macedo & Lopes, 2008).

The process of salinization occurs when evaporation and transpiration remove pure water (as vapor) from the ground, causing loss of water that raise the concentration of salts in the soil surface (Taiz & Zaiger, 2013), making more saline than the deeper layers. Thus, the seeds are more than salinized environment established seedlings, whose roots have the ability to absorb water from the deeper regions of the soil (Agboola, 1998).

The action of salts on the seeds varies widely among species and can exert toxic effects by causing damage before and/or after the onset of germination. For seeds that have integument permeable to salts, the presence of salinity may cause loss of germinability (Tobe et al., 2003). Generally, this stress triggers some common reactions, causing a water deficit in the plant, with a reduction in the rate of photosynthesis and energy overexposing the chloroplasts, leading to oxidative stress, that accelerates the production of reactive oxygen

species (ROS) and subsequently changes the balance between the formation and removal of such species, which has a negative effect on the structures and cellular metabolism (Sobhanian et al., 2011).

Studies involving the germination and vigor of tropical tree species such as *Libidibia ferrea* Mart ex Tul., in salt stress condition, are still scarce, thus justifying the development of studies, which may allow the definition of tolerance from one species to environmental constraints. The *L. ferrea* belongs to the Fabaceae family - Caesalpinoidae, occurring in shrub and tree Caatinga, and popularly known as jucá (Lorenzi, 2002). Also according to the author, it is an arboreal plant, wide dispersion and low population density, forming rounded crown, closed and dense. Its fruit is a vegetable, indehiscent; each fruit contains two to ten ellipsoids seeds, yellow or brown to very hard consistency.

Given the lack of information about the behavior of their seeds in saline environments aimed to evaluate the influence of different salts in water absorption and germination of *L. ferrea* seeds.

2. Material and Methods

The experiment was conducted in a completely randomized design with four replications, consisting of 25 seeds each. The treatments were arranged in a double factor (2 × 5), and consisted of a combination of two kinds of seeds (scarified and non-scarified) and five solutions (T1 - Distilled water [witness]; T2 - sodium chloride [NaCl], T3 - potassium chloride [KCl], T4 - calcium chloride [CaCl]; T5 - potassium phosphate solution [K_2HPO_4]), where all salt solutions had the conductivity adjusted to 5 dS m^{-1}.

For water-absorption curve, seeds were immersed in plastic cups containing 50 ml of the solution for each treatment, and the degree of absorption measured to stabilize the water absorption, so for the seeds non-scarified intervals of 0, 1, 2, 3, 6, 7, 8, 9, 24, 26, 30, 32, 48, 50, 54, 72 and 78 hours, and scarification stabilization, was given 54 hours. At the end of each period, the seeds were removed from the cups, dried with towel and heavy paper, resulting in the wet weight. Successive weighing was carried out to measure the wet weight gain during the period and time. As the water content absorbed in each time calculated by Equation 1, wherein, Wi and Wf are the initial and final weight of seeds at each time point, respectively.

$$\%\ \text{of absorbed water} = \left(\frac{\text{Wf} - \text{Wi}}{\text{Wi}}\right) \times 100 \tag{1}$$

To evaluate the water absorption of the seeds as a function of time, it was used the model proposed by Maia et al. (2009), and adapted according to the Equation 2, wherein A and A_{max} are the absorption of water by the seed in the time T and a maximum estimated, respectively, α and n are model parameters, set by nonlinear regression methodology, α in $hour^{-1}$ and n is the form factor and dimensionless.

$$A = A_{max} - \frac{A_{max}}{1 + (\alpha \times T)^n} \tag{2}$$

The absolute rates of absorption (ARA) and relative (RRA) of water were estimated by Equations 3 and 4, respectively. The time to maximum ARA ($T.ARA_{max}$) for germination 50% of maximum and the absolute maximum germination rate were calculated by Equations 5, 6 and 7, respectively.

$$T.ARA_{max} = \frac{1}{\alpha}\left[\frac{n}{n+1}\right]^{1/n} \tag{3}$$

$$RRA = \frac{n}{T \times \left[1 + (\alpha \times T)^n\right]} \tag{4}$$

$$T.ARA_{max} = \frac{1}{\alpha}\left[\frac{n}{n+1}\right]^{1/n} \tag{5}$$

$$T.A_{50\%} = \frac{1}{\alpha} \tag{6}$$

$$ARA_{max} = \frac{A_{max} \times \alpha^n \times (n+1)^2}{4n} \times \left(T.ARA_{max}\right)^{n-1} \tag{7}$$

At the end of the water absorption period, the seeds were put to germinate in paper towel, moistened with distilled water, and the solutions of the treatments, a volume equal to 2.5 times the weight of dry paper. The germination test was conducted in a germination chamber type Biochemical Oxygen Demand (BOD), set the temperature to 25 °C, with a photoperiod of eight hours of light, using four replications of 25 seeds for each treatment, and corresponds to the total percentage of seeds that gave normal seedlings (Brasil, 2009).

