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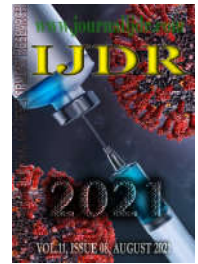
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TOLERANCE AND PRE-GERMINATIVE TREATMENTS IN JUCÁ SEEDS (*Libidibia ferrea* (Mart. ex Tul.) L.P. Queiroz var. *ferrea*) SUBMITTED TO SALINITY

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ABSTRACT

Germination and vigor of seeds of *Libidibia ferrea* (Mart. ex Tul.) L.P. Queiroz var. *ferrea*) may be compromised due to salinity of water or soil, conditions very common in semiarid regions. In this context, the objective was to evaluate the physiological potential of seeds of *L. ferrea* subjected to saline stress, as well as verifying the effects of hydropriming and osmopriming as mitigating this stress. For this, two experiments were carried out, the first being in a completely randomized experimental design, in a factorial scheme 2 x 6, two seed lots, submitted to six salinity levels (0, 4, 8, 12, 16 and 20 dS m⁻¹). The second experiment was also carried out in a completely randomized experimental design in factorial scheme 4 (priming) x 4 (salinity). In both experiments, four replicates of 25 seeds were used, and the variables analyzed were: first count, germination, seedling length (aerial part and root) and dry mass (aerial part and root). The response to salinity varied between lots the *L. ferrea*, and germination was negatively affected by increased salinity in only one of the lots, while vigor was impaired from 12 dS m⁻¹ in both lots. Seed hydropriming and osmopriming provided greater tolerance to saline stress up to 16 dS m⁻¹, compared to non-priming ones.

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INTRODUCTION

The Jucá (*Libidibia ferrea* Martius of Basilion *Caesalpinia* Mart. Ex Tul.), family Fabaceae, is native to Brazil with wide distribution in the North and Northeast regions. This species is used in civil construction, home medicine, landscaping and recovery of degraded areas (LORENZI, 2013). Usually produces abundant amount of seeds with dormancy of the tegumentary type (MAIA, 2012; DANTAS et al., 2015). Although jucá is a species adapted to the semiarid region, the germination of its seeds and plant growth can be compromised due to soil or water salinity, very frequent conditions in the Northeast region of Brazil. Salinity is a growing problem that affects the productivity of species in several regions of the world (SHABALA, 2013). The effect of dissolved solutes is similar to water deficiency in soil, whose plants in situations of excess salinity respond similarly to water deficit. Toxicity is another effect that can be observed when the soil has excess salt. High concentrations of total saicans in plant cells can inactivate enzymes and inhibit protein synthesis (TAIZ; ZEIGER,

2013). Considering that salinity is one of the factors that promotes inhibition in plant growth and productivity, it is necessary to search for alternatives that help mitigate the degenerative action of salt stress on crops (NÓBREGA et al., 2020). In this sense, the physiological priming of seeds has been shown to be a promising alternative, attenuating the negative effects of saline stress. In some species, this technique provides more uniform and synchronized germination, high emergence rate and seedling development, even under conditions of adverse abiotic stresses, in addition to high aerial part growth rate (ARUNKUMAR, JEGADEESWARI, USHAMALINI, 2019). Osmotic agents such as sodium chloride (NaCl), potassium nitrate (KNO₃), magnesium sulfate (MgSO₄), magnesium chloride (MgCl₂), potassium orthophosphate (KH₂PO₄), manganese sulfate (MnSO₄), mannitol and polyethylene glycol are the most used in seed priming of several species. In the laboratory, these osmotic agents simulate situations of water scarcity without penetrating the seed tegument due to the size of their molecules (MARCOS-FILHO, 2015). The priming technique is already a widely used practice to increase germination

speed and emergence uniformity in vegetable seeds, including many patented treatments for some species (PAPARELLA et al., 2015). Thus, the objective was to evaluate the tolerance of jucá seeds submitted to different salinity levels, as well as to verify the effects of pre-germinative treatments (physiological priming) on seeds submitted to salt stress.

