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Initial establishment of *Erythrina velutina* Willd seedlings under water deficit: physiological and biochemical aspects

Estabelecimento inicial de mudas de *Erythrina velutina* Willd sob déficit hídrico: aspectos fisiológicos e bioquímicos

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ABSTRACT

For plant species to establish and survive in semiarid regions, such as the Caatinga plant domain, they need to present biochemical versatility. Thus, the objective of this study was to evaluate through physiological and biochemical indicators the sensitivity of seeds and seedlings of *Erythrina velutina* Willd to water deficit, as this species is used in the recovery of degraded areas in the Brazilian semiarid region. The seeds were submitted to five osmotic potentials (-0.1; -0.2; -0.3; -0.4; and -0.5 MPa), evaluating the percentage of germination (normal seedlings), germination speed index, length of aerial parts and root system, total dry mass, the concentration of photosynthetic pigments (chlorophylls a, b, and carotenoids) in aerial parts and root system, soluble carbohydrates and proteins, proline, and the enzymes ascorbate peroxidase, catalase, superoxide dismutase, peroxidase, and polyphenoloxidase. The water deficit reduced the germination speed index and percentage, aerial parts and root length, aerial parts and root dry mass, and photosynthetic pigment content in seedlings; however, there was an increase in the levels of total carbohydrate, proline, and protein, and a reduction in superoxide dismutase. The water potential from -0,4 MPa was a limiting factor for the seedling and/or seedling formation process. The direct sowing of *E. velutina* would only be indicated up to the potential of -0,3 MPa for the regeneration of degraded areas in semiarid regions. The species in the seedling formation stage present less biochemical plasticity to overcome limiting conditions of water availability.

Keywords: antioxidant enzymatic activity; biochemical adaptations; drought; mulungu; physiological adaptations.

RESUMO

As espécies vegetais, para se estabelecerem e sobreviverem em regiões semiáridas como o domínio vegetal Caatinga necessitam apresentar versatilidade bioquímica. Assim, objetivou-se avaliar por meio de indicadores fisiológicos e bioquímicos a sensibilidade das sementes e mudas de *Erythrina velutina* Willd ao déficit hídrico, pois a referida espécie é usada na recuperação de áreas degradadas no semiárido brasileiro. As sementes foram submetidas a cinco potenciais osmóticos (-0,1; -0,2; -0,3, -0,4 e -0,5 MPa), avaliando-se a porcentagem de germinação (plântulas normais), o índice de velocidade de germinação, o comprimento da parte aérea e do sistema radicular, a massa seca total, a concentração de pigmentos fotossintéticos (clorofilas a, b e carotenoides) na parte aérea e no sistema radicular, carboidratos e proteínas solúveis, prolina e as enzimas ascorbato peroxidase, catalase, superóxido dismutase, peroxidase e polyphenoloxidase. O déficit hídrico reduziu o índice de velocidade e a porcentagem de germinação, o comprimento da parte aérea e da raiz, a massa seca da parte aérea e da raiz, o teor de pigmentos fotossintéticos nas plântulas; entretanto, houve aumento dos níveis de carboidrato total, prolina, proteína e redução da superóxido dismutase. O potencial hídrico a partir de -0,4 MPa é fator limitante para o processo de formação de plântulas e ou mudas. A semeadura direta de *E. velutina* só seria indicada até o potencial de -0,3 MPa para a regeneração de áreas degradadas em regiões semiáridas. A espécie na fase de formação de plântulas apresenta menor plasticidade bioquímica de superar condições limitantes de disponibilidade hídrica.

Palavras-chave: atividade enzimática antioxidante; adaptações bioquímicas; seca; mulungu; adaptações fisiológicas.

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Introduction

Brazil has great diversity related to its morphoclimatic domains. Among them, the Caatinga biome in the Northeastern stands out, characterized by an extensive semiarid area (Fernandes and Queiroz, 2018). In recent decades, the native vegetation of the Caatinga has been replaced by pastures and crops, with deforestation followed by fires being normally used, with negative interference in the flora, fauna, water quality, and soil-plant-climate balance (Medeiros et al., 2018). According to Dantas and Angelotti (2022), climate change tends to influence the distribution, migration, and extinction of plant species in the most diverse ecosystems in the world, and research is needed to better understand species' vulnerability to these changes, from seed germination to the production of a new generation of seeds. Water scarcity is one of the main factors limiting the development of most species, which forces plants to activate survival mechanisms, such as morphological, physiological, and biochemical adaptations (Brito et al., 2022). The impact of drought on the Caatinga biome undoubtedly affects the establishment of seedlings and reforestation (Vilela et al., 2018; Novaes et al., 2020), as well as the population dynamics of species, and the natural regeneration of the ecosystem. On the other hand, when natural regeneration occurs at a reduced rate, seedlings can be planted, or direct sowing can be used (Nunes et al., 2017; Silva et al., 2017). Relevant studies on the germinative process of seeds in artificial stress situations are of great relevance for ecophysiology and can be considered useful instruments to assess not only the limits of survival tolerance but also the adaptation of species to the stresses normally found in nature (Luo et al., 2022).

Among the forest species in the Caatinga biome, *Erythrina velutina* Willd (Fabaceae-Papilionoideae) stands out. In Brazil, it has already been recorded in the Northeast (Alagoas, Bahia, Ceará, Paraíba, Pernambuco, Piauí, Rio Grande do Norte, and Sergipe) and Southeast (Minas Gerais), and not only in the Caatinga but also in the Cerrado (Martins, 2020). However, Leite et al. (2022) found that *E. velutina* has a drought tolerance mechanism that rapidly reduces photosynthesis and leaf gas exchange and thus improves water use efficiency; but recovery is slow after water supply.

The *E. velutina* is important in popular medicine as a calming and anxiolytic remedy (Schiave and Pacheco, 2022); its flowers and leaves are rich in alkaloids, which have therapeutic properties with anxiolytic and sedative effects (Palumbo et al., 2016). It is a species indicated for mixed plantations in the restoration of degraded areas of permanent preservation (Carvalho, 2008). Rodrigues et al. (2018) found that the roots of *E. velutina* established a symbiotic association with α and ß rhizobia in natural regeneration in the Caatinga in the state of Pernambuco (PE), Brazil, which could provide tolerance to abiotic stresses, greater accumulation of nitrogen in the aerial part, in addition to favoring the growth of plants, highlighting its relevant ecological role in the plant community. Silva et al. (2020) found that the flowers of *E. velutina* are visited by urban birds; they considered that the presence of the referred species may contribute to the promotion of urban biodiversity.

