

Influence of the mass of *Luetzelburgia auriculata* (Allemão) Ducke seeds on tolerance to saline stress

Maria de Fátima de Queiroz Lopes^{1*}, Riselane de Lucena Alcântara Bruno¹, Ronimeire Torres da Silva¹, Francisco Hélio Alves de Andrade², Lucas Kennedy Silva Lima³, Leonardo Vieira de Sousa¹, William Santana Alves¹

¹Universidade Federal da Paraíba UFPB, Brazil

²Universidade Federal de Lavras UFLA, Brazil

³Universidade Federal do Recôncavo da Bahia UFRB, Brazil

*Corresponding author: fatimaqueiroz0@gmail.com

Abstract

Luetzelburgia auriculata is used in the reforestation of degraded areas in Brazil due to its adaptation to the conditions of the country's semiarid region. However, saline stress is one of the main abiotic factors with a negative impact on plant development. The aim of the present study was to evaluate the influence of the mass of *Luetzelburgia auriculata* seeds on tolerance to saline stress. The experiment was conducted with a completely randomized design in a 2 x 5 factorial scheme with two classes of seeds [light (< 0.35 g) and heavy (≥ 0.35 g)], from ten morphologically similar mother plants that showed natural variation in seed mass, and five salinity levels, distributed in four replicates of 25 seeds. We evaluated the following variables: germination rate; germination speed index; mean germination time; initial seedling growth; and fresh and dry mass of the shoots, cotyledons and roots. The data were submitted to analysis of variance. Means for seed classes were compared using the F-test and regression analysis was performed for NaCl levels. The increase in saline stress led to reductions in germination, root length, fresh and dry mass of the shoots, and the GSI, as well as a delay in MGT. Seedlings from heavy seeds (≥ 0.35 g) exhibited greater tolerance to salt stress than those from light seeds (< 0.35 g), due to lower occlusion caused by NaCl levels. Thus, heavy seeds of *L. auriculata* are more tolerant to salt stress and should be prioritized, especially in the presence of high salinity levels.

Keywords: Abiotic stress; Germination of seeds; Heavy seeds; Light seeds; Salinity; Semiarid region.

Abbreviations: WTS_weight of one thousand seeds; G_germination rate; FGC_first germination count; GSI_germination speed index; MGT_mean germination time; SL_shoot length; RL_root length; SFM_shoot fresh mass; RFM_root fresh mass; FMCot_cotyledon fresh mass; SDM_shoot dry mass; RDM_root dry mass; DMCot_cotyledon dry mass; RDM/SDM_root dry mass-shoot dry mass ratio; ANOVA_analysis of variance.

Introduction

Luetzelburgia auriculata (Allemão) Ducke belongs to the family Fabaceae and is mainly found in northeastern Brazil (Cardoso et al., 2014). This species is used in the reforestation of degraded areas due to its potential as a source of lumber and its adaptation to the conditions of the semiarid region (Lorenzi, 2008). The success of a large part of native plant species in northeastern Brazil for diverse exploitation and recovery purposes depends on their adaptability to the environment and tolerance to abiotic stress factors, such as drought and soil salinity (Mendonça et al., 2010).

Saline soils are common in the semiarid region of northeastern Brazil due to the low rainfall index and high evaporation rate, favoring the accumulation of salts, which are not lixiviated and consequently hamper the development of plants (Vasconcelos et al., 2013). Another common problem in the region is the low quality of the water used for irrigation of seedlings in nurseries, delaying growth and planting in the field (Guedes et al., 2015).