MATERIAL AND METHODS

Two experiments were carried out at the Seed Analysis Laboratory of the Center for Agrarian Sciences of the Federal Rural University of the Semi-Arid, Mossoró, RN, Brazil. The two batches of seeds of *L. ferrea* from two different locations. Lot I consisted of seeds, provided by the Center for Ecology and Environmental Monitoring of the Federal University of Vale do São Francisco in February 2019; lot II was obtained from ripe fruits on the campus of the Federal Rural University of the Semi-Arid, Mossoró campus (5° 11' of south latitude and 37° 20' of west longitude and average altitude of 18 m), collected between July/August 2019. Experiment I was carried out in a completely randomized design, with four replicates of 25 seeds, in a 2 x 6 factorial scheme, consisting of two lots and six levels of water salinity (0, 4, 8, 12, 16 and 20 dS m⁻¹). For this, solutions were prepared with sodium chloride (NaCl) and distilled water, whose levels were adjusted with the aid of a bench conductivity meter. For level 0 dS m⁻¹, distilled water was used only. Previously, seed dormancy was overcome according to the methodology of Alves et al. (2009). Soon after, the seeds were treated with fungicide Manfil® at a dose of 1.5 g/kg of seeds, to avoid possible contamination by fungi. Sowing was performed on three sheets of paper towels, two as a base and a third to cover, moistened with the equivalent of 2.5 times the dry weight of the papers with the solutions described above. After sowing, the papers were rolled and packed in a transparent and sealed plastic bag to reduce moisture loss and kept in a germination oven at 25 °C and eight hours for 20 days (BRASIL, 2013).

Experiment II was carried out with the most salinity-sensitive lot in experiment I. The design was completely randomized, in a factorial scheme 4 x 4, being four salinity levels (0, 12, 16 and 20 dS m⁻¹) and four types of priming (hydropriming, osmopriming at -0.2 and -0.4 MPa, and seeds without priming), with four replicates of 25 seeds. To determine the seed priming time, the imbibition curve was determined with four replications of 25 seeds placed between two sheets of paper towel, moistened with distilled water (hydropriming) and in polyethylene glycol osmotic solutions (PEG 6000) at potentials -0.2 and -0.4 MPa, prepared according to the table of Villela et al. (1991). The water absorption level was measured by weighing on an analytical scale every hour for 24 hours and then every 6 hours, until the radicle emission in at least 50% of the seeds of each repetition. The water content absorbed in each time was calculated by the following expression: %absorbed water = (PF - Pi) / Pi x 100, where PF = final weight; Pi = initial weight. After determining the priming times by the imbibition curve, a germination test was installed with four replicates of 25 seeds, consisting of no-priming seeds; -0.2 and -0.4 MPa for 60 and 84 hours, respectively; hydropriming for 36 hours. The sowing occurred similarly to experiment I. In both experiments were evaluated:

First germination count - the fifth day for this evaluation was considered, adopting as a criterion the emission of the primary root, whose results were expressed as a percentage.

Germination -the criterion was the formation of normal seedlings at 20 days after sowing (BRASIL, 2013), whose results were expressed as a percentage.

Lengths of aerial parts and primary root of normal seedlings - at the end of the germination test, all normal seedlings were measured with the aid of a ruler graduated in centimeters. The measurement of the aerial part was made from the insertion of the basal portion of the primary root to the apex of the aerial part; for the length of the primary root, the measurement was taken from the apical part to the

basal point of the primary root and the results expressed in centimeter per seedling (cm. seedling⁻¹).

Dry mass of seedlings - after measurements of the aerial and root parts, the seedlings were packed in paper bags separated by treatment and placed to dry in a forced air circulation oven at 60°C for 72 h. Then, it weighed on a precision scale and the results expressed in milligrams per seedling (g. seedling⁻¹). In both experiments the obtained data were submitted to variance analysis, and in experiment I, when the effect of the lots was significant, the means compared by the Tukey test (p<0.05). The levels of osmotic potentials were analyzed by regressions in the Excel 2010 program, and the f value corrected.

RESULTS AND DISCUSSION

Experiment I: In the first count of the germination test, it was found that lot I was not affected by the increase in salinity levels, while the II showed a significant reduction in germination from 12 dS m⁻¹ (Figure 1A). These results indicate that the lots have different behaviors, however, they have the same vigor. Seeds with high physiological potential are less affected under conditions of abiotic stress.

