

ORIGINAL ARTICLE

Seed mass modulates tolerance to water deficit in *Luetzelburgia auriculata* (Allemão) Ducke seedling

Massa da semente modula tolerância ao déficit hídrico em plântulas de *Luetzelburgia auriculata* (Allemão) Ducke

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Abstract

In semi-arid regions, water deficit occurs for long periods, but the adaptation mechanisms developed by endemic species are little known. The objective of this study was to determine the tolerance of *Luetzelburgia auriculata* (Allemão) Ducke seedlings to water stress regarding germination and seedling establishment related to seeds' mass. The experiment was conducted in 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 osmotic potential levels (-0.2; -0.4; -0.6; -0.8 and -1.0 MPa), distributed in four replications of 25 seeds per plot. The following parameters were evaluated: germination rate (GR), first germination count (FGC), germination speed index (GSI), mean germination time (MGT), root length (RL), fresh and cotyledon dry mass (CFM/CDM) and fresh and dry root mass (RFM/RDM). As the osmotic potential became more negative, there was a reduction in physiological variables. The heavy seeds (≥ 0.35 g) showed increases when compared to light seeds (<0.35 g), of 15.73, 22.94, 15.02, 23.33, 31.43, 21.43% for the variables GR, FGC, RL, CFM, CDM and RFM, respectively. Therefore, heavy seeds are more tolerant to water stress and should be prioritized for the recovery of degraded areas, especially in places with low rainfall.

Keywords: Osmotic potential; PEG 6000 solution; Semi-arid; Water stress.

Resumo

Em regiões semiáridas, o déficit hídrico ocorre por longos períodos, mas os mecanismos de adaptação desenvolvidos por espécies endêmicas são pouco conhecidos. O objetivo deste estudo foi determinar a tolerância de plântulas de *Luetzelburgia auriculata* (Allemão) Ducke ao estresse hídrico quanto à germinação e estabelecimento de plântulas em função da massa de sementes. O experimento foi conduzido em delineamento inteiramente casualizado em esquema fatorial 2 x 5, com duas classes de sementes (leves <0,35 g e pesadas ≥ 0,35 g) e cinco níveis de potencial osmótico (-0,2; -0,4; -0,6; -0,8 e -1,0 MPa), distribuídas em quatro repetições de 25 sementes por parcela. Os seguintes parâmetros foram avaliados: taxa de germinação (TG), primeira contagem de germinação (PCG), índice de velocidade de germinação (IVG), tempo médio de germinação (TMG), comprimento da raiz (CR), massa fresca e seca do cotilédono (MFC, MSC) e massa fresca e seca de raiz (MFR, MSR). À medida que o potencial osmótico se tornou mais negativo, houve redução das variáveis fisiológicas. As sementes pesadas (≥ 0,35 g) tiveram acréscimos em relação às sementes leves (<0,35 g), de 15,73, 22,94, 15,02, 23,33, 31,43, 21,43% para as variáveis TG, PCG, CR, MFC, MSC e MFR, respectivamente. Portanto, sementes pesadas são mais tolerantes ao estresse hídrico e devem ser priorizadas para plantios na recuperação de áreas degradadas, principalmente em locais com baixa pluviosidade.

Palavras-chave: Potencial osmótico; Solução PEG 6000; Semiárido; Estresse hídrico.

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INTRODUCTION

The genus *Luetzelburgia* is formed by eight species, among which *Luetzelburgia auriculata* (Allemão) Ducke, stands out due to its adaptation even to shallow and stony soils. In Brazil, the species is found in the semi-arid region in the states of Ceará, Maranhão, Piauí, Paraíba and Bahia (Mello et al., 2010). This species can be used in the afforestation of roads, recovery of degraded areas, and in agroforestry systems (Lopes et al., 2021). It also has apicultural potential, and its wood is used in the manufacture of furniture and in civil construction (Londe et al., 2014; Barroso et al., 2016).

The seasonality of rainfall is one of the limiting factors for the establishment and development of species in semi-arid regions (Alvalá et al., 2019). In this sense, the dry season can extend for months, followed by intense rains with irregular distribution, concentrated in three or four months, causing a phenomenon known as "green drought" (Londe et al., 2014). Water deficit is responsible for reducing plant development, through the formation of reactive oxygen species (ROS), causing damage to membranes through lipid peroxidation, which can be eliminated through efficient antioxidant mechanisms (Choudhury et al., 2016).

Among the strategies developed by plants to face water scarcity, the following stand out: escape, used by short cycle plants that complete the life cycle during the rainy season to avoid the dry season; maintenance of water in plant for photosynthetic production; and drought tolerance, involving stomatal opening for a shorter period of time (Salehi-lisar and Bakhshayeshan-Ağdam 2016).

In order for seed germination to occur, favorable conditions of temperature, light and water availability are required (Zaferanieh et al., 2020). Water scarcity is a limiting factor of seed germination, and seedling vigor is directly related to the osmotic potential of water in the soil, so its lack impairs germination capacity (Azerêdo et al., 2016).