For the germination speed index (GSI) was used the methodology described by Maguire (1962) and the length of normal seedlings was measured with the aid of a ruler graduated in centimeters, and the results are expressed in seedling^{-1} cm. The dry weight of normal seedlings was determined in an oven at 70 °C, where they remained until constant weight, and the results were expressed in mg seedling $^{-1}$.

The germination data were submitted to analysis of variance by F test ($p < 0.05$), and qualitative means compared by Tukey test using the SISVAR statistical software, version 5.3 (Ferreira, 2010).

3. Results and Discussion

From the values of the adjusted parameters, it was observed that the mathematical model used satisfactorily estimated water absorption of the seeds as a function of time for all treatments with high coefficients of determination, both intact seeds as for scarified within the different solutions evaluated (Table 1).

Table 1. Adjusted parameters of the model (A_{max}, α, n), coefficient of determination (R^2), time to absorb 50% of A_{max} (% TA50), time to maximum absorption rate ($T.ARA_{max}$) and estimated maximum absorption rate (ARA_{max}) for the evaluated treatments, T1 (witness distilled water); T2 (NaCl solution); T3 (KCl solution); T4 ($CaCl_2$ solution) and T5 (K_2HPO_4 solution)

	T_1	T_2	T_3	T_4	T_5
Not Scarified Seeds					
$A_{max\ (\%)}$	102.36	100.71	110.39	120.99	160.51
α	0.0327	0.0394	0.0243	0.0264	0.0127
n	2.0134	1.6048	1.3072	1.1537	1.2209
R^2	0.9990	0.9948	0.9948	0.9979	0.9969
$T.A_{50\%}$	30.52	25.35	40.43	37.77	78.58
$T.ARA_{max}$	17.77	10.21	8.65	3.83	11.087
ARA_{max}	2.18	2.42	1.73	2.26	1.36
Scarified Seeds					
A_{max}	181.31	154.54	165.09	168.80	150.31
α	0.0814	0.0946	0.0796	0.0731	0.0951
n	2.1368	2.8385	2.6455	2.3347	3.4828
R^2	0.9987	0.9993	0.9985	0.9955	0.9966
$T.A_{50\%}$	12.27	10.56	12.55	13.66	10.51
$T.ARA_{max}$	7.63	8.15	9.29	9.23	8.87
ARA_{max}	9.91	11.79	10.07	8.71	13.54

The A_{max} estimated for non-scarified seeds occurred in the T5 and for scarified seeds in T1 (Table 1), with values of 160.51 and 181.31%, respectively, showing the effect of mechanical scarification in facilitating the process of water absorption by the seeds. When comparing A_{max} of scarified seeds over non-scarified, it has increased these values in scarified on average 0.77; 0.53; 0.50 and 0.40% for T1, T2, T3 and T4, respectively. However, for T5 decreased by 0.07%.

According to Marcos Filho (2005), a considerable amount of water abstraction is essential for the resumption of metabolic activities of the seed. Water has several very important functions, helping to soften the seed coat, intensify respiratory rate, encourage gas exchanges, induce the synthesis and activity of enzymes and hormones and contribute significantly to the regularity of digestion, translocation and assimilation of reserves and growth subsequent.

The concentration of salts in the substrate determines the reduction in water potential, resulting in lower capacity for absorption of water by the seeds, which can often influence the germination and seedling development (Lopes & Macedo, 2008). Verslues et al. (2006), report that the presence of salts cause different types of stress, including altering the absorption of nutrients, especially K^+ ions and Ca^+, accumulation of toxic ions such as Na^+, osmotic and oxidative stress. This fact justifies the more time for water absorption of the T5 on intact seeds, in addition to these present a natural barrier to water entry, which is its seed coat, and to the extent that it ruptured by mechanical scraping, is facilitated water inlet, even when added salts.

In non-scarified seeds, although the A_{max} have been given for T5, it was found that the time to absorb 50% of A_{max} (TA50%) was higher compared to the other treatments (78.58 h), inferring that despite the high maximum absorption, a longer time was needed to absorb 50% of water. As for the scarified time to absorb 50% of water, it was approached from all treatments, averaging 12.26 h (Table 1).