For lot II, the increase in the saline concentration of the substrate caused the reduction in water absorption capacity by the seeds, negatively influencing the radicle emission capacity. In general, seeds require longer contact time with the solution to occur the process of imbibition and absorbing enough water for the beginning of the germination process. And the presence of sea in the middle may have caused changes in seedling metabolism, further reducing the process speed (CRUZ et al., 2020). Seed germination of lot I was also not affected by increased levels of the seed. This result agrees with Dantas (2018), stating that, with the exception of *Urundeuva Myracrodruon*, most native species of caatinga are highly tolerant to salinity, presenting high germination in electrical conductivity above 10 dS m⁻¹. On the other hand, lot II was negatively affected by the increase in salinity levels, and it was observed that in the absence of salt stress the highest germination percentages were obtained (Figure 1B). For this lot, in the situation of salt stress, there was a significant reduction from the salinity level of 4 dS m⁻¹. Seed germination quickly, uniformly and in a higher percentage is an important characteristic when it comes to sowing in the field, since this process when it happens slowly, the seeds are arranged to the adverse conditions of the environment (FERREIRA et al., 2013). Some species, when exposed to certain salt levels, present physiological disorders, such as reduced water absorption by seeds, which leads to a series of metabolic changes, including altered enzymatic activities, general reduction of hydrolysis and, consequently, decreased germination (NEDJIMI, MOHAMMEDI & BELKHEIRI, 2014). The different behaviors between lots may be due to lot I being more tolerant to salinity than lot II for variable germination, since the response to salinity varies between species (SILVA et al., 2019), and between populations of the same species (MEGDICHE et al., 2007; GHARS, DEBEZ, ABDELLEY, 2009; DEL VECCHIO et al., 2012; SANTO et al., 2014; SANTO et al., 2017).

The results of this work indicated that there was a difference in germination behavior for the different plots evaluated, in which lot I presented tolerance up to the level of 20 dS m⁻¹. Lot II responded negatively to germination by increasing the salinity level from the level of 4 dS m⁻¹. The reduction of the values of the first count and the final germination can be attributed to the low water potential of the substrate, promoted by the increase of the concentration of sodium chloride, inducing a lower capacity of water absorption by the seeds due to the osmotic and toxic effects of salt (SECCO et al., 2010). When comparing the results of the first count with the germination counts, it was verified that the data of the first variable were the most affected with the increase of the electrical conductivity of the water (Figures 1A and B). This fact is expected because germination speed is the first variable affected by reduced water availability.

