Activity of antioxidant enzymes and proline accumulation in *Erythrina velutina* Willd. seeds subjected to abiotic stresses during germination[1]

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ABSTRACT - The aim of this study was to evaluate the effect of different abiotic stresses on the activity of antioxidant enzymes and on accumulation of proline in *Erythrina velutina* Willd. seeds during germination. Mulungu seeds were scarified and placed to germinate at constant temperatures of 15, 25, and 35 °C, moistened with distilled water, and exposed to 12 h of light. Other seeds were exposed to solutions of NaCl (EC of 0, 4, and 8 dS.m^{-1}) and polyethylene glycol (osmotic potentials of 0.0, -0.2, and - 0.6 MPa) and maintained in a germination chamber set at 25 °C and 12 h photoperiod for seven days. At the end of each period of imbibition, the embryonic axis and cotyledons of the seedlings were collected separately and used to quantify proline content and the activity of antioxidant enzymes. These were detected in both the cotyledons and embryonic axis of the mulungu seeds. Antioxidant activity varied depending upon the type and degree of stress applied. It was concluded that under the aspect of the detoxification process, the mechanism found in mulungu seeds is more efficient when subjected to different temperatures followed by salt stress and water stress.

Index terms: antioxidative metabolism, water stress, salt stress, heat stress, mulungu.

Atividade de enzimas antioxidantes e acúmulo de prolina em sementes de *Erythrina velutina* Willd. submetidas a estresses abióticos durante a germinação

RESUMO - Objetivou-se com este trabalho avaliar o efeito de diferentes estresses abióticos na atividade das enzimas antioxidantes e no acúmulo da prolina em sementes de *Erythrina velutina* Willd. durante a germinação. Sementes de mulungu foram escarificadas e colocadas para germinarem nas temperaturas constantes de 15, 25 e 35 °C, umedecidas com água destilada e com 12 h de luz. Outras sementes foram expostas a soluções teste de NaCl (condutividade elétrica 0, 4 e 8 dS.m^{-1}) e soluções de polietilenoglicol (potencias osmóticos 0,0; -0,2 e -0,6 MPa) e mantidas em câmaras de germinação ajustadas a 25 °C, fotoperíodo de 12 h durante sete dias. Os cotilédones e eixo embrionário de plântulas foram coletados e utilizados para quantificar o teor de prolina e a atividade de enzimas antioxidantes. Estas foram detectadas nos cotilédones e eixo embrionário de sementes de mulungu. A atividade antioxidante foi variável dependendo do tipo e nível de estresse aplicado. Os resultados encontrados permitem concluir que sob o aspecto do processo de desintoxicação, o mecanismo encontrado nas sementes de mulungu é mais eficiente quando submetidas a diferentes temperaturas, seguido pelo estresse salino e estresse hídrico.

Termos para indexação: metabolismo antioxidativo, estresse hídrico, estresse salino, estresse térmico, mulungu.

Introduction

Some plant species native to the Caatinga (a xeric shrubland and thorn forest in Brazil) are of great biological importance due to their potential for popular use, evaluated through ethnobotanical surveys. *Erythrina velutina* Willd. (Leguminosae–Papilionoideae)*, a forest species native to the Caatinga of the brazilian Northeastern region, has shown tolerance or adaptation mechanisms regarding abiotic stresses, especially thermal, saline, and water stresses (Ribeiro-Reis, 2012). This species produces a large quantity of viable seeds annually, and this is its main form of propagation. It is a plant that grows drought and high temperature conditions over most of the year (Carvalho, 2008).

Success in establishment of seedlings is mostly dependent on seed quality (viability and vigor). High resistance to abiotic stresses is a characteristic arising from seeds; however,

knowledge of these properties for *E. velutina* is not yet known as a potential source for granting tolerance to seedlings or adult plants. In addition, there are no published studies that deal with the physiological and biochemical changes in seeds from species native to the Caatinga, with a focus on the relation among enzyme activities in the different parts of the seeds during germination under different abiotic stresses. Most of these stresses, such as drought, salinity, heat, and cold may disturb the metabolic balance of cells, resulting in increased production of reactive oxygen species (ROSs), such as the superoxide radical ($.O_2^-$), hydrogen peroxide (H_2O_2), and hydroxyl radical (OH.). Plants have developed an elaborate and efficient network of mechanisms for elimination that allow them to overcome the toxicity of ROSs (Bailey-Serres and Mittler, 2006; Foyer and Noctor, 2005).