According to Parvin et al. (2014), if the drought deficit exceeds the tolerance limits of the seeds, not only a delay in germination will be observed but also the total compromise of the germinative process. Stavi et al. (2015) considered that the lack of water during the dry season can negatively interfere with the recruitment and survival of seedlings, becoming the main factor responsible for their mortality. During the evolution process, plants developed survival strategies against stressful situations, such as the drought deficit, which upon reaching a critical value, triggers not only morphological and physiological changes but also biochemical, as the production of reactive oxygen species (ROS), which unleashes metabolic adjustments for the preservation of life (Cruz de Carvalho, 2008). Ribeiro et al., when submitting *E. velutina* seeds to water potentials of up to -0.8 MPa (2012) and -0.6 MPa (2014), evaluated biochemical characteristics and those related to germination (primary root protrusion) and verified the sensitivity of the species to water deficit, finding that above -0.6 MPa root protrusion no longer occurred. It is important to highlight that in both researches, 2012 and 2014, germination was not considered in terms of the formation of normal seedlings (agronomic criterion); therefore, length, dry mass, concentration of chlorophylls a, b, and carotenoids in seedlings were not evaluated, as well as there was no evaluation of the activity of antioxidant enzymes peroxidase, superoxide dismutase, and polyphenoloxidase.

Thus, aiming to verify the sensitivity of the *E. velutina* to drought deficit and its behavior for the recovery of areas in the semiarid region of Brazil, the present study aimed to identify by means of physiological and biochemical indicators, the behavior of the seeds and seedlings of species submitted to drought deficit simulated with polyethylene glycol (PEG).

Material and Methods

The experiment was conducted at the Seed Analysis Laboratory, belonging to the Federal University of the Agreste of Pernambuco (UFAPE), in Garanhuns (PE). The seeds of *E. velutina* were collected from dried pods of 22 matrices plants located in the rural zone of Sanharó (PE), at 8°22'46.9" S 36°30'0,7.6" W. After harvesting, the seeds were selected manually, separating the plant parts and the damaged seeds, leaving only the intact ones. Following selection, they were stored in plastic containers, with subsequent storage in the refrigerator at temperatures ranging from 2–5°C for 18 months.

Effect of osmotic potential

The seeds were sown on two sheets of paper towel and covered with a third sheet for later preparation of the rolls. The substrate was previously moistened with distilled water (0.0 MPa) and PEG 6000 solutions at -0.1; -0.2; -0.3; -0.4; and -0.5 MPa osmotic potentials (Villela et al., 1991), with a volume equivalent to 2.5 times the weight of dry paper (Brasil, 2009). Then, the paper rolls were placed in germination chambers of the type Biochemical Oxygen Demand (BOD) at 25°C (Ribeiro et al., 2012) and a constant photoperiod with light.

On the 14th day after sowing, the germination percentage and speed index, the aerial part and root system length, the aerial part and root total dry mass, and photosynthetic pigments concentration (chlorophylls a, b, totals, and carotenoids) were evaluated.

Germination percentage and speed index

The number of normal seedlings was considered (germination criterion – with radicle and aerial part formed) on the 14th day after sowing. For the germination speed index, normal seedlings were counted daily between the 5th and the 14th day after sowing, according to Maguire (1962).

Seedling length

On the 14th day, the normal seedlings (with radicle and aerial part formed) of each repetition were measured, using a ruler graduated in centimeters, for the aerial part and root length. The results were expressed in cm plants^{-1} (Krzyzanowski et al., 1999).

Seedling dry mass

After removing the cotyledons and sectioning in the aerial part and root system, the aerial part and the root system of each repetition were packed in paper bags and placed in a regulated lab oven at 65°C for 72 hours, until a constant dry mass was reached. After this period, the samples were weighed on an analytical balance with an accuracy of 0.001g, and the results expressed in g plants^{-1}.

Quantification of chlorophylls and carotenoids

Primary leaves were used to determine the levels of chlorophyll (a, b, and total) and carotenoids by the acetone extraction method, described by Lichtenthaler and Wellburn (1983). The quantification of the pigments did not occur in the -0.5 MPa treatment because there was no germination when the seeds were submitted to this treatment.

On the 14th day after sowing, plant material of the aerial part (primary leaves) was collected to evaluate osmoregulators (carbohydrates, proline, and soluble proteins) and enzymes (ascorbate peroxidase, catalase, superoxide dismutase, peroxidase, polyphenoloxidase). For biochemical analysis, each of the five replicates was represented by a simple extract dosed in triplicate for the readings.

Quantification of soluble carbohydrates

Extracts were obtained from 10 mg samples of lyophilized leaf tissue, macerated in a marble mortar with 2 mL of distilled water. The mixture remained at rest for one hour, being stirred every 15 minutes, and then centrifuged at 3,000 × g, for 15 minutes. The precipitate was discarded, and the supernatant (extract) was used to determine soluble carbohydrates through the method described by Dubois et al. (1956). The results were expressed in mmol g^{-1} MS.

Proline quantification

Proline content was determined by homogenizing 50 mg of lyophilized plant material, with 4 mL of 3% sulfosalicylic acid, remaining at rest for one hour at 25 °C, and then stirred every 15 minutes, with subsequent centrifugation at 3,000 × g for 5 minutes. Only the supernatant (extract) was used for the determination of free proline, according to Bates (1973). Pure proline was used as the standard and the results were expressed in µmol g^{-1} MS.

Quantification of soluble proteins

The extracts were obtained from 20 mg samples of lyophilized leaf tissue, macerated in a mortar with 2 mL of buffer solution 0,01 M Na-K-phosphate, at potential of hydrogen [pH] 7.6, containing 0.1 NaCl M, and kept for one hour at 4°C, under periodic stirring (every 15 minutes). Subsequently, they were centrifuged at 3,000 x g for 5 minutes at 4°C. The precipitate was discarded and supernatant was used. The protein determination was in accordance with Bradford (1976). As a standard, bovine serum albumin (BSA, Sigma Chemical Company) was used, and the results were expressed in mg g^{-1} MS.