The seed mass/size influences several aspects, such as seedling establishment and germination percentage (Souza et al., 2017). Large seeds usually have higher content of reserves and produce seedlings more tolerant to some unfavorable conditions, such as drought, defoliation, shade, and competition with other plants, ensuring higher survival rates as was observed in *Prunus persica* (Souza et al., 2016). On the other hand, lighter seeds have a greater dispersion efficiency because they are carried over longer distances by runoff and also by secondary dispersers (Souza et al., 2017).

The introduction of tree species in degraded areas is commonly carried out by planting seedlings. However, direct sowing is a promising alternative in the sense of simplifying and reducing the expense of the restoration process, due to the elimination of costs to maintain nurseries and transport seedlings. In addition, some of the root systems of some species can be restricted and damaged in the containers in which seedlings are produced, in addition to presenting acclimatization problems if the environmental conditions are very different from the nursery conditions (Palma & Laurance 2015).

Several studies have been performed to verify the degree of tolerance to water stress in native and exotic species in Brazil's semi-arid region, such as *Piptadenia moniliformis* Benth. (Azerêdo et al., 2016); *Amburana cearensis* (Allemão) AC Smith (Almeida et al., 2014); *Poincianella pyramidalis* (Tul.) LP Queiroz and *Anadenanthera colubrina* (Vell.) Brenan (Santos et al., 2016); *Salvia hispanica* L. (Simon et al., 2017), and *Robinia pseudoacacia* (Giuliani et al., 2019), but few have taken into account seed mass (Almeida et al., 2014).

However, to the best of our knowledge, no studies with *L. auriculata* have been published assessing tolerance to water stress the effects of seed mass, so there is a need to investigate the response of this species in the initial growth phase, when the plants are most vulnerable to abiotic stresses, to enable determining the ideal mass for the formation of seedlings, as well as to relate the mass with water deficit.

Thus, the objective of this study was to determine the tolerance of *Luetzelburgia auriculata* (Allemão) Ducke seedling to water stress regarding germination and seedling establishment as a function of seeds mass.

MATERIAL AND METHODS

Seed collection and processing

The pods of *L. auriculata* were collected manually, free of symptoms of pests and diseases, from ten vigorous trees, in the municipality of Caridade, Ceará (4°13'35" S, 39°12'1" W, 140 m). The climate in the region, according to the Köppen classification, is BSw'h', that is, very hot semi-arid, with a rainy season in summer and total annual precipitation less than 750 mm. The seeds were extracted, processed manually and placed to dry in the shade for three days, and then packed in polyethylene bags and stored in a cold chamber (12 ± 2 °C and 50% RH) until establishment of the experiment.

Beforehand, each seed group was individually weighed on an analytical balance (accuracy of 0.0001 g) to divide the sample into two classes of seeds according to their specific mass, defined as light (< 0.35 g) or heavy (≥ 0.35 g), based on the frequency of occurrence of seed weight (Figure 1) obtained from the random weighing of 400 seeds.

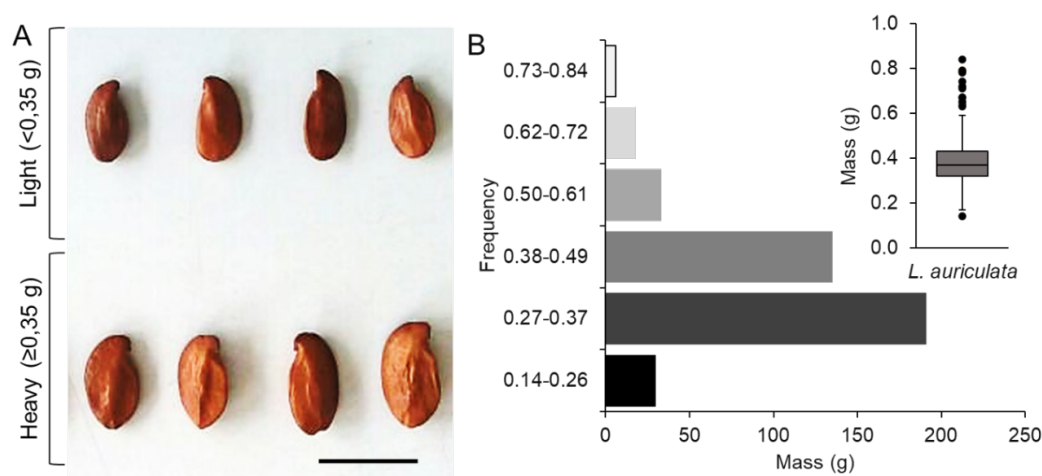


Figure 1. Representation of the distribution of classes of light (< 0.35 g) and heavy (≥ 0.35 g) (A) seeds and mass frequency in histogram with box plot distribution for a sample of 400 seeds of *Luetzelburgia auriculata* (B). Bar = 2 cm.

Experiment establishment and conduction

Before the implementation of the experiment, the seeds were disinfected by immersion in 0.5% sodium hypochlorite solution for 5 minutes and then washed in distilled water. The seeds were subjected to water stress, using solutions of polyethylene glycol (PEG 6000, Dinamica®), prepared according to Villela et al. (1991).

For each seed class, four replications of 25 seeds were used, placed on germitest® paper, in the form of rolls moistened with distilled water with 2.5 times the weight of the paper (control) or the PEG 6000 solution at different potentials (-0.2, -0.4, -0.6, -0.8 and -1.0 MPa), and then packed in transparent plastic bags to avoid water loss through evaporation.