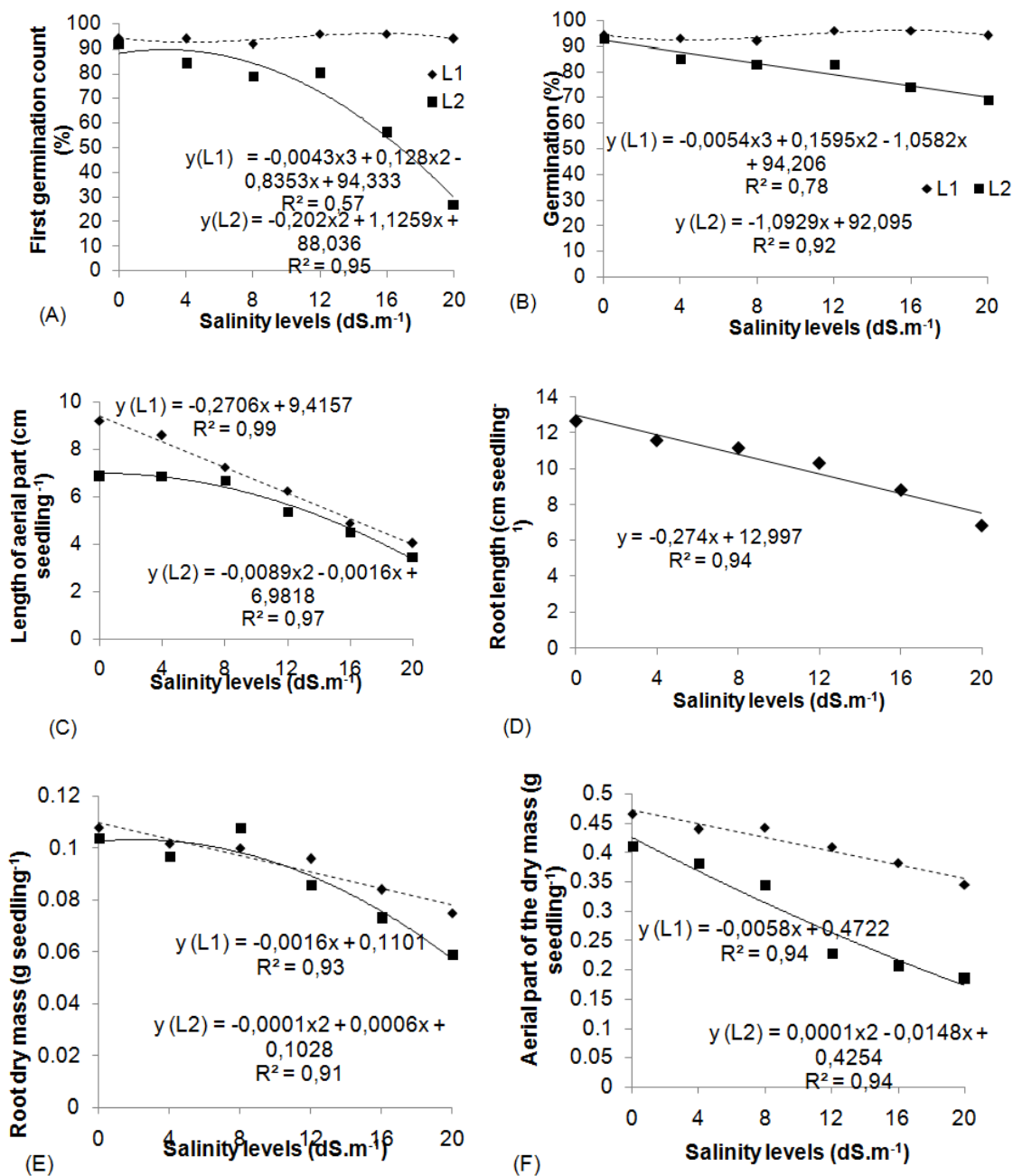


Figure 1. First count (A), germination (B), aerial part length (C), root length (D), root dry mass (E) and aerial part dry mass (F) of seedlings, of two lots (L1 and L2) of jucu seeds (*Libidibia ferrea* (Mart. ex Tul.) L.P. Queiroz var. *ferrea*), subjected to different levels of salinity

The dry masses of the aerial and root parts also regressed with increasing salinity, and those from lot II were more sensitive, presenting lower dry mass accumulation compared to I (Figures 1E and F). Therefore, it is verified that salinity causes changes in the plant's ability to absorb, transport and use ions necessary for growth, in addition to reducing the metabolism rate of assimilation and the activity of enzymes responsible for respiration and photosynthesis (NOBRE et al., 2010). Thus, it decreases the acquisition of energy for cell growth and differentiation in tissues and, therefore, reduces embryonic axis elongation and dry matter production (NOBRE et al., 2010). Second PEDÓ et al. (2014), the lowest dry matter mass allocation may be the result of the effect of the high concentration of sodium chloride on the mechanisms of hydrolysis and mobilization of reserves for the seedling. In general, it is perceived that the germination of seeds of *L. ferrea* submitted to salt stress showed a certain tolerance, with germination above 70% in both lots, however, seedling vigor decreased as the water salinity level increased.

Experiment II: The initial water content of the seeds was 9.0%. Regarding the imbibition curve, regardless of the type of priming, there was rapid water absorption in the initial twelve hours of imbibition, with an average increase in water content of approximately 3.0% per hour (Figure 2). Between 12 and 36 hours, there was a reduction in water absorption in hydropriming seeds, while for osmopriming seeds (-0.2 MPa) this decrease was between 12 and 60 hours and, for osmopriming seeds (-0.4 MPa), this interval was longer, occurring between 12 and 84 hours. The radicle emission, in at least 50.0% of the seeds, occurred after 48, 72 and 96 hours for the hydropriming seeds, osmopriming (-0.2 MPa) and (-0.4MPa), respectively. The curves of the three types of priming presented the three-phase pattern proposed by Bewley et al. (2013). Phase I was characterized by rapid water absorption and increased seed metabolism; in II, the imbibition remained constant during the occurrence of metabolic events necessary for embryo development; and finally, in phase III, the seeds returned to absorb water quickly and then the emission of the primary root occurred in viable and non-

dormant seeds. The duration of each phase of instilling is directly influenced by the speed of water absorption by the seed, and may vary according to the thickness of the integument, water availability, chemical composition, temperature, contact area, physiological condition of the seed (CARVALHO; NAKAGAWA, 2012).