In addition to the delay in treatment of water absorption with non-scarified seeds, T5 also caused an increase in the time for maximum absorption rate ($T.ARA_{max}$), suggesting that there may be interference with the uptake of water by seeds in the presence of K_2HPO_4. As for the scarified seeds, T. ARA_{max}, showed a slight variation treatments, ranging from 7.73 to 9.29% (Table 1). The estimated maximum absorption rate (ARA_{max}) for non-scarified seeds on average ranged from 1.36% to 2.42 day^{-1}, while, for seeds scarified the highest rate occurred in T5, with 13.54% day^{-1}.

For the water absorption curve of the non-scarified seeds regardless of treatment, it was found that the physiological stage I imbibition occurred in about 21 hours when there was a greater increase in water absorption, and phase II was extended to about 79 hours, with small increases in absorption (Figure 1A) to reach stabilization. There was a phase III, because the seeds after stabilization of absorption were forwarded to the germination test. For scarified seeds, is that the phase I went up to approximately 13 hours when there was a greater increase in water absorption, since phase II was extended up to 57 hours, until the stabilization of absorption (Figure 1B). Lima et al. (2006) observed for *L. ferrea* scarified seeds high percentage of water gain during the first 24 hours of the start of imbibition, providing a mass increase of 70%. According Bewley & Black (1994) in orthodox seeds, the gain of water during imbibition reaches high values, which may vary in other species of 40 to 100% relative to the initial weight (Cabral et al., 2003).

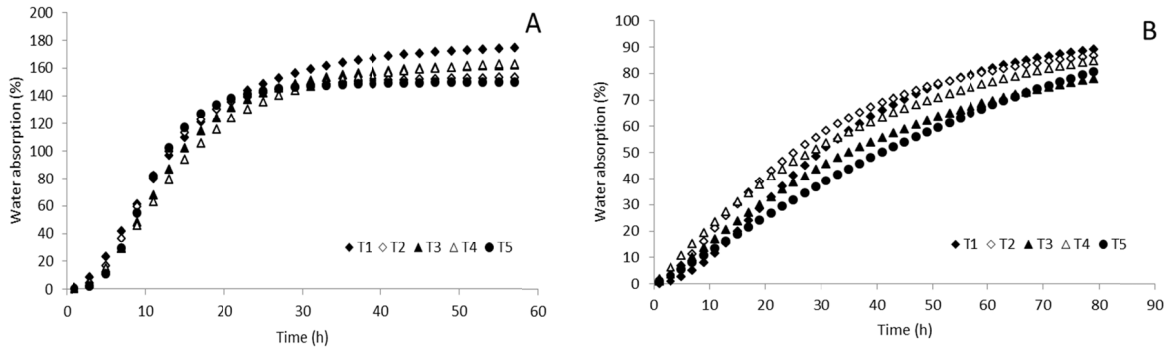

Figure 1. Percentage of water absorption in non-scarified (a) and scarified (b) jucá seeds for the evaluated treatments, T1 (witness distilled water); T2 (NaCl solution); T3 (KCl solution); T4 ($CaCl_2$ solution) and T5 (K_2HPO_4 solution) versus time (h)

Both for the non-scarified as the scarified seeds, it has the same behavior in all treatments to ARA, wherein the seeds intact most ARA was 2.42% h^{-1} (T2) and lowest for T5 (Figure 2B). However, it has to be at the end of 78 hours, T2 had the lowest ARA, while T5 had a higher rate of absorption to other (0.62% h^{-1}). In scarified seeds, it was found that to obtain the highest rate at T5, with 13.54% h^{-1} (Figure 2A), but over time it has been reduced.