The test was conducted in a BOD (biochemical oxygen demand) chamber at a constant temperature of 30 °C with a 12-hour photoperiod (Nogueira et al., 2012). During the germination evaluation the paper rolls could lose water by evaporation, changing the osmotic potential of the solutions. Thus, once a week the paper rolls were rehydrated with the respective PEG6000 solutions for each treatment.

Experimental design

The experiment was conducted in 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 levels of osmotic potentials (-0.2, -0.4, -0.6, -0.8 and -1.0 MPa), distributed in four replications of 25 seeds in the plot.

Environmental variables

Germination rate (GR) was evaluated daily, with seeds that had a radicle measuring 2 mm being considered germinated. The first germination count (FGC) was performed eight days after the beginning 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 the mean germination time (MGT) was calculated using the formula described by Labouriau (1983). 25 days after the beginning of the experiment, the length of normal seedlings that exhibited well-formed essential structures was measured (Brasil, 2009).

All told, 15 seedlings were selected at random to measure the root length with a millimeter ruler. The results were expressed in cm seedling⁻¹. The seedlings were then sectioned into root and shoot to determine the fresh mass on an analytical scale (precision: 0.0001 g). At the lower water potentials (-0.8 and -1.0 MPa) the presence of epicotyl was not recorded and therefore the fresh and dry mass was performed only with the cotyledons, which are the reserve structures and may be associated with tolerance to water deficit. The structures were then placed separately in Kraft paper bags and dried in a forced air oven at 65 °C for 72 h for the subsequent determination of the dry mass of the root (RDM) and cotyledons (CDM) on an analytical scale (0.001 g). The results were expressed in g seedling⁻¹.

Data analysis

The Shapiro-Wilk test was used to determine the distribution (normal or non-normal) of the data. Values of FCG and GR were transformed to Arcsine $(X/100)^{1/2}$ and CFM, CDM, RFM and RDM were transformed to $\log(x + 1)$ to satisfy the assumptions of normality and variance homoscedasticity. The data were submitted to analysis of variance (ANOVA) and 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 osmotic potential of -1.0 MPa was not used in the analysis because germination of both light and heavy seeds was completely inhibited. The SISVAR 4.3 program was used for the statistical analysis (Ferreira, 2019). In order to visualize the germination of light and heavy *L. auriculata* seeds as related to different water potentials over time, line graphs between seed masses were plotted.

In addition to ANOVA, the data were also submitted to principal component analysis (PCA), and treatments were plotted in relation to the first two components (PC1 and PC2). The group number was defined based on the dispersion in the first two components (PC1 and PC2) by cluster analysis. Heat maps were created to visualize the relationship between all treatments and physiological and biometric variables of *L. auriculata*, and the best group was determined based on the average Gower distances. According to this method, each rectangle represented the contribution of each variable to the formation of *L. auriculata* seedlings. The scale ranged from lilac to yellow, corresponding from low to high contribution, respectively. The analyses were performed using the software R v.4.0.1 (R Core Team, 2020).

RESULTS AND DISCUSSION

This study presents the first report of the effect of variation of water potential on the physiological quality of *L. auriculata* seeds with different masses. In both classes of seeds (light < 0.35 g and heavy ≥ 0.35 g), the water content was 9.0%. The 1000-seed weight of the heavy seeds was 487 g and of the light seeds was 317 g. A significant difference was found between the isolated factors and/or interaction for most of the variables evaluated (Table 1). The seed mass influenced seven of the nine variables evaluated, with the exception of the germination speed index (GSI), and root dry mass (RDM).

Table 1. Analysis of variance (ANOVA) results of the physiological and morphological characteristics of light (<0.35 g) and heavy (≥ 0.35 g) seeds of *Luetzelburgia auriculata* submitted to different levels of water stress

FV	FGC	GR	GSI	MGT	RL	CFM	CDM	RFM	RDM
Mass (M)	46.09**	32.09**	0.08 ^{ns}	6.62*	30.88**	34.59**	49.94**	31.48**	1.90 ^{ns}
Level (L)	138.79**	74.13**	321.28**	7.07**	365.43**	5.19**	14.22**	98.29**	9.18**
M x L	10.33**	4.70**	22.05**	0.66 ^{ns}	15.79**	5.80**	2.67 ^{ns}	3.67**	1.37 ^{ns}
Error	58.12	52.66	0.11	1.04	0.66	0.01	0.009	0.0004	0.0001
CV (%)	10.37	5.29	10.02	19.69	9.17	3.67	3.78	1.53	1.07
Regression									
Linear	978.84**	976.89**	2008.99**	38.14**	2388.59**	16.36**	8.18**	565.18**	83.13**
Quadratic	20.73**	150.66**	57.18**	164.54**	59.10**	2.74 ^{ns}	13.30**	137.98**	8.04**

**, * and ns significance at 1%, 5% and not significant, by the F-test, respectively. FGC: first germination count, GR: germination rate, GSI: germination speed index, MGT: mean germination time RL: root length, CFM: cotyledon fresh mass, CDM: cotyledon dry mass, RFM: root fresh mass, RDM: root dry mass, of light (<0.35 g) and heavy (≥ 0.35 g) seeds of *Luetzelburgia auriculata* submitted to water stress. **, * e ns significância a 1%, 5% e não significativa, pelo teste F, respectivamente. PCG: primeira contagem de germinação, G: germinação, IVG: índice de velocidade de germinação, TMG: tempo médio de germinação CR: comprimento da raiz, MFC: massa fresca do cotilédone, MSC: massa seca do cotilédone, MFR: massa fresca da raiz, MSR: massa seca da raiz, de sementes leves (<0,35 g) e pesadas (≥ 0,35 g) de *Luetzelburgia auriculata* submetidas ao estresse hídrico.