Extraction of enzymes

For enzymatic activities, the aerial part of the seedlings was collected, identified, and immediately frozen in liquid nitrogen, and samples were then stored in an ultra-freezer for two weeks. A 0.05-gram portion of leaf tissue was subjected to extraction in solution containing 300 mM potassium phosphate buffer (pH 7.5), 2 mM of ethylenediamine tetraacetic acid (EDTA) and 20 mM ascorbic acid with the addition of 0.3 g of polyvinylpolypyrrolidone (PVPP) (Gomes-Junior et al., 2006), making a final volume of 2,000 µL. The extract was centrifuged at 15,000 × g, for 10 minutes at 4°C. The collected supernatants were divided into 2 mL aliquots and stored in an ultra-freezer at a temperature of -80°C, for three days until the beginning of the quantification analyses of total soluble proteins (Bradford, 1976). Bovine serum albumin was used as standard activity, besides the enzymes ascorbate peroxidase (APX) (Nakano and Asada, 1981), catalase (CAT) (Havir and McHale, 1987), superoxide dismutase (SOD) (Giannopolitis and Ries, 1977), peroxidase (Urbanek et al., 1991) and polyphenoloxidase (Kar and Mishra, 1976). The enzymes were expressed in mg-1 protein units.

The experimental design used, both for the germination test and biochemical analysis, was completely randomized, with six treatments and four replications of 25 seeds each. The data of the measured variables were subjected to analysis of variance by the F-test. Means were compared using standard error and by Tukey's test, and submitted to regression analysis, applying the linear or quadratic model, through the SISVAR statistical program (Ferreira, 2011).

Results and Discussion

The drought stress reduced the germination speed index and percentage (Figures 1A and 1B) of *E. velutina* seeds. From zero water potential to -0.1 MPa, there was a reduction in germination speed index from 11.02 to 3.84, and the germination percentage from 68% to 45%, in a linear decreasing form. At a potential of -0.5 MPa, there was no germination.

In this study, the seeds of *E. velutina* were found to be quite sensitive to drought stress, since less normal seedlings and less speed in their establishment were observed from -0.1 Mpa. Probably the high viscosity of the osmotic agent employed (PEG) of high molecular weight, together with the low O_2 diffusion rate, contributed to the reduction and delay of seedling formation (Braccini et al., 1996). Also, the more negative the osmotic potential, the more time it will take for the seed to absorb water, for the occurrence of enzymatic digestion and the translocation of soluble and diffusible substances to the growth points of the embryonic axis, resulting in a smaller number of seedlings emerged per day, culminating in a lower germination speed (Bewley and Black, 1994).

The world's semiarid regions experience low availability of moisture during germination, and the behavior of *E. velutina* in soils with potentials from -0.4 MPa indicates that the referred species is highly vulnerable, so its planting is not recommended. According to Ribeiro et al. (2012, 2014), the germination percentage is a factor less sensitive to the effects of osmotic stress than the germination speed index. In the results of this research, both the germination percentage and the speed rate showed to be sensitive to accuse the deleterious effects of hydric limitations.

Differences in the behavior of the same species in the face of drought stress may be due to the number of days destined for the germination process and mainly due to the procedure adopted to evaluate the germination process, that is, different germination criteria used. The present research considered the formation of normal seedlings as a germination criterion in contrast to Ribeiro et al. (2012, 2014), who considered the protrusion of the primary root. It is thus evident that the adopted germination criteria may result in different responses in relation to the sensitivity of the species to drought stress. Pelegrini et al. (2013) when also adopting root protrusion as a germination criterion, found that in *Erythrina falcata* Benth. (Fabaceae), there was a drastic inhibition of the percentage and speed index and an increase in the average germination time from potentials below -0.4 MPa. It can be highlighted as well, that tolerance to drought stress can be related to environmental conditions and genetic diversity presented by mother trees of the same species.

Polyethylene glycol (PEG) reduced and delayed the germination of *Pinus sylvestris* var. *mongolica* (Zhu et al., 2005) and *Picea asperata* Mast. (Yang et al., 2010) as the concentration of the osmotic agent increased. The intensity of the germinative response to water deficit varies among seeds of different species, and for each one there is an external water potential value below which germination does not occur. Seeds of *E. velutina*, for example, have their germination and index of speed greatly affected at -0.4 MPa. The reduction in germination power, compared to the control, serves as an indicator of the tolerance index of the species to water deficit.

By submitting seeds of xerophytic species, such as *Caragana korshinskii* Kom., *Artemisia sphaerocephala* Krasch,, and *Hedysarum scoparium* Fischer & C. A. May. (collected from semiarid regions of northwest China), and *Reaumuria soongorica* (Pall.) Maxim. and *Zygophyllum xanthoxylum* (Bunge) Engl. (harvested in the arid regions), under drought stress induced by osmotic potentials of PEG 6000, Zeng et al. (2010) found that *Z. xanthoxylum* had a higher sensitivity to water availability among the species studied, characterizing an adaptive germination mechanism.

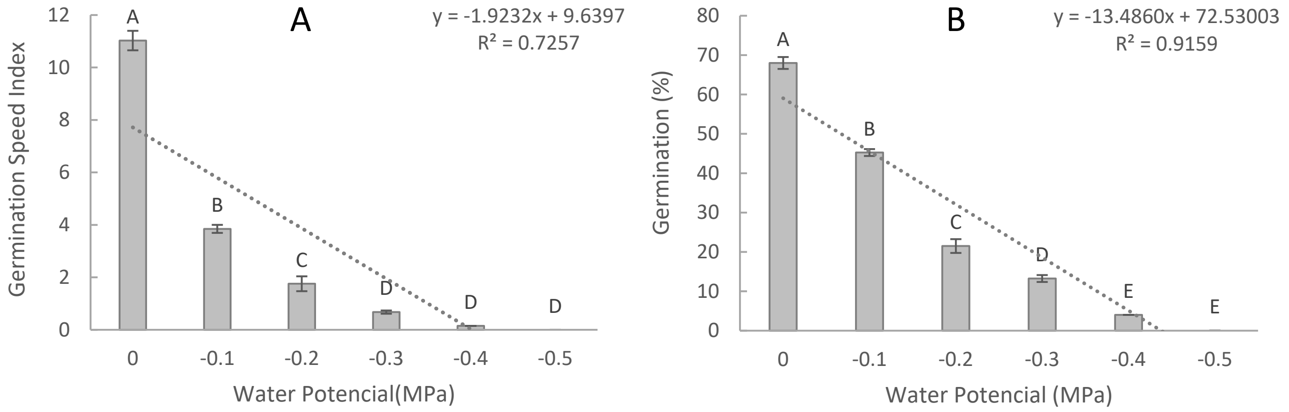

Figure 1 – Germination speed index (A) and germination percentage (B) of *Erythrina velutina* Willd seeds submitted to different levels of osmotic potentials (0.0; -0.1; -0.2; -0.3; -0.4; and -0.5 MPa). Equal letters indicate no statistical differences by Tukey's test at the 5% probability level.
y: fitting equation; R^2: correlation coefficient.