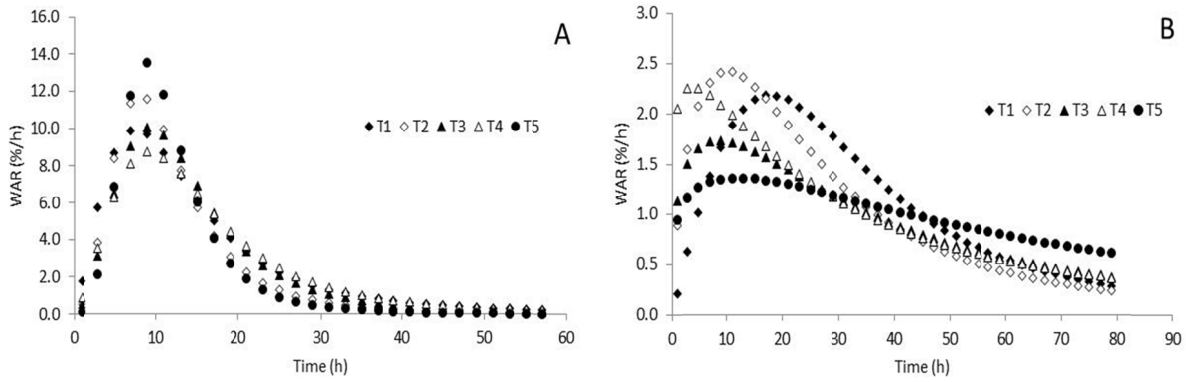

Figure 2. Water absorption rate in non-scarified (a) and scarified (b) jucá seeds for the evaluated treatments, T1 (witness distilled water); T2 (NaCl solution); T3 (KCl solution); T4 ($CaCl_2$ solution) and T5 (K_2HPO_4 solution) versus time (h)

By comparing the relative absorption of distilled water (T1) with the other treatments in both types of seeds, it is that for the scarified seeds (Figure 3A), the relative absorption values at T5 were higher than T1, in the period of 10 to 20 h, after that period, there was a decrease, making less than the other treatments. Further, it was found that all treatments increased absorption until it reaches the maximum point, and then decrease except T4 that after the maximum absorption became constant. As for the non-scarified seeds, there was a higher absorption at first, until approximately 30 h, reducing the ratio to the end of the cycle, but values greater than or equal to T1 (Figure 3B).

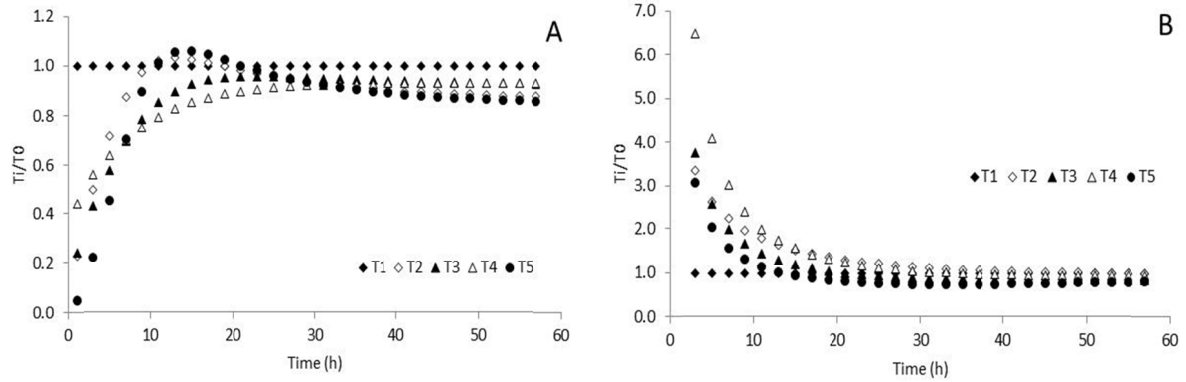

Figure 3. Relationship of water absorption in non-scarified (a) and scarified (b) jucá seeds for the evaluated treatments, T1 (witness distilled water); T2 (NaCl solution); T3 (KCl solution); T4 ($CaCl_2$ solution) and T5 (K_2HPO_4 solution) versus time (h)

Regarding the germination of seeds in different treatments there was a higher percentage of germination in scarified seeds, than in non-scarified, and this superiority, on average, 0.23, 0.42, 0.30, 0.52 and 0.27% for T1, T2, T3, T4 and T5, respectively. Since the germination of non-scarified and scarified seeds they differed significantly in all treatments (Table 2), confirming Lima et al. (2006) who obtained percentage of average germination of 94%, on paper substrate moistened with distilled water, when they were scarified mechanically. Possibly the smallest percentage of germination of non-scarified seeds occurred due to physical barriers imposed by the seed coat, giving the seed cutaneous numbness, as well as smaller gain of water during soaking, hampered by the presence of salts. In this case, the rupture of the integument is necessary for there to seed by water absorption to an appropriate level of hydration, restarting its metabolic activities, thus initiating the process.