As for the levels of osmotic potential, all variables were affected and interactions were significant for the first germination count (FGC); germination rate (GR), germination speed index (GSI), root length (RL), cotyledon fresh mass (CFM), and root fresh mass (RFM) (Table 1).

The heavy seeds (≥ 0.35 g) showed increases of 15.02, 23.33, 31.43 and 21.43% for RL, CFM, CDM and RFM in relation to light seeds (<0.35 g), respectively, leading to the belief that heavy seeds are more tolerant to water deficit. This same trend was observed for FGC and GR (Figure 2A-G). Regarding the data amplitude, the heavy seeds exhibited less amplitude than the light ones, with an opposite behavior only for CDM (Figure 2G).

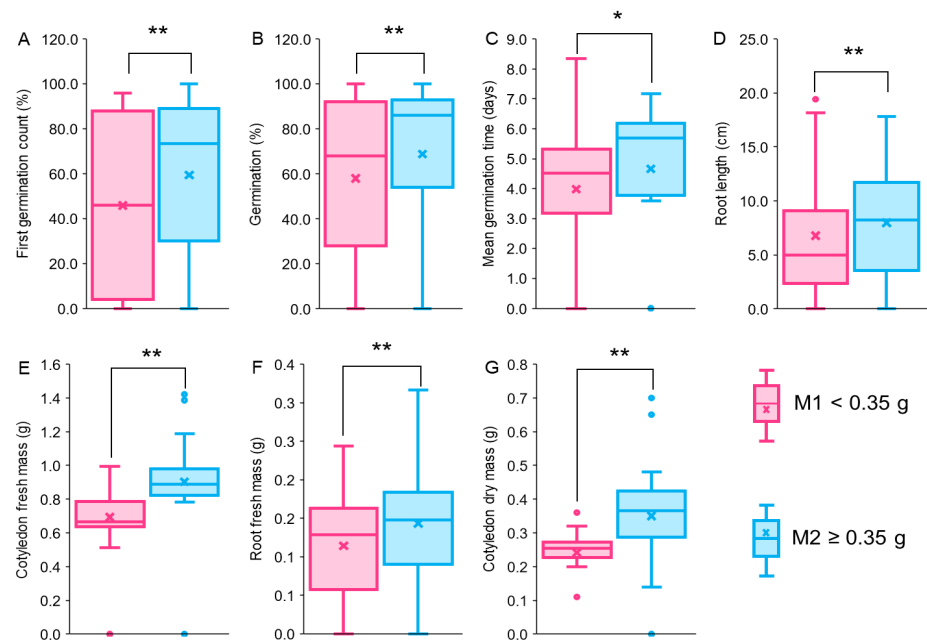


Figure 2. Physiological and morphological characteristics of seedlings of *Luetzelburgia auriculata* from light and heavy seeds subjected to different levels of water stress and evaluated as a function of mass: light (M1 < 0.35 g) and heavy (M2 ≥ 0.35 g). A: first germination count; B: germination rate; C: mean germination time; D: root length; E: cotyledons fresh mass; F: root fresh mass; G: cotyledon dry mass. * and ** indicate variation at 5 and 1% by the F-test of analysis of variance. X corresponds to the average of each characteristic and • corresponds to outlier values.

The mean germination time (MGT) can also be strongly affected by water availability. In this study, heavy seeds showed a MGT 14.6% higher than light seeds (Figure 2C). This behavior can be considered an evolutionary strategy, since seeds start germinating only under specific conditions that can guarantee the establishment of the species (Wang et al., 2019) or due to the greater mass of the seed, requiring more time to start the process of mobilizing reserves (Taiz et al., 2017). This is commonly observed due to the relationship between environmental conditions, genes and phenotypic expressions as a consequence of natural selection and local adaptation. Santos et al. (2016) found the highest MGT values in *Poincianella pyramidalis* [Tul.] and LP Queiroz in *Anadenanthera colubrina* (Vell.) under water stress (-0.8 MPa). At more negative values of osmotic potential, there was no germination, as also identified in the present study.

An increase in time to the beginning of germination was observed from the potential of -0.2 MPa, while germination was only compromised from the potential -0.4 MPa onward and was more pronounced in light seeds. This trend was also observed for the potential -0.8 MPa (Figure 3A-F). These results indicate that light seeds are more sensitive in relation to heavy ones, however, in the water restriction of -1.0 MPa both classes of seeds did not germinate and this can be considered as a strategy of establishment of the species only under suitable conditions for its perpetuation.