Within this context, Bewley and Black (1994) emphasized that drought stress delays seed germination, thereby causing seedling establishment and development to occur only under favorable environmental conditions. The species under study is commonly found in the semiarid landscape and is described as a drought-resistant plant; however, its seeds can be considered sensitive to water deficit, since at a water potential of -0.2 MPa only 20% of the seeds germinate.

The germination of *Sophora davidii* (Franch.) Pavol. was evaluated under different osmotic potentials (0.0 to -1.0 MPa), where the highest rates occurred under potentials ranging from 0 to −0.4 MPa (Wang et al., 2016). Similarly, the seeds of *Caesalpinia echinata* Lam. germinated until the osmotic potential of 0.4 MPa (Rodrigues et al., 2020). Both germination and seed vigor of *Handroanthus impetiginosus* (Mart. Ex DC) were negatively affected from -0.6 MPa (Santos et al., 2018). *Aspidosperma polyneuron* Muell. Arg. seeds had germination capacity reduced as the osmotic potential became more negative (Rodrigues et al., 2019), not germinating below -0.6 MPa. In turn, the germination of *Chamaecytisus palmensis* (Christ) F.A. Bisby & K.W. Nicholls seeds (Zhenyi et al., 2019) was only completely inhibited under the drought stress of -1.20 MPa. Shaygan et al. (2017) found that even exposed to -1.5 MPa osmotic potential, *Atriplex halimus* L. seeds germinated 31%, indicating that they are highly tolerant to drought stress.

The failure to regenerate natural forests, due to drought induced by climate change (McLaren and McDonald, 2003; Springer, 2005; Carón et al., 2015; Zhang et al., 2015), favors the decline in the size of plant populations. It is emphasized that in the dry season, seedlings tend to die when the soil moisture is below a critical limit of water potential (McDowell et al., 2008), which varies according to plant species. Wit in this context, Jeltsch et al. (2008) and Milbau et al. (2009) highlighted that for the development of conservation strategies, as well as for the acquisition of knowledge about survival mechanisms and population dynamics, studies are needed to assess how drought stress acts on plant regeneration.

The length of the aerial part and the root system of the seedlings were influenced by drought stress, with decreasing linear adjustment (Figures 2A and 2B). The greater length of the aerial part (A) and root (B) was found in the control, statistically differing from the other treatments. For potentials -0.1; -0.2; -0.3; and -0.4 MPa, the aerial parts of *E. velutina* seedlings did not differ among themselves.

In seedlings of *E. velutina*, it was found that in potentials -0.1; -0.2; and -0.3 MPa, the aerial part growth and the length of the root were similar regarding the adaptive characteristic of the species to these conditions because even with the reduction of the water potential, it kept the growth of the root. The continuity of root growth, under conditions of less water availability, depends on the maintenance of a minimum turgor pressure in the cells, which is sufficient to allow cellulosic wall elongation and cell growth (Hsiao and Xu, 2000).

A reduction in the length of the root system and the hypocotyl of seedlings of *P. sylvestris* was verified as a function of stress subjected to PEG of -0.20 MPa, indicating the sensitivity of growth in the face of drought stress simulated by PEG (Zhu et al., 2005). Still, the same authors attributed drought stress as a possible cause of inhibition of the natural regeneration of the Mongolian pine in sandy lands. In the same context, Zhang et al. (2015) considered that the drought induced by climate change is responsible for suppressing the germination of seeds and the growth of *Nyssa yunnanensis* W.Q.Yin ex H.N.Qin & Phengklai seedlings, compromising the process of natural regeneration of the habitat of this species.

From the water potential of -0.2 MPa (PEG 6000), the length of the stem of seedlings of *H. impetiginosus* declined (Santos et al., 2018). Both the growth of the primary root and the hypocotyl after the germination of *C. palmensis* seeds were restricted mainly under osmotic potentials below -0.12 MPa (Zhenyi et al., 2019). These authors emphasize that the reduction in the length of the primary root and the hypocotyl results in the decline in the number of root hairs, negatively interfering with the water absorption capacity of young roots. Therefore, the natural regeneration of *C. palmensis* plantations in sandy lands can be compromised by drought stress.

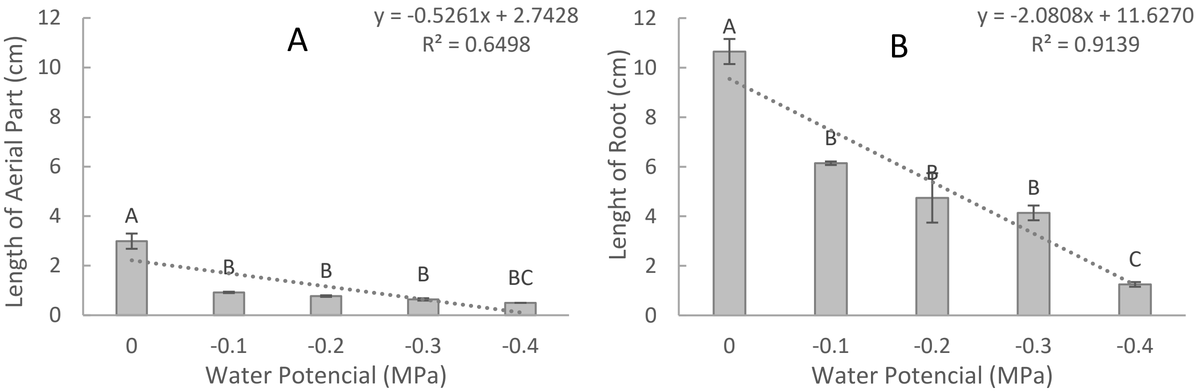

Figure 2 – Length of aerial part (A) and root system (B) of seedlings of *Erythrina velutina* Willd submitted to different levels of osmotic potentials (0.0; -0.1; -0.2; -0.3; and -0.4 MPa) in germination. Averages followed by equal letters do not differ by Tukey's test at the 5% probability level.
y: fitting equation; R^2: correlation coefficient.