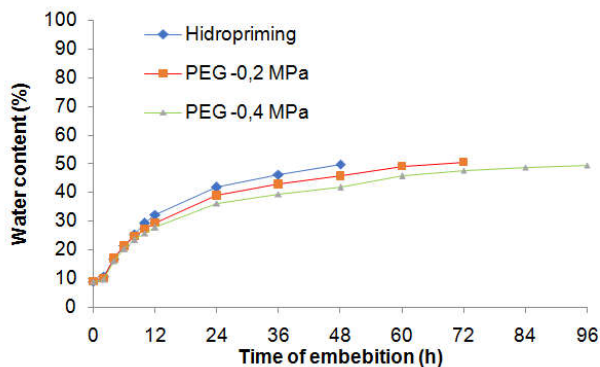
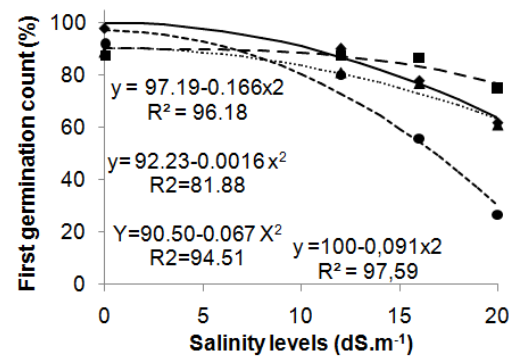


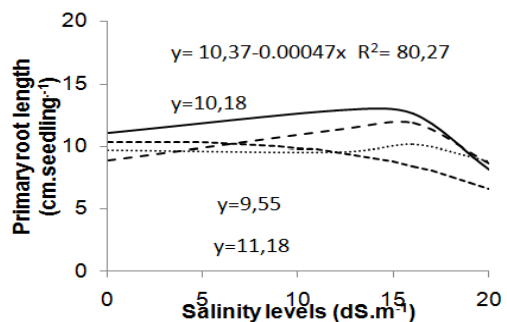
Figure 2. Embebtion curve in jucá seeds (*Libidibia ferrea* (Mart. ex Tul.) L.P. Queiroz var. hydropriming, osmoprimering in PEG -0.2 MPa and -0.4 MPa at 25 °C

In addition, seeds with high physiological potential are more effective in mobilizing their energy reserves, providing fast and uniform germination in adverse conditions, and thus higher quality plants. Also in this sense, seeds of high physiological quality allow greater translocation of reserves of cotyledons or endosperm to the embryonic axis during the germination process, resulting in more vigorous seedlings (PÉGO; NUNES; MASSAD, 2011). The increase in salinity levels resulted in a gradual decrease in seedling growth, expressed as a reduction in root and aerial part length (Figures 1C and D). But specifically, the length of the shoot and root was differentially affected by salinity, with the roots being more drastically affected, even with a low level of salinity. This is attributed to the fact that the roots are directly exposed to salinity (OUJI et al., 2015). Regarding aerial part length, lot I obtained a significant linear reduction and quadratic lot II, according to the increase in salinity levels. Regarding seedling root length, the results of the analysis showed a decreasing linear effect with increasing NaCl concentrations, indicating a reduction in root length in both lots. The high concentration of salts, because it reduces the osmotic potential and provides the action of ions on the protoplasm, is considered a stress factor for plants (ANDRÉO-SOUZA et al., 2010). Thus, it can limit germination, slowing or hindering seedling growth and development and its chances of survival (PEREIRA et al., 2012). Organ growth depends on cell elongation (expanding growth), cell division, and cell differentiation. By decreasing about -0.1 MPa in osmotic potential, there is an appreciable decrease in cell growth, and consequently in root growth (PEIXOTO, 2020). The results of this research show that the growth of seedlings of *L. ferrea* are affected in a saline environment, however, the reduction of the aerial part is greater when compared to the root, probably because salt stress reduces the availability of water necessary for seedling growth and, they invest more in root growth in order to improve the efficiency of water absorption and, consequently, to ensure its survival, which is therefore a characteristic of adaptation of this species to water deficiency and other environmental factors (SILVA et al., 2019). In the seeds without priming there was a decrease in the values of the first germination count with increasing salinity. Up to 12 dS m⁻¹, there was no significant difference between seeds without priming and the priming, however, in 16 and 20 dS m⁻¹, priming methods were efficient compared to no priming seeds, since they presented higher values (Figure 3A).