Salinity exerts a negative influence on the germination of seeds, causing a reduction in water availability due to solutes. When in excess, these solutes have toxic effects that result in the phenomenon known as physiological drought, as well as an imbalance in ionic homeostasis (Silva et al., 2011). Saline stress also limits soil water uptake by the plant, which induces osmotic stress and thus nutritional imbalance (such as K and Ca) due to the high concentrations of potentially toxic salts in soil and plant cells (Shrivastava and Kumar, 2015). The accumulation of ions at toxic levels interferes in physiological processes such as photosynthesis, respiration, starch metabolism and nitrogen fixation (Farooq et al., 2015; Lima et al., 2020). By reducing leaf area and photosynthesis, these effects can result in reduced plant biomass production and consequently lower crop yield (Atieno et al., 2017; Win et al., 2018; Lima et al., 2020). In response to salt stress, plants simultaneously employ various physiological, biochemical, anatomical, morphological and genetic tolerance mechanisms, but these responses depend on signal perception, integration and

processing (Hanin et al., 2016). Among the genetic and biochemical responses associated with salt stress in plants are those involved in the activation of signaling pathways. Reactive oxygen species (ROS), despite their potential toxicity, have the advantage of being versatile signaling molecules within cells. Nitrogen-activated protein kinase (MAPK) can trigger plant response to biotic and abiotic stresses by activating antioxidant enzymes. ROS signaling is closely associated with cellular homeostasis and highly integrated with hormonal signaling networks, allowing plants to regulate development processes, as well as adaptive responses to environmental constraints (Miller et al., 2010; Hanin et al., 2016). In this sense, heavy seeds (higher levels of reserves) can trigger homeostatic balancing mechanisms more efficiently than light seeds and thus enable greater tolerance to salt stress. However, lighter seeds tend to have a larger surface/volume ratio than heavier seeds because of their smaller size, which facilitates the germination process, but with a lower quantity of reserves (Kopper et al., 2010).

Studies have been conducted to investigate the tolerance of forest species to saline stress, such as *Physalis angulata* L. (Souza et al., 2011), *Tabebuia aurea* (Mart.) Bur (Silva et al., 2014), *Gallesia integrifolia* (Spreng.) Harms (Lopes et al., 2015), and *Erythrina velutina* Willd. (Ribeiro et al., 2017). However, studies of *L. auriculata* are scarce and have evaluated saline stress only in the seedling phase (Bessa et al., 2017). Moreover, no studies have determined whether there is a relation between seed mass and tolerance to saline stress. Such information is fundamental for the determination of the degree of salt tolerance of this species (Araújo et al., 2016). Thus, the aim of the present study was to evaluate the influence of the mass of *Luetzelburgia auriculata* seeds on tolerance to saline stress.

Results and Discussion

Water content and weight of one thousand seeds

The moisture content was around 9.0% in both classes of *L. auriculata* seeds [light (< 0.35 g) and heavy (\geq 0.35 g)], demonstrating that mass exerted no influence on hygroscopic balance. In the determination of the 1000-seed weight, the difference between classes was 170 g (Table 1). The variation in seed mass and size is an attribute used as an adaptation and conservation strategy of the species for perpetuation in different environments (Oliveira et al., 2006).

Analysis of variance

ANOVA (Table 2) revealed effects for isolated factors and interactions between factors. Interactions were significant for the following factors: germination rate (G), first germination count (FGC), germination speed index (GSI), mean germination time (MGT), root length (RL), and cotyledon fresh mass (FMCot). Differences between seed classes were found for the majority of characteristics. The exceptions were root length (RL), shoot fresh mass (SFM), cotyledon fresh mass (FMCot), and shoot dry mass (SDM). The heavy seeds (\geq 0.35 g) had higher values than the light seeds (< 0.35 g) for the majority of variables analyzed. In contrast, the light seeds had higher values for root fresh mass (2.64 g) and mean germination time (4.9 days) in comparison to the heavy seeds (2.08 g and 3.87 days,

respectively) (Table 2). However, a shorter germination time signifies greater seed vigor, which is an important characteristic for the early acquisition of viable seedlings.

Physiological evaluations

In the analysis of the electrical conductivity of the solution and seed mass, germination, evaluated 25 days after the onset of the experiment, declined with the increase in saline concentration for the light seeds, whereas the mean germination rate remained constant (93%) for the heavy seeds (Fig. 1A). Studying *Chorisia glaziovii* O. Kuntze seeds submitted to saline stress, Guedes et al. (2011) also found an approximate 50% reduction in germination with the increase in saline concentration of the solution up to 6 dSm⁻¹. Silva et al. (2014) found a maximum germination rate of 55% for *Tabebuia aurea* (Mart.) Bur. when the electrical conductivity of the solution corresponded to 1.79 dSm⁻¹ and found a decreasing germination rate with the increase in the salt concentration.