The accumulation of dry mass in the aerial part was greater for seedlings that were not subjected to drought stress (control), and there was a reduction in treatments under the effect of stress, without showing statistical differences between them (Figure 3A). The root dry mass was higher in the control, followed by the water potential -0.1 MPa; the lowest values came from seedlings submitted to treatments -0.2; -0.3; and -0.4 MPa (Figure 3B). The highest value of total dry mass was also observed in the control treatment, with a decrease by half recorded in the treatment -0.1 MPa (Figure 3C).

The photosynthetic pigments of primary leaves were negatively influenced by the decrease in osmotic potential, presenting an adjustment to the linear equation (Figure 4). Both the content of chlorophyll a and b, as well as the total chlorophyll (Figures 4A, 4B and 4C) showed the same behavior, with a higher content in the control followed by the treatment -0.1 Mpa, which did not differ statistically from the treatments -0.2 and -0.3 Mpa. In turn, the greatest reduction for these characteristics was observed in the potential -0.4 Mpa, not differing from the osmotic potentials -0.2 and -0.3 Mpa. As the osmotic potentials became more negative, there was a reduction in chlorophyll a (Figure 4A) and chlorophyll a /chlorophyll b ratio (Figure 4D), with statistical differences between treatments. Carotenoids in seedlings submitted to different osmotic potentials (-0.1; -0.2; -0.3; and -0.4 Mpa) did not show statistical differences between them (Figure 4E).

In seedlings (*E. velutina)*, a reduction of chlorophyll a, b, chlorophyll a/b, and total were observed with a decrease in water potential, showing that the water deficit contributed decisively to the reduction of photosynthetic pigments. As the water deficit intensified, the levels of chlorophyll were reduced due to the formation and, consequently, the action of ROS. Possibly, the cause of gradual damage to the chlorophyll molecules and the reduction of the levels of chlorophyll b is related to chloroplast degradation.

A decline in chlorophylls during the period of exposure to drought can reduce the light pressure of photosystem II (PSII), which according to Pompelli et al. (2010), can be observed as a form of protection of the photosynthetic apparatus of the mentioned PSII. There may be a correlation between the degradation of photosynthetic pigments, resulting from stress, with the accumulation of ROS, which are responsible for oxidative stress (Nxele et al., 2017). Lipid peroxidation results from the degradation of thylakoid membranes in chloroplasts by ROS (Pottosin and Shabala, 2016; Foyer, 2018).

In other forest species, there was a significant decline in total chlorophyll in *Rhus tripartita* (Ucria) Grande seedlings, as well as the chlorophyll a as the aridity intensified (Zouaoui et al., 2019). While *Prosopis juliflora* (Sw.) DC. had higher chlorophyll/carotenoid and chlorophyll a/b ratios than *Anadenanthera colubrina* var. cebil (Vell.) Brenan, in addition to suffering less oxidative stress, it used water more efficiently in environments with reduced availability (Teixeira Oliveira et al., 2014).

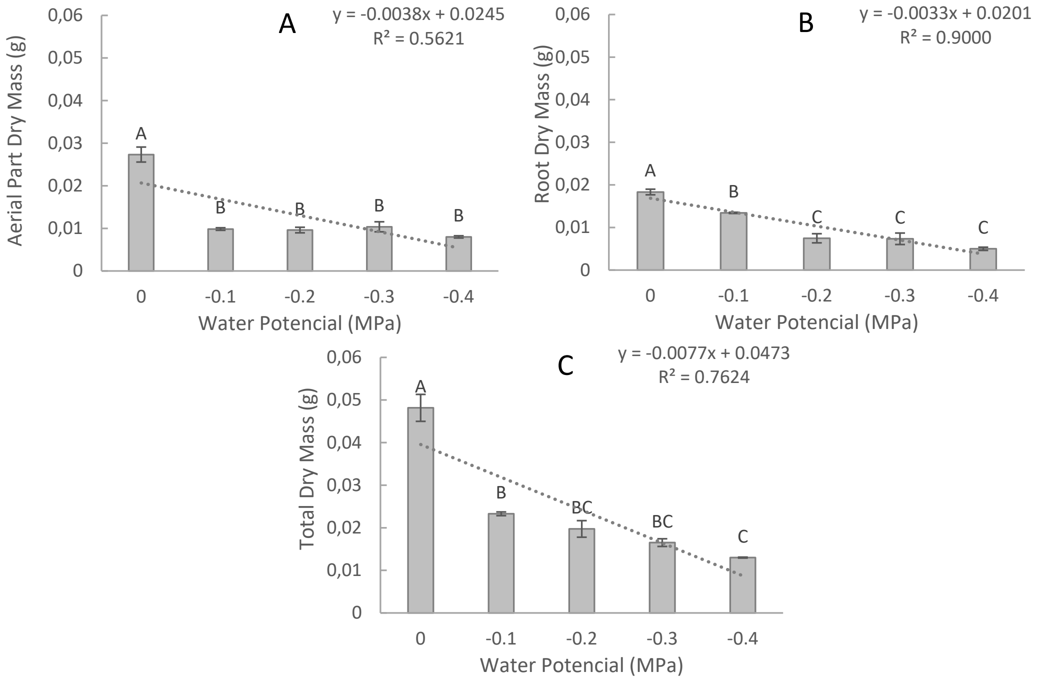

Figure 3 – Dry mass of aerial part (A), root (B), and total (C) of *Erythrina velutina* Willd seedlings submitted to different levels of osmotic potentials (0.0; -0.1; -0.2; -0.3; and -0.4 MPa) on germination. Averages followed by equal letters do not differ by Tukey's test at the 5% probability level.
y: fitting equation; R^2: correlation coefficient.