The reduction of germination with increasing salinity happens because salts cause cytotoxicity, dehydration and reduces metabolic activity and synthesis of new tissues, due to decreased water availability, resulting in lower germination speed or even loss of germination capacity (TAIZ, ZEIGER, 2013; MARCOS-FILHO, 2015).



(A)



(B)

--- No Priming - - - Hydropriming 36 horas PEG -0.2 Mpa
 ——— PEG -0.4 MPa

Figure 3. First count of germination (A), primary root length (B) and aerial part length, from jucá seeds (*Libidibia ferrea* (Mart. ex Tul.) L. Queiroz var. *ferrea*) submitted to different salinity levels and types of physiological priming

In this sense, Nogueira et al. (2018) found that the effects of salinity on the germination of *Mimosa Ophthalmocentra*, also caused a reduction in germination from 16 dS m⁻¹. On the other hand, priming can modify the energy state of water, changing its distribution in different seed tissues, resulting in higher germination speed after treatment, as there is greater availability of water and increased hydration level of macromolecules that participate in the germination process, also reducing the availability of injury during pregnancy (BATISTA et al., 2018; RIBEIRO et al., 2019). Moreover, based on the soaking curve, it is verified that the hydro and osmo priming seeds are physiologically closer to the beginning of the third phase of the curve and, thus, to the root emission, presenting higher values of germination speed (ATAÍDE et al., 2016). Through physiological priming, it is observed that seedling emergence occurs more rapidly, thus favoring their vegetative development, as well as decreasing the exposure of seeds to possible unfavorable conditions of the environment (MARCOS-FILHO, 2015).

As the scarcity of water availability is the first phase of salt stress, due to osmotic stress, one of the main mechanisms to improve seed germination and growth is controlled seed intake through priming (PIRASTEH-ANOSHEH; HASHEMI, 2020). With regard to root length (Figure 3B), there was a significant reduction from 16 dS m⁻¹ for no priming seeds. High salinity levels reduces first of all cell elongation and division due to the osotic effect created around the root. Secondly, it causes imbalance and toxicity of the Na⁺ ion, which damages the cells of the perspirant leaves. Consequently, seedling growth decreases in environments with high salt content (SHAH; HOUBORG, HOUBORG, MCCABE, 2017). The roots derived from hydropriming seeds and osmoprimering at -0.4 MPa showed a

significant reduction only in 20 dSm⁻¹. However, osmopriming at -0.2 MPa ensured root growth, with no reduction in values even in high salinity conditions (Figure 3B). Analyzing the isolated effect of the priming methods in relation to aerial part length (Table 2) it was found that the hydropriming and osmopriming seeds presented statistically higher values, comparing the no priming seeds.

Table 2. Isolated effect of physiological priming types on aerial part length of jucá seedlings (*Libidibia ferrea* (Mart. ex Tul.) L.P. Queiroz var. *ferrea*)

Types of priming			
No priming	Hydro 36h	PEG -0.2 MPa	PEG -0.4 MPa
5.08 b	7.37 a	7.70 a	7.70 a

*Averages followed by the same letter do not differ from each other by the Tukey test at 5% probability.; ; Hydropriming; PEG= polyethylene glycol

The results of the isolated effect of salt stress under aerial part length show that increased salinity levels cause the gradual reduction of the aerial part of the seedlings from the level of 12 dS m⁻¹ (Figure 4). High salinity levels slow sows the metabolism and transport of embryo reserves, thus affecting seedling development (BEWLEY et al., 2013).