The detoxification process involves control and removal of ROSs in the different cellular compartments (Prisco and Gomes-Filho, 2010), which is performed by antioxidants of an enzymatic nature, such as the enzymes superoxide dismutase (SOD, EC 1.15.1.1), ascorbate peroxidase (APX, EC 1.11.1.11), glutathione peroxidase (GPX, EC 1.11.1.9), guaiacol peroxidase (GOPX, EC 1.11.1.7) and catalase (CAT, EC 1.11.1.6); non-enzymatic antioxidants may also be included, such as α-tocopherol (vitamin E), β-carotene, ascorbate (vitamin C), and reduced glutathione (GSH), which plays an important role in the regulation of cell homeostasis amid ROSs (Foyer and Noctor, 2005).

Proline has also been studied as a plant response to abiotic stresses. Many plants accumulate proline under conditions of water deficit, salinity, and extreme temperatures, in addition to activation of antioxidant enzyme activity (Chen et al., 2001). Proline plays an important role in osmoregulation and osmotolerance; however, its exact role in exercising resistance to abiotic stresses such as salinity continues to be under controversy (Azooz et al., 2004; Demiral and Turkan, 2006). In these situations of abiotic stresses, including drought, salinity, and extreme temperatures, proline acts as an osmoprotector, located in the cytoplasm, as a stabilizer of enzymes, of the macromolecule and organelle structure, and as a remover of reactive oxygen species (ROSs) (Demiral and Turkan, 2006).

The ROSs are of growing interest in seed physiology, and their contributions have been related to the role of ROSs in the loss of vigor and viability during prolonged storage of orthodox seeds (Bailly et al., 2004). Seeds may be exposed to severe stresses also during the germination process, including drought and high temperatures, and, in this sense, there are few reports on biochemical mechanisms of response to stress and, more specifically, on the activity of antioxidant enzymes. Thus, the aim of this study was to verify the activities of the enzymes related to oxidative stress and proline accumulation during germination of *Erythrina velutina* seeds subjected to abiotic stresses.

Materials and Methods

Obtaining seeds: E. velutina seeds were harvested, in October 2009, from parent plants at the municipality of Jutaí (Lagoa Grande, PE, Brazil) with geographical coordinates of 37º 18′ 03″ W and 90º 52′ 92″ S collected. The seeds were processed manually, placed in transparent plastic bags (0.15 mm thickness), and kept at ambient temperature (30 °C ± 5 ºC, 56 ± 6% RH) in the laboratory.

Prior to the physiological trials, mechanical scarification of the outside seed coat was carried out with the assistance of a mini-rotary tool (Western R-40). The seeds were then subjected to asepsis using a commercial 2% sodium hypochlorite solution for two minutes, and then washed in distilled water.

Evaluation was performed daily, considering germinated seeds those with at least two mm of length of the radicle. At the end of the test period, the seedlings were collected for biochemical analyses. The variables evaluated were mean percentage and time of germination (Labouriau, 1983), mean speed of germination (Kotowski, 1926), and germination speed index (Maguire, 1962).

Effect of temperature: seeds were placed on rolls of towel paper, moistened with distilled water corresponding in volume to 2.5 times the weight of the substrate. The rolls containing the seeds were incubated in BOD germinators set at constant temperatures of 15, 25 and 35 °C and a 12 h photoperiod for a period of seven consecutive days.

Effect of osmotic potential: seeds were placed in rolls of towel paper, moistened with test solutions of PEG 6000 at different osmotic potentials (0.0, -0.2, and -0.6 MPa), prepared according to Villela et al. (1991), corresponding in volume to 2.5 times the weight of the substrate. The rolls containing the seeds were incubated in germinators at 25 °C and a 12 h photoperiod for a period of seven consecutive days.