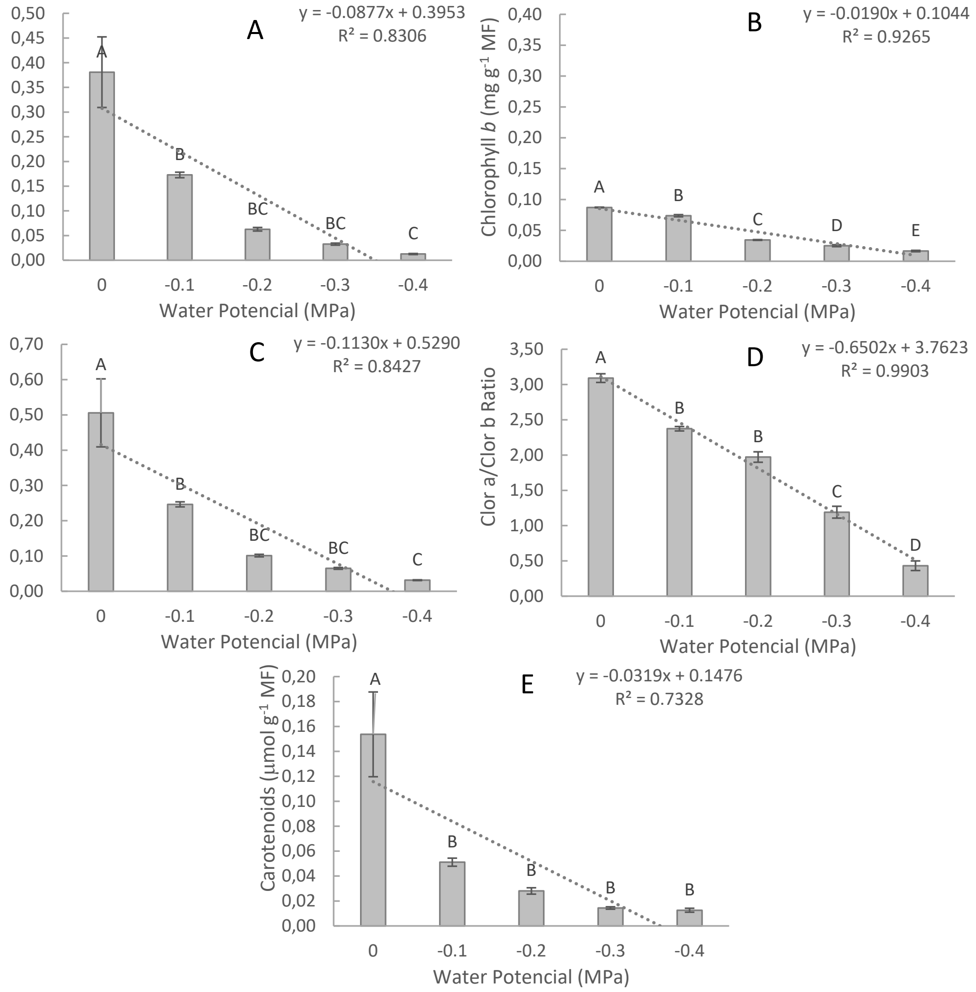

Figure 4 – Chlorophyll a (A), chlorophyll b (B), total chlorophyll (C), chlorophyll a/b ratio (D), and carotenoids € of primary leaves of *Erythrina velutina* Willd seedlings submitted to different levels of osmotic potentials (0.0; -0.1; -0.2; -0.3; and -0.4 MPa) on germination. Averages followed by equal letters do not differ by Tu'ey's test at the 5% probability level.
y: fitting equation; R^2: correlation coefficient.

In the species *Pyracantha fortuneana* (Maxim.) H.L.Li. and *Rosa cymosa* Tratt., and four trees *Broussonetia papyrifera* (L.) Vent., *Cinnamomum bodinieri* H.Lév., *Platycarya longipes* Y.C.Wu., and *Pteroceltis tatarinowii* Maxim., drought stress significantly decreased the pigment content, but increased the proportion of carotenoids to total chlorophylls (Liu et al., 2011).

The carotenoids are also accessory pigments that, besides being associated with chlorophyll molecules, contribute to promoting the coloring of plant tissues, and play an essential role in photoprotection, preventing damage caused by excessive solar radiation, which induces excitation of chlorophyll molecules (Taiz et al., 2017).

The observations of carbohydrate and proline were only carried out up to the water potential of -0.3 MPa, since from -0.4 MPa there was not enough plant material for evaluation (Figures 5A and 5B). The carbohydrate concentration was higher when the seeds were subjected to drought stress, with no statistical differences between the osmotic potentials (Figure 5A). The osmotic adjustment, due to the concentration of proline, was greater in the potential -0.2 MPa in relation to the control and treatment -0.1 MPa; however, it did not differ from the -0.3 MPa potential, which in turn, did not differ from the -0.1 MPa potential (Figure 5B). Water deficit changes the quantity and quality of sugars found in leaves, which can be a sign of stress (Chaves and Oliveira, 2004). It is highlighted that the signaling role of sugars may have an adaptive character and be related to osmoregulation (Singh and Gautam, 2013). It was also found that seedlings exposed to water deficit present an increase in sugars, which can be seen as a purpose of maintaining the water level of the leaf; thus, osmotic adjustment leads to cellular osmotic balance (Kerbauy, 2019; Singh and Gautam, 2013).