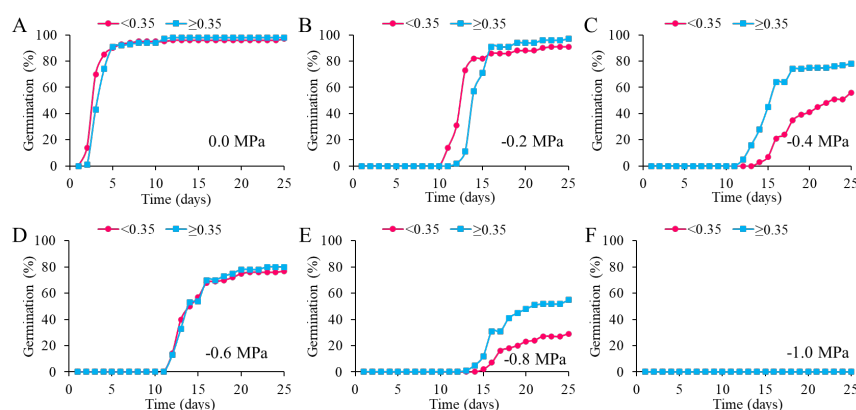


Figure 3. Germination of light (< 0.35 g) and heavy (≥ 0.35 g) seeds of *Luetzelburgia auriculata* submitted to different levels of water stress. A: control treatment (0.0 MPa); B: -0.2 MPa; C: -0.4 MPa; D: -0.6 MPa; E: -0.8 MPa; F: -1.0 MPa.

Regarding the interaction between the factors, both classes of seeds showed a decrease in the first germination count due to the reduction of the osmotic potential ($p < 0.01$), (Figure 4A, Table 2). Between the two classes of seeds, the heavy ones stood out with increases of 77.42 and 67.19% at the potentials of -0.8 MPa and -0.4 MPa in relation to light seeds (Figure 4A, Table 2). The germination rate was reduced in both seed classes, but the heavy seeds adjusted to a quadratic regression model ($p < 0.01$), with a reduction after the potential of -0.38 MPa, while the light seeds showed linear reduction of germination (Figure 4B). In the comparison within each potential, the heavy seeds stood out at the potentials -0.4, -0.6 and -0.8 MPa, with germination rates of 28.2, 13.48 and 47.27% higher than for the light seeds. On the other hand, considering the variables that showed interaction, the osmotic potential of -0.4 MPa caused a significant reduction in the physiological quality of *L. auriculata* seeds in both classes (Table 2).

The germination values (Figure 3, Figure 4A, B) indicated that under natural conditions, heavy seeds of *L. auriculata* probably start the germination process with less water content in the soil; different from species that need a high amount of water for germination, because even with low precipitation they can be established more efficiently than light seeds, since germination was the same between light and heavy seeds in the control treatment (0.0 MPa) (Table 2), indicating a greater tolerance to water deficit in this species in heavy seeds compared to light seeds. The hormonal regulation that stimulates the synthesis of gibberellins is responsible for activation of α -amylase, which is the main enzyme responsible for the

degradation of starch. This degradation releases oligosaccharides contained in the cotyledons of heavy seeds, which seem to be more efficient than light seeds (Damaris et al., 2019).

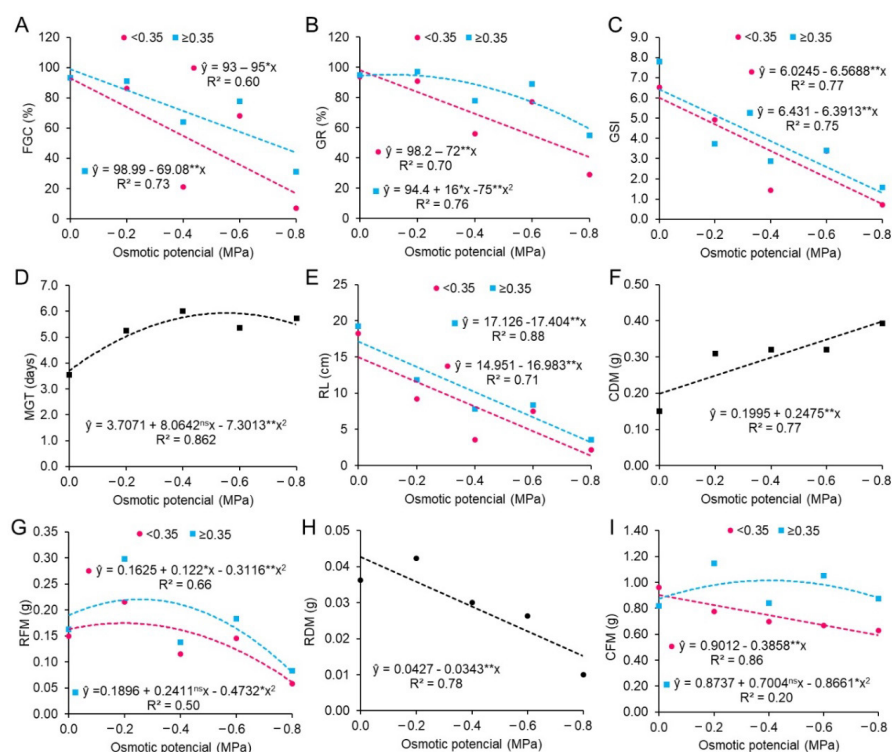


Figure 4. Physiological and morphological variables of seedling and seeds light (<0.35 g) and heavy (≥ 0.35 g) of *Luetzelburgia auriculata* submitted to different levels of osmotic potential. A: first germination count (FGC); B: germination rate (GR); C: germination speed index (GSI); D: mean germination time (MGT); E: root length (RL); F: cotyledon dry mass (CDM); G: root fresh mass (RFM); H: root dry mass (RDM); I: cotyledon fresh mass (CFM). * e ** significant at 5 and 1% by the F test.