Table 2. Germination, germination speed index (GSI), length and dry mass of seedlings from scarified and non-scarified seeds of *Caesalpinia ferrea* for the treatments, T1 (witness distilled water); T2 (NaCl solution); T3 (KCl solution); T4 ($CaCl_2$ solution) and T5 (K_2HPO_4 solution)

Types seeds	Treatments (%)*				
	T_1	T_2	T_3	T_4	T_5
Germination (%)					
No-scarifieds	63 bBA	53 bC	53 bC	54 bCB	70 bA
Scarifieds	84 aA	81 aA	78 aA	78 aA	83 aA
CV (%)	6.74				
GSI					
No-scarifieds	8.6 bAB	5.6 bC	7.5 bBC	6.5 bBC	10.8 bA
Scarifieds	11.8 aA	9.3 aB	12.1 aA	13.1 aA	13.3 aA
CV (%)	11.8				
Length (cm)					
No-scarifieds	17.5 aC	17.0 aC	18.4 aAB	20.1 aA	19.0 aAB
Scarifieds	18.8 aB	14.2 bC	17.6 aB	18.0 aB	21.7 aA
CV (%)	5.68				
Dry Mass (g/seedlings)					
No-scarifieds	0.030 aA	0.025 bA	0.025 bA	0.030 aA	0.030 aA
Scarifieds	0.028 aA	0.030 aA	0.030 aA	0.030 aA	0.030aA
CV (%)	10.53				

Note. *: Means followed by the same uppercase and lowercase in the lines in columns do not differ significantly by Tukey test at 5% probability.

The interaction between the different salts within each type of seed (non-scarified and scarified) was significant, and for non-scarified seeds, the K_2HPO_4 solution has highest percentage of germination compared to the others, then the control (Table 2). For scarified seeds no significant difference between treatments.

So much for the non-scarified as for scarified seeds, the action of salt on seed germination was less pronounced, possibly because the electrical conductivity of 5 dSm^{-1} not be enough to affect the germination of jucá seeds.

The unavailability of water is a limiting factor in the early stages of establishment of various species, suggesting that these mechanisms of tolerance to salt stress are acting during the early stages of germination. According to Kishor et al. (2005), there are many cellular mechanisms by which organisms can tolerate salt stress environment, including the proline accumulation, which has been reported for various plants for acting in the stabilization of proteins, membranes and subcellular structures, in addition to removing species reactive oxygen.

GSI was higher in the scarified seeds were significantly different in all treatments when compared with non-scarified (Table 2), since when he realized the comparison between treatments, only T2 differed from the other treatments, with the lowest rate, when seeds were scarified. The seeds had not been scarified, the SGI of T2 continued to show the lowest rate, while the highest was recorded in T5, coinciding with the highest value displayed for germination.

Ribeiro et al. (2008) working with *Mimosa caesalpiniaefolia* Benth, found that with the gradual increase of the concentration of salts there was a reduction in germination rate, unlike the species studied here, which presented no decrease in germination rate on the basis of salts.

For the length of intact seedlings in scarified and non-scarified seeds, there was statistical difference only for T2. Compared the treatments in non-scarified seeds, it was observed that T3, T4 and T5 were not statistically different, with the longer lengths. In gliricidia seeds (*Gliricidia sepium* (Jacq.) Steud), Holland et al. (2007) observed its sensitivity to salinity, and when compared to the neem (*Azadirachta indica* A. Juss.) and a turco (*Parkinsonia aculeata* L.) where the height values were lower.

For seedling dry matter, only T2 and T3 showed differences between the scarified and non-scarified seeds, and compared the treatments within each type of seed, no differences were observed. Usually in sensitive plants, salinity decreases the emergence rate and reduces dry and fresh weight of shoot and root system (Shannon et

al., 1998). However, despite not having determined the tolerance of *L. ferrea* salinity, this can also be a tolerant species to salinity in electrical conductivity used in this study (5 dSm^{-1}).

According to Larcher (2000), a moderate degree plant's salt resistance is useful in trying to use salt affected soil in dry regions. Whereas about one-third of Earth's land surface is arid or semi-arid, and that this area is estimated that, half is affected by salts, it is important to invest in research aimed at identifying species able to germinate and survive well in these conditions.

4. Conclusions

The saline solution composed K_2HPO_4 influenced by the water absorption of the non-scarified seeds of *L. ferrea*, showing high maximum absorption compared to absorption of other studied and distilled water solutions, while for the scarified there was no interference of the solutions salt in absorption.

Scarified and non-scarified Seeds of *L. ferrea* have different times for the physiological phases of water absorption, where the intact seeds needed a longer time to maximum absorption, when compared to the scarified.

The action of salts in the germination and non-scarified and scarified seed vigor, did not significantly reduce these figures, even with significant differences, a fact due to electrical conductivity of 5 dSm^{-1} not be enough to affect the germination of *L. ferrea* seeds.

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