The negative effect of saline stress on the germination of light seeds was possibly due to the lower quantity of reserves these seeds have in comparison to heavy seeds. This response is important from the ecological standpoint, demonstrating that heavy seeds exhibit greater tolerance to abiotic stress and possibly adapt better to unfavorable environments.

The light seeds presented a reduction in the first germination count (FGC) with increase in the NaCl concentration, ranging from 93% in the control to 78% at the highest salt concentration (Fig. 1B). Despite this reduction, the species demonstrates potential tolerance to saline environments, as larger reductions have been found in other species that occur in the *Caatinga* biome (dry scrubland forest), as reported by Guedes et al. (2011), who found considerable reductions in the FGC for *Chorisia glaziovii*, with no germination at saline concentrations of 4.5 and 6.0 dSm⁻¹. This finding can be attributed to the harm caused by the high osmotic pressure due to the excess salt, which leads to a reduction in water availability to seeds (Sá et al., 2016). In contrast, the heavy seeds were not influenced by the increase in salt in the solution, maintaining a mean FGC of 90% (Fig. 1B). This demonstrates that heavier seeds exhibited greater tolerance to the reduction in water availability and the harm caused by NaCl.

Regarding the germination speed index (GSI), differences were found between the two classes of seeds, with linear decrease in performance from 6.6 to 4.5 dSm⁻¹ for the light seeds and quadratic performance for the heavy seeds (Fig. 1C) with the increase in saline concentration. Evaluating the effect of saline stress on the germination of *Myracrodruon urundeuva* (Allemão) Engl. seeds, Oliveira et al. (2007) also found a reduction in the GSI at higher saline concentrations, with a lower GSI in comparison to the control beginning at a concentration of 20 dSm⁻¹.

Mean germination time (MGT) increased linearly with the light seeds, going from 3.7 days at a concentration of 0.0 dSm⁻¹ to 4.7 days at a concentration of 6.0 dSm⁻¹ (Fig. 1D). Dutra et al. (2014) found a larger increase in the MGT with the increase in salt concentration in an experiment involving *Jacaranda pteroides* Cham. seeds, rising from 11.4 to 27.24 days, a 41.85% increase. The behavior of the heavy seeds fit the quadratic regression model, with the largest MGT (4.18 days) being observed when the saline concentration was 3.92 dSm⁻¹.

Table 1. Moisture content and 1000-seed weight (WTS) in the two mass classes of *Luettelburgia auriculata* (Allemão) Ducke seeds

Mass class	Moisture (%)	WTS (g)
Light (< 0.35g)	9.76a	316.6b
Heavy (≥ 0.35g)	9.16a	487.2a
CV (%)	4.33	4.38

Same letters do not differ significantly from each other (ANOVA F-test at 5% probability).