Effect of salinity: seeds were placed in rolls of torvel paper, moistened with NaCl solution, corresponding in volume to 2.5 times the weight of the substrate, at different electrical conductivity (EC), verified at 0, 4, and 8 dS.m^{-1} in an electrical conductivity meter. The rolls containing the seeds were incubated in BOD germinators set at constant temperatures of 25 °C and a 12 h photoperiod for a period of seven consecutive days. At the end of this period in the abiotic stress trials, the cotyledons and embryonic axis were collected for biochemical analyses.

Enzymatic extraction and biochemical analyses: 1 g of fresh matter of the samples collected was homogenized in 10 mL of 100 mM potassium phosphate buffer, pH 7.5; containing 1 mM of EDTA (ethylenediamine tetraacetic acid), 3 mM of DTT (dithiothreitol) and 5% of PVPP (polyvinylpolypyrrolidone) (Gomes-Junior et al., 2006). The macerate was centrifuged at 10,000 x g for 30 min at 4 °C. The supernatant collected was divided into aliquots of 1.5 mL and stored in the freezer at –80 ºC up to the time of analyses of total soluble protein contents (Bradford, 1976) and activity of catalase antioxidant enzymes (Azevedo et al., 1998), ascorbate peroxidase (Nakano and Asada, 1981), guaiacol peroxidase (Matsuno and Uritani, 1972), and glutathione-S-transferase, which was determined according to the specifications of the Glutathione S-Transferase Assay Kit (CS0410 – Sigma).

Quantification of Proline: this was performed through homogenization of 0.5 g of fresh matter in 10 mL of 3% sulfosalicylic acid (w/v), which was centrifuged at 3000 rpm for 10 min. The supernatant was divided into aliquots and stored in a freezer at –20 ºC up to the time of analysis (Bates, 1973).

Design and Statistical Analysis: a completely randomized experimental design was used, consisting of three treatments and four replications of 25 seeds for each stress applied. The data on germination percentage, mean time, mean speed and germination speed index were analyzed through analysis of variance using the Assistat program (Silva and Azevedo, 2009). The differences between the mean values obtained were compared by the Tukey test at 5% probability. For effect of analysis of variance, the data on germination percentage were transformed by the function $(x + 0.5)^{0.5}$. For the enzyme data, the standard error of the mean (SEM) was established.

Results and Discussion

The percentage of seed germination remained at high levels with the increase in temperature, reduction of osmotic potential, and increase in salt concentration in the germination medium (Table 1). When compared to the other treatments of temperature and osmotic potential, there was only a significant difference between seeds subjected to 35 °C and to the potential of -0.6 MPa, with a reduction of 18.2% and total inhibition of germination, respectively.

The effect of the temperature and water stresses was also verified in mean time, mean speed, and germination speed index. At the temperature of 15 °C, there was a significant increase in mean time and significant reduction in mean speed and germination speed index. At 35 °C, the difference for these variables was seen only in the germination speed index (Table 1).

Table 1. Germination (G, %), Mean time (Mt, days), Mean speed (Ms, days^{-1}) and germination speed index (GSI, radical protrusion.day^{-1}) of *Erythrina velutina* seeds subjected to different temperatures, osmotic potentials, and concentrations of NaCl solution during germination.

Treatments	G	Mt	Ms	GSI
Temperature (°C)				
15	92 a	4.54 a	0.22 a	5.99 b
25	99 a	2.99 b	0.34 a	9.04 a
35	81 b	2.94 b	0.34 a	7.43 b
PEG 6000 (MPa)				
0.0	100 a	4.43 a	0.23 a	2.49 a
-0.2	97 a	5.59 a	0.18 b	1.76 b
-0.6	0 b	0.00 b	0.00 c	0.00 c
NaCl (EC dS.m^{-1})				
0	100 a	4.67 a	0.22 a	2.42 a
4	100 a	4.90 a	0.20 a	2.20 a
8	100 a	4.73 a	0.21 a	2.30 a

Mean values followed by the same small letter in the column for each treatment do not differ among themselves by the Tukey test at 5% probability.