Table 2. Analysis of the interaction between seed classes and water stress levels in *Luetzelburgia auriculata*.

First germination count (%)					
	0 MPa	-0.2 MPa	-0.4 MPa	-0.6 MPa	-0.8 MPa
< 0.35 g	93.33 a	86.00 a	21.00 b	68.00 a	7.00 b
≥ 0.35 g	93.00 a	91.00 a	64.00 a	77.50 a	31.00 a
Germination rate (%)					
< 0.35 g	94.00 a	91.00 a	56.00 b	77.00 b	29.00 b
≥ 0.35 g	95.00 a	97.00 a	78.00 a	89.00 a	55.00 a
Germination speed index					
< 0.35 g	6.52 b	4.93 a	1.43 b	3.38 a	0.73 b
≥ 0.35 g	7.80 a	3.73 b	2.88 a	3.39 a	1.57 a
Root length (cm)					
< 0.35 g	18.28 a	9.24 b	3.60 b	7.52 a	2.16 b
≥ 0.35 g	19.22 a	11.85 a	7.82 a	8.37 a	3.56 a
Root fresh mass (g)					
< 0.35 g	0.15 a	0.22 b	0.12 a	0.15 b	0.06 a
≥ 0.35 g	0.16 a	0.30 a	0.14 a	0.18 a	0.08 a
Cotyledon fresh mass (g)					
< 0.35 g	0.96 a	0.77 b	0.70 a	0.67 b	0.63 b
≥ 0.35 g	0.82 a	1.15 a	0.84 a	1.05 a	0.88 a

Equal letters in the columns do not differ by the F test at 5% probability.

The germination speed index showed behavior similar to FGC, with a linear decrease due to reduction of water availability for heavy and light seeds (Figure 4C). In the comparison between the classes of seeds, the heavy ones stood out at the potentials of -0.8 MPa (53.50%) and -0.4 MPa (50.35%), and in the control treatment (16.41%), while the light seeds were better at the potential of -0.2 MPa (24.35%); while at the potential of -0.6 MPa there was no variation (Table 2). This result indicates that heavy seeds of *L. auriculata* can establish themselves more efficiently under water deficit conditions compared to light seeds. For the mean germination time, there was no interaction ($p > 0.05$), but an effect of water potential was observed, with a quadratic model having a peak at 6.42 days at the potential of -0.43 MPa (Figure 4D).

Better tolerance may be associated with the greater energy reserves contained in large seeds, causing increased tolerance to water stress (Zepeda & Martorell, 2019). On the other hand, *L. auriculata* germination was inhibited considerably from the water potential -0.6 MPa (Figure 4B). A study evaluating the effect of water stress on the germination of *Piptadenia moniliformis* Benth. also showed a reduction in germination rates at osmotic potentials between -0.6 and -1.2 MPa (Azerêdo et al., 2016). It is likely that the high molecular weight of polyethylene glycol, which has high viscosity, limited the uptake of water by seeds by simulating water deficit (Oliveira et al., 2010).

The data for cotyledon fresh mass (CFM) fit a quadratic regression model for the heavy seeds, with a maximum value of 0.45 g at a potential of -0.55 MPa, while for the light seeds there was increasing linear behavior, with a value of 0.12 g in the control (0.0 MPa) (Figure 4I). Regarding the classes of seeds, at osmotic potentials of -0.2, -0.8 and -1.0 MPa, the heavy seeds produced CFM values 33, 36 and 28% greater than the light seeds (Table 2).

The reduction in water availability decreases water absorption by seeds and consequently reduces mobilization of reserves, resulting in less growth or inhibition of germination (Taiz et al., 2017; Pinheiro et al., 2017). In this study, this effect was more evident in light seeds (Table 2, Figure 5). The lower water availability also hampers Phase I of the three-phase water absorption process, in which dry seeds absorb water quickly through imbibition, where the matrix potential arises from the contact of water with solid surfaces, such as the micro-capillaries of cell walls and surfaces of proteins and other macromolecules. The rehydration of cell macromolecules activates the basal metabolic processes, including breathing, transcription and translation. Imbibition ceases when all potential water binding sites become saturated, and the Ψ_m becomes less negative (Taiz et al., 2017).

A quadratic regression model fit the RFM data in both mass classes. Heavy seeds had a maximum RFM value of 0.21 g at the potential of -0.23 MPa, while for light seeds the maximum value was 0.17 g at the potential of -0.16 MPa (Figure 4G). The heavy seeds stood out in relation to the light ones at the potentials of -0.2 and -0.6 MPa, with increases of 26.67 and 16.67%, respectively (Table 2).