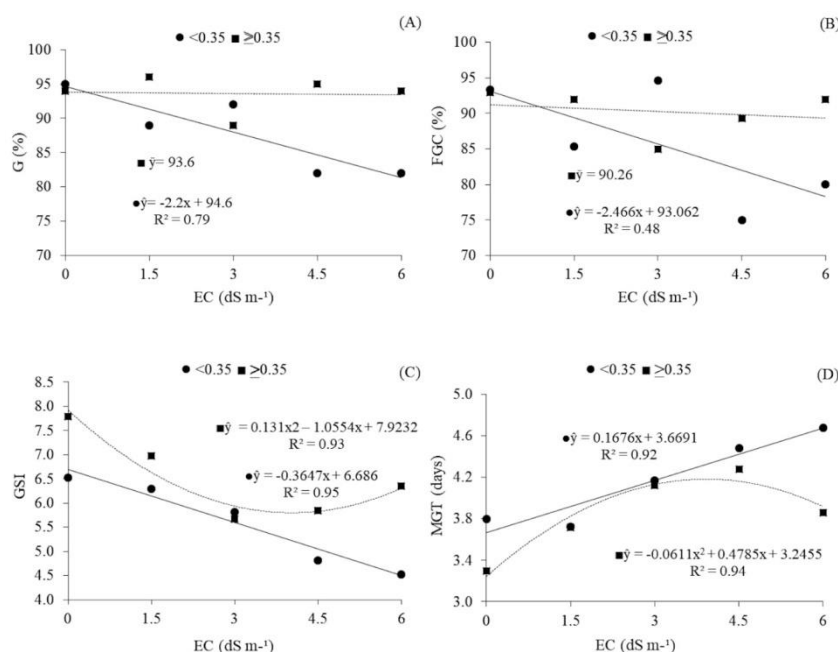


Fig 1. Physiological variables of light (< 0.35 g) and heavy (≥ 0.35 g) *Luettelburgia auriculata* (Allemão) Ducke seeds submitted to different electrical conductivities of solution (dS m⁻¹). G: germination rate (A), FGC: first germination count (B), GSI: germination speed index (C), MGT: mean germination time (D).

Table 2. ANOVA results for biometric and physiological characteristics of light (< 0.35 g) and heavy (≥ 0.35 g) *L. auriculata* seeds submitted to differing electrical conductivity of NaCl solution (EC_{sol}).

FV	G	FGC	GSI	MGT	SL	RL	SFM
Mass (M)	12.7**	10.12**	50.8**	27.7**	160.1**	0.13 ^{ns}	12.2**
EC _{sol} (N)	2.11 ^{ns}	5.81**	30.6**	30.6**	4.46 ^{ns}	20.8**	3.3*
M x N	3.40*	5.86**	6.69**	5.67**	1.1 ^{ns}	4.85**	1.5 ^{ns}
Error	28.26	24.61	0.16	0.03	0.10	1.20	1.36
CV (%)	5.86	5.68	6.69	4.60	12.78	6.59	15.19
Regression							
Linear	8.2**	15.23**	108.2**	107.8**	17.2**	58.5**	11.9**
Quadratic	0.18 ^{ns}	1.41 ^{ns}	10.94**	6.32*	0.31 ^{ns}	6.94 ^{ns}	0.29 ^{ns}
Mass							
< 0.35	87.80b	84.71b	5.60b	4.19a	1.88b	16.51a	8.42a
≥ 0.35	93.80a	90.12a	6.51a	3.87b	3.17a	16.65a	7.00b
FV							
	RFM	FMCot	SDM	RD	DMC	RDM/SDM	
Mass (M)	9.2**	0.6 ^{ns}	2.7 ^{ns}	6.8*	155.9*77	7.09**	
EC _{sol} (N)	2.0 ^{ns}	5.7*	7.6**	2.9*	5.67**	0.93 ^{ns}	
M x N	2.3 ^{ns}	3.0*	0.58 ^{ns}	0.45 ^{ns}	0.16 ^{ns}	2.19 ^{ns}	
Error	0.23	2.51	0.01	0.001	0.10	0.01	
CV (%)	20.51	12.29	11.38	20.73	12.74	21.29	
Regression							
Linear	0.001 ^{ns}	0.045 ^{ns}	30.74**	11.41**	21.69**	0.03 ^{ns}	
Quadratic	1.24 ^{ns}	12.43*	0.03 ^{ns}	0.86 ^{ns}	1.42 ^{ns}	0.02 ^{ns}	
Mass							
< 0.35	2.64a	12.66a	1.13a	0.42b	1.88b	0.37b	
≥ 0.35	2.08b	13.08a	1.20a	0.51a	3.19a	0.44a	

**significance at 1%, *significance at 5%, ns – non-significant by ANOVA F-test; G: germination; FGC: first germination count; GSI: germination speed index; MGT: mean germination time; SL: shoot length; RL: root length; SFM: shoot fresh mass; RFM: root fresh mass; FMCot: cotyledon fresh mass; SDM: shoot dry mass; RDM: root dry mass; DMCot: cotyledon dry mass; and RDM/SDM: ratio between root and shoot dry mass, of light (< 0.35 g) and heavy (≥ 0.35 g) *Luettelburgia auriculata* (Allemão) Ducke seeds submitted to different electrical conductivities of the solution.