According to Carvalho and Nakagawa (2012), the greater the temperature is, the quicker germination will be and the more efficient the process will be, up to a certain limit. Species which are native to or well adapted to the Caatinga, in spite of having seeds with different ideal temperature ranges, have high tolerance limits, in line with the hot conditions of the region. *Prosopis juliflora* is able to germinate at higher temperature limits, such as those normally registered in the Caatinga ecosystem (Miranda et al., 2013). Seeds from *Cereus jamacaru* have greater germination capacity and shorter mean time when germinated at 30 °C, in comparison to other temperatures (Meiado et al., 2010). In seeds from aroeira (*Myracrodruon urundeuva*) collected in the Caatinga, the best temperature for germination, with greater speed, is 30 °C, and the temperature limit, at which the speed of germination of this species is harmed, is above 35 °C (Guedes et al., 2011). Seeds of *E. velutina* show a similar response, with a tolerance limit of 35 ºC, with 25 °C being the optimum temperature for germination (Ribeiro-Reis, 2012).

As well as having a strong influence on the speed and final germination percentage, in an intracellular manner, temperature influences the biochemical reactions that determine the germination process since their enzymatic systems have specific thermal requirements (Marcos-Filho, 2005).

Evaluating the effect of osmotic potential (water restriction) for *E. velutina* seeds, the −0.2 MPa solution led to an increase in mean germination time from 4.43 (control) to

5.59 days, though this was not significant. Nevertheless, for the other variables, there were significant reductions in mean speed and in the germination speed index. This information indicates GSI as a factor which is more sensitive to the effects of osmotic stress than germination percentage.

The increase in salt concentration did not have a significant effect on the germination percentage and other germination variables in *E. velutina* seeds. In contrast, in *Chorisia speciosa* seeds (Fanti and Perez, 2004), a high limit of tolerance to salt stress was not seen, with moderate tolerance to NaCl. This has also been observed in *Jatropha curcas* (Andreo-Souza et al., 2010) and *Carthamus tinctorius* (Dantas et al., 2011).

The reduction in the germination percentage and the

delay in the beginning of the germination process with the increase in salt concentration that occurred in these species may be related to the physiological dryness produced. In this condition, in which external water potentials are very low due to the salt concentration, water absorption by the seed is restricted, inhibiting metabolic events that culminate in seedling emergence (Custódio et al., 2009).

Catalase activity (CAT) showed variations in accordance with the stress applied (Figure 1). It was seen that at the temperature of 15 °C, there was no difference in the activity of this enzyme between the cotyledons and the embryonic axis. With the increase in temperature to 25 °C, there was an increase in activity, especially in the axis, remaining constant at the temperature of 35 °C (Figure 1A).
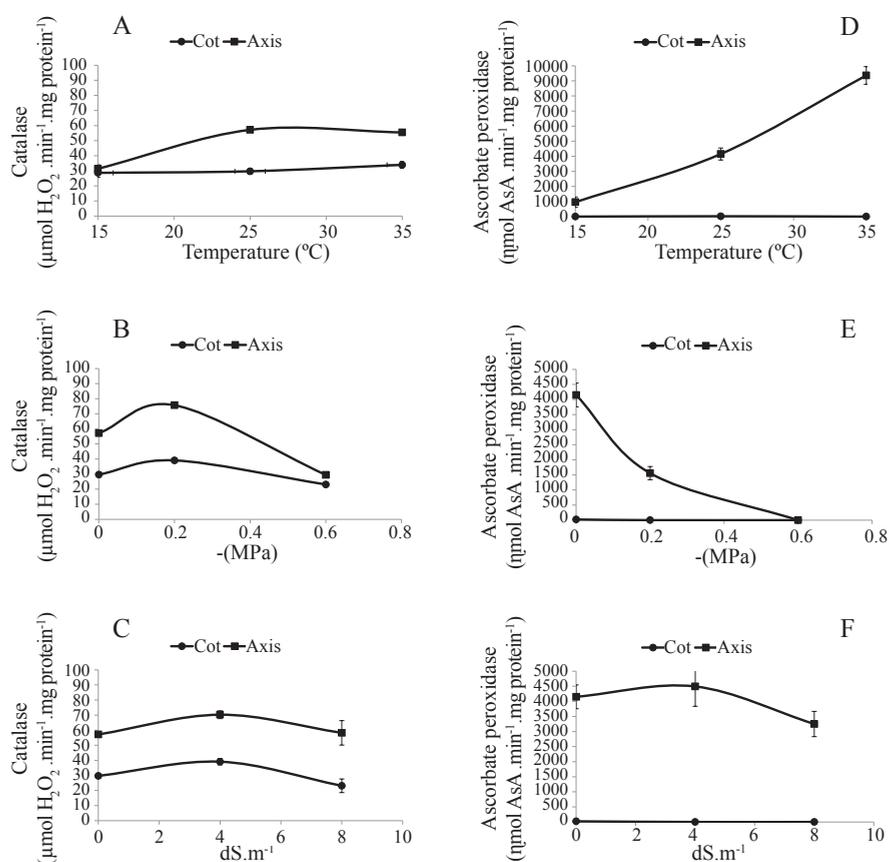