Regarding RDM, a linear model explained most of the variation ($R^2 = 0.78$), with values close to 0.05 g in the control treatment (0.0 MPa), reaching lowest mass at the most negative potential (-0.8 MPa) (Figure 4H). For the CFM, a linear model explained most of the variation ($R^2 = 0.86$) for light seeds, with values close to 0.9 g in the control treatment (0.0 MPa), while the heavy seeds showed low adjustment to the regression model (Figure 4I).

Regarding root length (RL), linear reductions ($p < 0.01$) were observed in both classes of seeds (Figure 4E), where the heavy seeds (> 0.35 g) stood out in relation to light seeds, with increases of 22.03, 53.96, and 39.33% at potentials of -0.2, -0.4 and -0.8 MPa, respectively (Table 2). This sharp drop in growth can be attributed to a reduction in cell expansion and turgidity (Finch-Savage & Bassel, 2016). The RL of heavy seeds, even with a decrease due to negative osmotic potential, still stood out in relation to light seeds, which can be explained by the higher content of reserves available for development of the embryonic axis (Steiner et al., 2019).

The limited water availability in the lower osmotic potentials resulted in less mobilization of reserves contained in the cotyledons, so the dry mass of the cotyledons (CDM) increased as the water potential decreased (Figure 4F). The mobilization of reserves for the formation of seedlings was low, with reduced root length, fresh and dry mass (Azerêdo et al., 2016). On the other hand, Silva & Carvalho (2008), analyzing the effect of seed size associated with water

stress on the germination of *Clitoria fairchildiana* R. Howard, found that in large seeds, the dry mass of the cotyledons remained constant, but for medium seeds, cotyledon dry mass decreased the more negative osmotic potential (Silva & Carvalho, 2008).

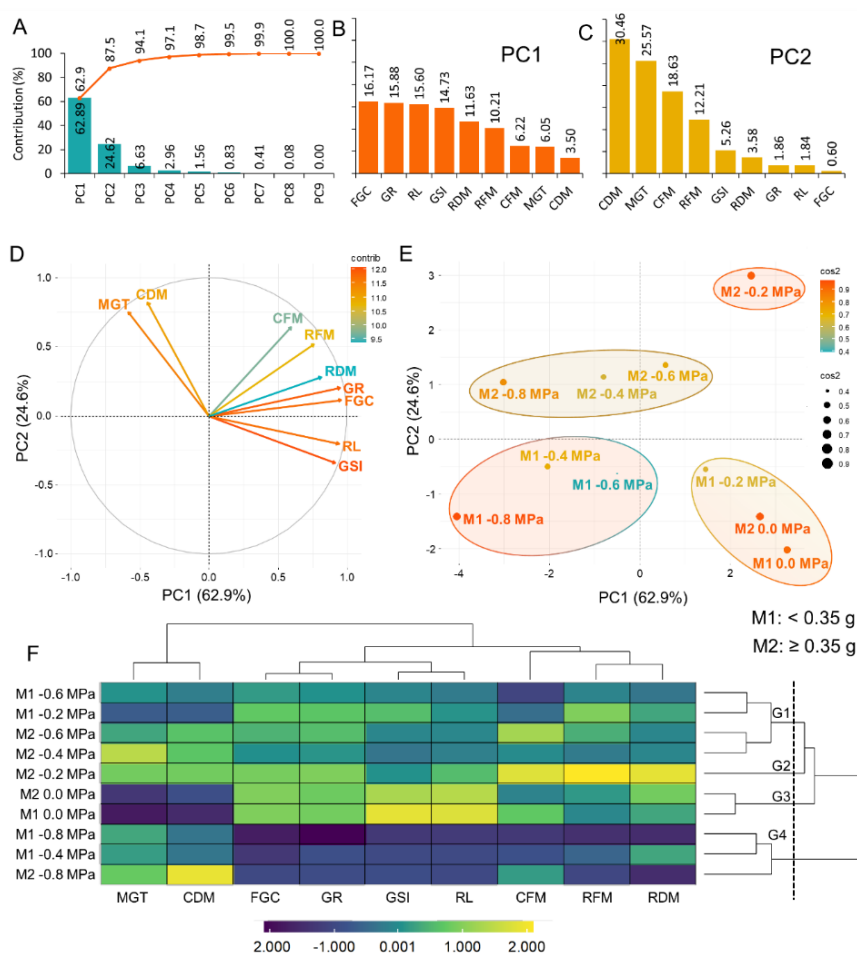


Figure 5. Multivariate analysis for the physiological and morphological variables of light and heavy seeds of *Luetzelburgia auriculata* submitted to different levels of osmotic potential. A: Individual and accumulated contribution of each principal component; B: character contribution to principal component 1 (PC1); C: character contribution to principal component 2 (PC2); D: Correlation circles of variables in function PC1 and PC2. The arrows represent the direction of the characteristic and its gradient colors represent the contribution, blue (low) to red (high), of each characteristic to the two components; E scatter plot for light and heavy *Luetzelburgia auriculata* seeds subjected to different osmotic potentials as a function of physiological and biometric characters; F: Heat map of light and heavy seeds of *L. auriculata* submitted to different osmotic potentials. Each rectangle represents the contribution of each characteristic to the performance of the seedlings as a function of seed mass and osmotic potential. The scale ranges from dark blue to yellow (low to high contribution). FGC: first germination count; GR: germination rate; GSI: germination speed index; MGT: mean germination time; RL: root length; CFM: cotyledon fresh mass; CDM: cotyledon dry mass; RFM: root fresh mass; RDM: root dry mass; M1: < 0.35 g and M2: ≥ 0.35 g.