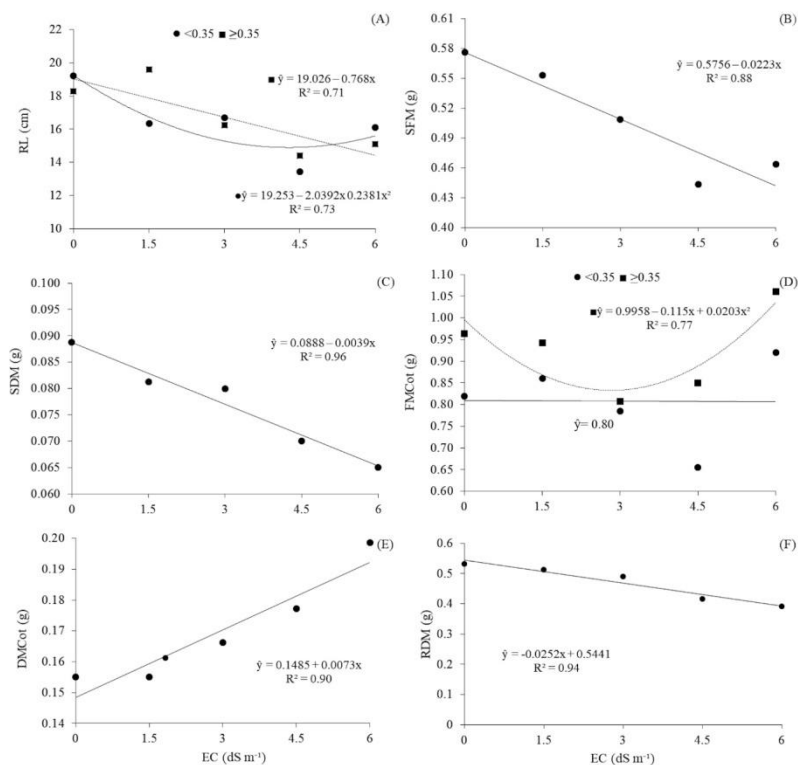


Fig 2. Morphological variables of seedlings from light (< 0.35 g) and heavy (≥ 0.35 g) *Luetzelburgia auriculata* (Allemão) Ducke seeds submitted to different electrical conductivities of solution (ECsol) (dSm⁻¹). RL: root length (A), SFM: shoot fresh mass (B), SDM: shoot dry mass (C), FMCot: cotyledon fresh mass (DE), DMCot: cotyledon (E), RDM: root dry mass (F).

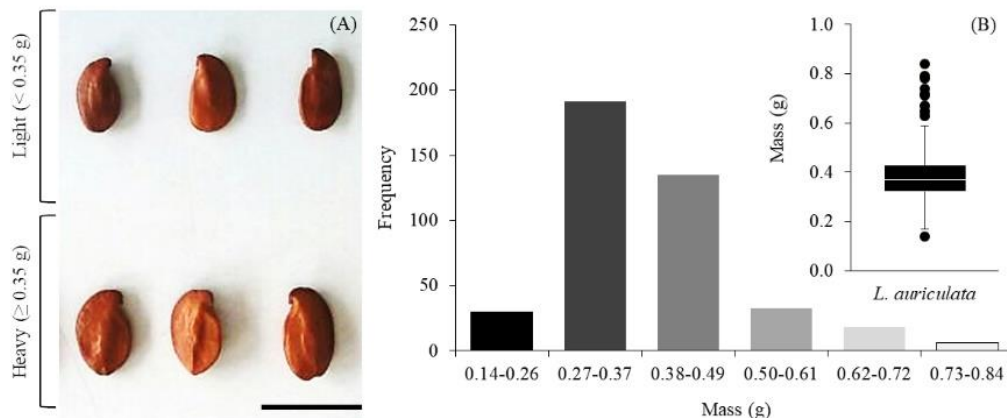


Fig 3. Representation of classes of light (< 0.35g) and heavy (≥ 0.35 g) seeds (A) and distribution of mass of *Luetzelburgia auriculata* (Allemão) Ducke seeds (B), bar = 2 cm.