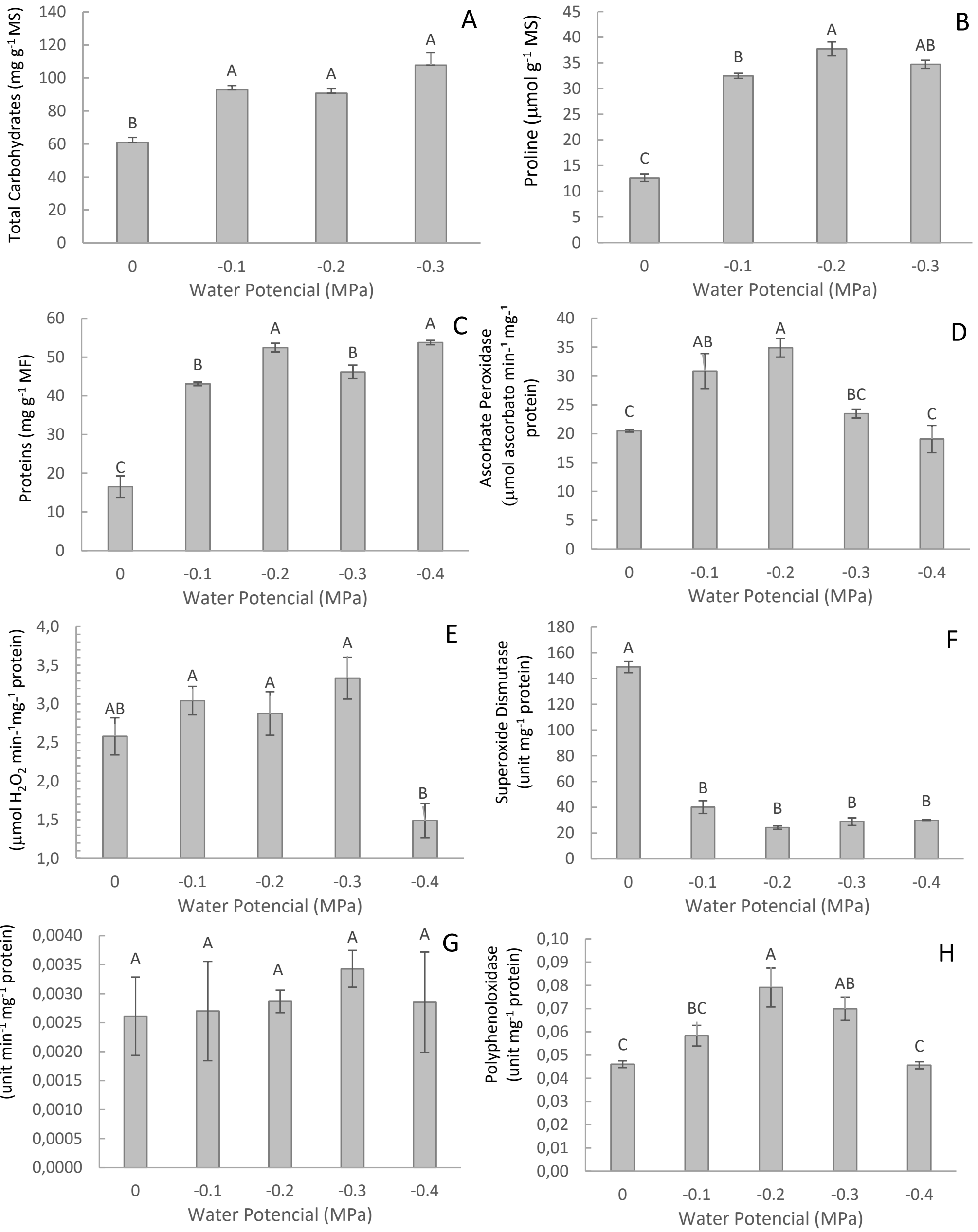

Figure 5 – Soluble carbohydrates (A), free proline (B), soluble proteins (C), ascorbate peroxidase (D), catalase (E), superoxide dismutase (F), peroxidase (G), and polyphenoloxidase (H) from the aerial part of *Erythrina velutina* Willd seedlings submitted to different levels of osmotic potentials (0.0; -0.1; -0.2; -0.3; and -0.4 MPa) on germination. Averages followed by equal letters do not differ by Tukey's test at the 5% probability level.

The aerial part of the seedlings submitted to drought stress showed a higher concentration of proteins (Figure 5C); where the lowest value was found in the absence of stress and the highest protein values were found in the potentials of -0.2 and -0.4 MPa, which did not differ statistically from each other.

In the present study, the seedlings of *E. velutina* under drought stress, regardless of osmotic potential, suffered an increase in soluble carbohydrates and soluble proteins in the aerial part, as a way of triggering their defenses against stress. This behavior is linked to the purpose of maintaining the water level in the cell medium, and the induction of leaf starch degradation to increase the concentrations of free carbohydrates in the cell cytoplasm, aiming at osmotic balance. Therefore, the increase in the levels of proline and total soluble carbohydrates is probably because both act as osmotic regulators in plants with low water potential in the leaf. The osmotic regulation is strongly dependent on the accumulation of soluble carbohydrates (Martin et al., 1993; Ivanov et al., 2019). In woody species such as *Eucalyptus microtheca* F. Muell. (Chunyang, 1998) and *Quercus ilex* L. (Pesoli et al., 2003) there was an increase in soluble sugars from plants under drought stress. There was a considerable increase in osmotic regulation substances (content of free proline and soluble sugar) and lipid peroxidation in seeds of *P. asperata* Mast subjected to drought stress, as the osmotic potential became more negative (Yang et al., 2010).

An accumulation of organic solutes (soluble sugars and proline) was found in the leaves and roots of young plants *Rhus tripartita* (Ucria) Grande, when they were exposed to drought stress (Zouaoui et al., 2019). The accumulation of soluble sugars in the cells is seen as an adaptive strategy of plants to resist environmental adversities. This accumulation would be due to the increase in starch hydrolysis (Taiz et al., 2017). To minimize the harmful effects of water deficit and thus maintain cell turgidity, plants begin to accumulate organic or inorganic solutes, which results in osmotic adjustment (Silva et al., 2019).

Under more negative water potentials in *A. polyneuron* seeds, Rodrigues et al. (2019) observed a change in the degradation of reserves, with sugars being degraded more slowly and soluble proteins more quickly. Still, prolamin was the least abundant protein, while glutelin was the most present.

The production of proline in the aerial part of *E. velutina* seedlings was higher in the three osmotic potentials (-0.1; -0.2; and -0.3 MPa) in relation to the use of pure water (0.0). However, for the same species, Ribeiro et al. (2014) found that proline levels in cotyledons did not vary between the exclusive use of water and osmotic potentials (-0.2 and -0.6 MPa); while in the embryonic axis, there was a small increase between 0.0 and -0.2 MPa, declining from this potential. Even though they are the same species, the tissue evaluated to verify the activity of the osmoregulator diverged, which may have affected the response to the formation of proline.

In this research, an increase in the activity of the APX enzyme was observed in the aerial part of *E. velutina* seedlings in the osmotic potentials of -0.1 and -0.2 MPa in relation to the absence of stress (Figure 5D).

It was recognized that oxidative stress, when damaging tissues, causes an increase in the activity of antioxidant systems, such as greater production of CAT and APX (Kilic and Kahraman, 2016). Zhang et al. (2017) emphasized that the high activity of APX and CAT under environmental stress conditions is essential, aiming to balance the intracellular environment between the formation and removal of H_2O_2 (hydrogen peroxide). Sartori et al. (2023) observed a significant increase in APX activity when the wheat seeds were subjected to high negative water potentials and an increase in temperature; however, for some combinations (water potential and temperature), there were no differences related to the absence of deficiency water.