In order to group all the information generated and identify the traits that most contributed to the discrimination of the *L. auriculata* response according to the seed mass and water potential, principal component analysis was carried out, where 87.5% of the data variability was explained by the first two components (PC1 and PC2) (Figure 5A).

We found that the reduction of osmotic potential made it difficult for the embryos to emit epicotyls, even at the smallest potentials. This may be associated with a reduction in the activation of enzymes responsible for mobilizing the reserves contained in cotyledons and making them available to the embryonic axis. Almeida et al. (2014), evaluating the effect of

water stress on seedlings of *Amburana cearenses* (Allemão) A.C. Sm., observed greater root growth in relation to the aerial part of the seedling, with greater allocation of biomass to the root region. The authors attributed this behavior to a drought tolerance mechanism.

According to the factor loadings, the parameter with the greatest potential for discrimination for the first component were FGC, GR, RL, GSI, RDM and RFM with 84.22% (Figure 5B) and for the second component were CDM, MGT, CFM and RFM with 86.87% (Figure 5C). Considering the PCA analysis, the main characteristics that contributed to water deficit tolerance were the fresh and dry mass of the cotyledons and the fresh mass of the root. From the circles, it was possible to observe positive correlations of high magnitude for the interaction of the physiological traits GR x FGC ($r = 0.96$); FGC x GSI ($r = 0.91$); GR x GSI ($r = 0.84$) and for the seedling traits RL x RDM ($r = 0.78$) and RFM x CFM ($r = 0.77$). Low contribution to both components was observed for RDM (Figure 5D). RL and GSI contributed most to the response of the control treatments while CFM, RFM, MGT and CDM explained most of the variation of the heavy seeds, varying from -0.2 to -0.8 MPa, and the negative effect of the traits explained most of the other treatments (Figures 5D, E).

The identification of easy-to-measure morphological markers for water deficit tolerance in forest species is important, since it can facilitate the selection of more tolerant genotypes for incorporation in environmental restoration or landscaping programs. Therefore, the variation in the mass of the seeds is a characteristic that can be explored for selection of plants more tolerant to water stress and as a strategy for adaptation and conservation of the species in different environments. However, the response to water stress as a function of seed mass can vary among species, as reported by Almeida et al. (2014) for *Amburana cearensis*, in which light and medium mass seeds were more tolerant to water deficit compared to heavy ones, and the potential of -0.6 MPa was a strictly limiting condition for the formation of normal seedlings. Here, we observed that the heavy seeds of *L. auriculata* were more tolerant to the reduction of water potential, since they presented greater PCG, GER, GSI, RL, CFM values at the potential of -0.8 MPa in relation to the light seeds. However, in the general context, only the potential of -0.2 MPa showed a behavior similar to the control, being considered the potential that does not interfere in the establishment of seedlings of *L. auriculata* originating from any seed weight (Figure 5).

From the heat map analysis, the formation of four groups was observed, with lower performance, represented by the dark blue color, of the light seeds with osmotic potentials of -0.4 and -0.8 MPa, and for the heavy seeds at the osmotic potentials of -0.8 MPa (group 4-G4). The control treatment of both classes of seeds formed an isolated group (Group 3-G3), standing out in relation to the other treatments. On the other hand, the heavy seeds (≥ 0.35 g) with osmotic potential of -0.2 MPa stood out for CFM, RFM and RDM, represented by shades of yellow in the heat map (Figure 5F).

The inhibition and unevenness of seed germination can be seen as a strategy for adaptation and conservation of species in arid and semi-arid environments. This phenomenon consists of an intrinsic temporal block, providing an additional period for seed dispersal at greater geographic distances, or for the seasonal dormancy cycle in the soil seed bank (Taiz et al., 2017). Based on the results presented here, it is likely that heavier seeds are better able to overcome the unfavorable conditions of the environment. Therefore, heavy *L. auriculata* seeds should be prioritized by nurserymen and seedling producers, whether aimed at the recovery of degraded areas or landscaping, as they have greater tolerance to water stress compared to light seeds.

However, further studies are needed to verify whether, after long-term water stress, seeds have the ability to germinate when subjected to favorable conditions, indicating that the species has adaptive mechanisms to deal with abiotic stress and whether the response depends of the seed mass.

CONCLUSIONS

Luetzelburgia auriculata seeds were sensitive to water stress, which had a negative effect on most of the variables analyzed, especially in the case of the light seeds. The heavy seeds

(≥ 0.35 g) of *L. auriculata* were more tolerant to water stress and should be prioritized for the recovery of degraded areas in places with low rainfall.

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