The results found for the GSI and MGT show that germination became slower with the increase in salinity, demonstrating the need for a longer hydration time in the soaking phase of the seeds for the reactivation of metabolic processes during the development of the embryonic axis (Marcos Filho, 2015).

Morphological evaluations

Root length (RL) values for the light seeds fit a quadratic model, with the shortest length (14.89 cm) found at a saline concentration of 4.28 dSm⁻¹ and the largest length (19.25 cm) in the control treatment. The heavy seeds exhibited decreasing linear behavior with the increase in the electrical conductivity of the solution, going from 19.79 in the control treatment to 14.41 cm at a saline concentration of 6.0 dSm⁻¹ (Fig. 2A). Likewise, the RL of *Chorisia glaziovii* plantlets was

also reduced with the increase in the electrical conductivity of the solution (Guedes et al., 2011).

Mean shoot fresh matter (SFM) and shoot dry matter (SDM) values fit a linear model. SFM went from 0.58 to 0.44 g, corresponding to a 24% decrease, while SDM went from 0.088 g to 0.065 g, a 26% decrease (Fig. 2B and C, respectively). It is possible that shoot development was impaired by the increase in the saline solution due to the reduction in water availability for plantlet growth (Medeiros et al., 2007).

The change in FMCot for the heavy seeds fit a quadratic regression model, with the lowest mass (0.83 g) found when the saline concentration was 2.83 dSm⁻¹ and the highest mass (1.03 g) found when the concentration was 6.0 dSm⁻¹ (Fig. 2D). In contrast, this variable did not fit the proposed regression models for the light seeds, maintaining a mean of 0.80 g. The breakdown of the mobilization of reserves was

not affected until approximately 3.0 dSm⁻¹, with a reduction in mobilization at higher electrical conductivities of the solution. This was also demonstrated by other variables, such as GSI and MGT.

A significant effect for DMCot (Fig. 2E) only occurred for level of salinity, with a linear increase in mass accompanying the increase in saline concentration. This occurred because the reserves did not translocate to the plantlet at higher concentrations. DMCot was 0.15 g at 0.0 dSm⁻¹ and 0.19 g at 6.0 dSm⁻¹, corresponding to a 26.66% increase. Studying the effects of saline stress on the germination of cashew seeds, Marques et al. (2011) found a similar response, in which the increase in saline concentration in the growth medium inhibited the loss of cotyledon reserves, leading to an increase in dry mass in these organs.

Root dry mass decreased with rising salinity, with the lowest values found when the concentration was 6.0 dSm⁻¹ (Fig. 2F). Silva et al. (2005) reported a similar finding in *Cnidoculus phyllacanthus* Pax & K. Hoffm, describing a 66.80% reduction in RDM with the increase in the salinity of the solution. In the present study, the effect was greater in the light seeds (< 0.35 g), which can be attributed to the lower quantity of reserves accumulated in lighter seeds (Carvalho & Nakagawa, 2012).

Materials and Methods

Plant materials

L. auriculata fruits were collected by hand from ten vigorous plants propagated from seeds free of signs of pests and disease. On average, 200 fruits were collected per mother plant in June 2016 from the Poço da Pedra farm in the municipality of Caridade, state of Ceará, Brazil (04° 13' 56" S 39° 11' 33" W). According to the Köppen classification, the climate in the region is BSw'h' (very warm semiarid), with a rainy season in summer and annual precipitation less than 750 mm.

The mother plants (ten) were morphologically and phenologically similar and developed under the same edaphoclimatic conditions, with heterogeneity in seed mass (Fig. 3) being an intrinsic characteristic of the species (Cardoso et al., 2014; Barroso et al., 2016). In this context, the object of this study was to investigate whether there is variation in salt stress tolerance as a function of seed mass, which is a variable trait between and within genotypes.