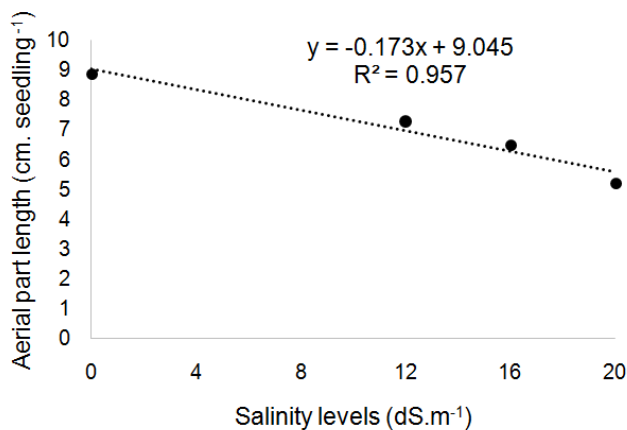


Figure 4. Isolated effect of salinity levels on aerial part length of jucá seedlings (*Libidibia ferrea* (Mart. ex Tul.) L.P. Queiroz var. *ferrea*)

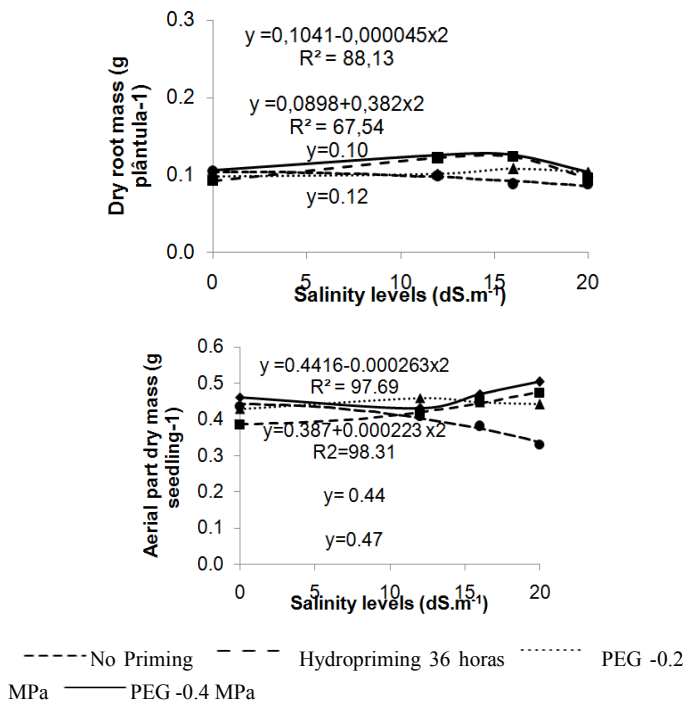


Figure 5. Dry root mass (A) and aerial part dry mass (B) of jucá seedlings (*Libidibia ferrea* (Mart. ex Tul.) L.P. Queiroz var. *ferrea*) different salinity levels and types of physiological priming

The results of the dry mass of the root indicated that for the seeds without priming, as well as the osmopriming at -0.2 MPa, they showed no difference for the different salinity levels tested. However, it is possible to verify that there was a decrease in the dry mass of the root for the seeds without priming. For osmopriming patients, there was an increase in mass when compared to the control, and this mass increase was more expressive for those that were priming in PEG -0.4 MPa (Figure 4A).

The dry mass of the aerial part of the no priming seeds decreases significantly as the stress of the saline increased. When the seeds were osmoconditioned, there was an increase in dry mass, especially for those no priming with PEG -0.4 MPa (Figure 4B). During osmotic priming, increases in soluble protein content and specific enzymes occur, which provides a higher concentration of solutes, resulting in faster growth and, consequently, greater biomass accumulation (SMITH; COBB, 1991). The results of this study showed that treatments with hydropriming and osmotic priming with PEG6000 mitigated the harmful effects of salinity on the vigor of seeds of *L. ferrea* and favored seedling growth under saline concentration.

CONCLUSIONS

There is a difference in salinity tolerance between lots, and lot 1 is more tolerant to increased salinity. The lot I started the physiological quality reduction with 8.0 dS m⁻¹ and lot I at 4.0 dS m⁻¹. The hydropriming and osmopriming of *L. ferrea* seeds provide greater tolerance to saline stress compared to no priming ones, up to 16 dS m⁻¹.

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