It was considered in the present research that the enzymatic antioxidant defense apparatus may have collapsed from the osmotic potential of -0.34 MPa, and that potential germination was extremely low (Figure 1). In turn, its activity was not observed in the cotyledons of *E. velutina* seeds subjected to drought stress, while in the embryonic axis, there was a reduction of this enzyme until it was inhibited in the potential of -0.6 MPa (Ribeiro et al., 2014). These authors, when considering that APX is an alternative way to remove H_2O_2 from the medium, assumed that the water potentials used (-0.2 and -0.6 MPa), during germination, kept ROS production low in the seeds, resulting in extremely low levels of the enzyme.

The activity of CAT (Figure 5E) in seedlings that developed in the absence of drought stress did not differ in relation to the various osmotic potentials, but between them, the greatest activity was found in the potentials -0.1; -0.2; and -0.3 MPa to the detriment of the potential -0.4 MPa.

Normally, CAT activity under stress conditions tends to increase (Barakat et al, 2013); however, Mhamdi et al. (2012) highlighted that negative regulation can occur when stress intensifies or even maintain enzymatic activity without significant changes. This fact was confirmed by Sartori et al. (2023) when observing that, although the CAT was similar between the combination of 32°C and -0.444 MPa and the others, the germination of wheat seeds was lower. A similar situation was observed in the present research, where, despite the absence of statistical differences in CAT activity between water potentials, there was a reduction in germination and germination speed from -0.1 MPa.

According to Ribeiro et al. (2014), CAT activity may have been sufficient to maintain the levels of H_2O_2 at a tolerable level. In turn, we did not find this association, since there was no difference between the exclusive use of water (0.0 MPa) and osmotic solutions (-0.1; -0.2; -0.3; and -0.4 MPa) in relation to CAT activity. It is evident that the activity of the enzyme depends not only on the osmotic potential but also on the part of the seed or seedling that is being evaluated. *E. velutina* embryonic axes showed an increase in CAT activity in the osmotic potential -0.2 MPa, when germination (protrusion of the primary root) was 97%, reducing under the potential of -0.6 MPa, where there was no germination (Ribeiro et al., 2014).

The SOD activity reduced with stress, with no differences between the osmotic potentials. However, peroxidase activity was not influenced by drought stress, to which *E. velutina* seedlings were subjected (Figure 5F). An inefficiency or insufficient activation of the enzymatic antioxidant system was observed when the *E. velutina* seeds were subjected to suboptimal water conditions; there was even a smaller SOD activity. With reduced water availability, an increase in SOD activity would be expected, because this enzyme is the first line of defense against oxidative stress, followed by CAT, APX, and glutathione peroxidase (Zandalinas et al., 2018). The same authors emphasized that the greater capacity for protection against oxidative damage may be due to the increased activity of antioxidant enzymes, resulting in better tolerance to water deficit. When O_2 is produced, it is dismutated into H_2O_2 by the action of SOD, so that H_2O_2 is eliminated by the action of APX, CAT, and glutathione peroxidase, converting it into water and oxygen (Barbosa et al., 2014). Thus, the greater the efficiency of antioxidant enzyme activity, the lower the oxidative stress level, culminating in a balance between O_2- and H_2O levels (Guimarães et al., 2018).

A greater activity of the polyphenoloxidase enzyme was verified in the aerial part of *E. velutina* seedlings submitted to the osmotic potentials of -0.2 and -0.3 MPa, to the detriment of the other potentials (Figure 5H). In the absence of drought stress, the polyphenoloxidase content did not differ from those found in the potentials of -0.1 and -0.4 MPa. When submitting seedlings of *Cassia angustifolia* Vahl. and *Cassia auriculata* Linn. to drought stress, Agarwal and Pandey (2003) associated the increased tolerance to oxidative stress with the high activity of polyphenoloxidase. When *P. asperata* seeds were subjected to drought stress, it was observed that the activity of antioxidant enzymes (APX, CAT, and peroxidase) increased significantly with decreased water potential (Yang et al., 2010). The authors considered this species insensitive to drought stress because there was an increase not only in enzymes but also in osmoregulators, and lipid peroxidation under the osmotic potential of -0.6 MPa in relation to the 0.0 and -0.2 MPa potentials.

In this context, it can be inferred that *E. velutina* seeds are quite sensitive to water deficit. Regarding the absence of water deficit (0.0 MPa) there was an increase in the activity of the enzymes APX only at water potentials -0.1 and -0.2 MPa, and polyphenoloxidase at -0.2 and -0.3 MPa. A decline in SOD activity was observed when the seeds were exposed to negative water potentials. Furthermore, in general, there were no significant differences in the activity of the CAT and peroxidase enzymes between the different water potentials. Zheng et al. (2009) emphasized that there is a dependence on plant tolerance to stressful conditions in relation to the synchronized activity of enzymes responsible for eliminating ROS; and as in the present research the defense system was possibly not activated efficiently, it is considered that *E. velutina* seeds are poorly tolerant to water deficit.

The fact that *E. velutina* seeds, submitted to drought stress during the germination process, had lower proline accumulation and lower activities of antioxidant enzymes, it was considered by Ribeiro et al. (2014) as an indication of the low tolerance of seeds to drought stress. Although Leite et al. (2022) verified that *E. velutina* had a drought tolerance mechanism, in which it rapidly reduces photosynthesis and foliar gas exchange, improving water use efficiency, its recovery was slow after water supply.

Conclusions

The water potential from the level -0,4 MPa is a limiting factor for the germinative process of *E. velutina* seeds to result in the formation of normal seedlings.

Direct sowing of *E. velutina* would only be indicated up to the potential of -0,3 MPa for the regeneration of degraded landscapes in semiarid regions, and from this potential, it is not indicated for planting in semiarid regions due to its vulnerability.

Increased water deficit in seeds simulated with PEG reduces photosynthetic pigments and increases osmotic regulators in *E. velutina* seedlings.

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ALMEIDA, D.T.R.G.F.: conceptualization, funding; data curation, formal analysis. SILVA, M.A.D.: data curation, formal analysis; writing – original draft. GONÇALVES, E. P.: conceptualization, writing – review & editing. ALMEIDA, F.F.A.: data curation; visualization; investigation. SILVA, J.C.A.: data curation; visualization; investigation. RODRIGUES, C.M: data curation; visualization; investigation. VIANA, J.S.: data curation, formal analysis.

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