The seeds were extracted, sorted manually, and placed to dry in the shade for three days. Next, the seeds were put in polyethylene bags and stored in a cold chamber (temperature: ±12 °C; relative humidity: 50%) until the onset of the experiment. Before the experiment, each seed was weighed individually on an analytical scale (precision: 0.001 g). The seeds were then divided into two classes based on mass: light (< 0.35 g) and heavy (≥ 0.35 g). The categories were determined based on the frequency of occurrence of seed mass (Fig. 3) determined from the random weighing of 400 seeds.

Experimental design

The experiment was conducted with a completely randomized design in a 2 x 5 factorial scheme with two classes of seeds [light (< 0.35 g) and heavy (≥ 0.35 g)] and five salt (NaCl) concentrations in the solution, measured by

electrical conductivity (0.0, 1.5, 3.0, 4.5, and 6.0 dS m⁻¹), distributed in four replicates of 25 seeds.

Experimental procedures

The experiment was conducted at the Seed Analysis Lab of the Center for Agrarian Sciences of Federal University of Paraíba, Campus II, in the city of Areia, state of Paraíba, Brazil (06° 57' 48" S 35° 41' 30" W). Before the tests, the seeds were disinfected by immersion in a 0.5% sodium hypochlorite solution for five minutes, followed by rinsing in distilled water. The moisture content was determined using two samples (5.0 g) of each class of seeds (light and heavy), which were placed in aluminum recipients in a force-air oven at 105 °C for 24 hours. Next, the weight of 1000 seeds was determined (Brasil, 2009).

The seeds were submitted to saline stress with different solutions of sodium chloride (NaCl). Four repetitions of 25 seeds were used for each mass class. The seeds were rolled in a paper towel (Germitest®) moistened with an amount equivalent to 2.5 times the dry mass of the paper with either distilled water (control) or the NaCl solution in different concentrations, providing increasing mean electrical conductivity of the solution (1.5, 3.0, 4.5, and 6.0 dSm⁻¹). The rolls were placed in transparent plastic bags to avoid moisture loss by evaporation. The test was conducted in a B.O.D. (biochemical oxygen demand) germinator at a constant temperature of 30 °C, with a 12-h light/dark photoperiod (Nogueira et al., 2012). Further moistening of the paper was performed in all treatments with the respective solution when judged necessary.

Variables

Germination was evaluated daily. Seeds with a radicle measuring 2 mm were considered germinated. The percentage of normal plantlets was determined. The first germination count was performed eight days after the start of the experiment and the last count was performed at 25 days (Nogueira et al., 2012). The germination speed index (GSI) was determined using the formula described by Maguire (1962) and mean germination time (MGT) was calculated using the formula described by Labouriau (1983). At 25 days after the onset of the experiment, we measured the length of normal plantlets that exhibited well-formed essential structures (Brasil, 2009). Fifteen randomly selected plantlets were used for the measurement of the shoots and roots with the aid of a centimeter ruler. The results were expressed as cm.plantlet⁻¹.

The plantlets were then sectioned into roots and shoots (epicotyl and cotyledons) for the determination of fresh weight on an analytical scale (precision: 0.001 g). The structures were then placed separately in kraft paper bags and dried in a forced-air oven at 60 °C for 48 h for the subsequent determination of the dry mass of the roots, shoots, and cotyledons on an analytical scale (precision: 0.001 g). The results were expressed as g.plantlet⁻¹. The root/shoot ratio was calculated by dividing the total dry mass of the roots by the total dry mass of the shoots.

Data analysis

The Shapiro-Wilk test was used to determine the distribution (normal or non-normal) of the data. The data were submitted to analysis of variance (ANOVA). The F-test

was used to compare means of the qualitative data ($P \leq 0.05$). When significant, quantitative data were submitted to polynomial regression analysis ($P \leq 0.05$). The SISVAR 4.3 program was used for the statistical analysis (Ferreira, 2011).

Conclusions

Light seeds (< 0.35 g) of *L. auriculata* are more sensitive to saline stress, with the increasing level of NaCl affecting the majority of variables analyzed. Therefore, heavy seeds (≥ 0.35 g) are more tolerant to salt stress and should be prioritized in reforestation projects in semiarid regions.

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