Figure 1. Activity of Catalase (A, B, C) and Ascorbate peroxidase (D, E, F) in the cotyledon (COT) and embryonic axis (AXIS) of *Erythrina velutina* seeds subjected to different temperatures (A, D), osmotic potentials (B, E), and concentrations of NaCl solution (C, F) during germination. Mean value of four replications ± Standard Error of the Mean (SEM).

Enzymes that remove ROSs are considered of great importance for concluding the germination process (Bailly, 2004) since the production of ROSs during seed germination is considered as a cause of stress that may affect the success of germination. Detection of the activity of these enzymes during germination has been observed in seeds of

some species, such as *Picea omorika* (Prodanovic et al., 2007), *Medicago sativa* (Cakmak et al., 2010), and *Jatropha curcas* (Cai et al., 2011); nevertheless, there are no published studies with seeds of forest species subjected to abiotic stresses.

An increase in CAT activity in osmotic potential (-0.2 MPa) was observed, mainly in the embryonic axis,

subsequently decreasing with the increase in water restriction (-0.6 MPa). This reduction proved to be more accentuated in the axis, arriving at levels equivalent to that found in the cotyledons (Figure 1B).

An increase in CAT activity at 4 dS.m^{-1} was seen in the seeds subjected to the different concentrations of the NaCl solution; moreover, a decrease in enzyme activity in the cotyledons was observed with the increase in salt concentration (Figure 1C). The variations found in the responses of CAT activity to the treatments applied show that this enzyme is more sensitive to water restriction in the embryonic axis of the *E. velutina* subjected to -0.6 MPa, compared to the other treatments.

Water restriction caused by this potential may have interfered in these defense mechanisms, blocking germination, without there being damages from deterioration to the seeds since, according to Marcos-Filho (2005), the increase in activity of these enzymes indicates the evolution of deterioration, due to the need for more intense activity of the enzymes participating in the antioxidant complex. According to Hendry (1993), seeds are more sensitive to water stress because the free radicals tend to accumulate more, since removal systems are not effective in dehydrated organisms.

Catalase (CAT) is a variety of peroxidase that catalyzes the breakdown of hydrogen peroxide (Gill and Tuteja, 2010; Sharma et al., 2012). Changes in the balance of ROS-detoxifying enzymes induce compensatory mechanisms in the tissues. For example, when catalase is reduced, protector enzymes, like ascorbate peroxidase (APX) and glutathione peroxidase (GPX), are expressed in greater quantities, as a compensatory effect (Apel and Hirt, 2004).

Activity of the enzyme APX was not seen in the cotyledons of the seeds subjected to stresses by temperature, water, and salt in *E. velutina* (Figures 1D and F). Activity in the embryonic axis increased in response to rise in temperature, ranging from 1000 to around 10,000 ηmol when the *E. velutina* seeds were incubated at 15 and 35 °C, respectively (Figures 1D, E and F). A contrasting result was seen in the seeds subjected to water and salt stresses, in which APX activity was inhibited with the decrease in osmotic potential of the solution from 0.0 to -0.6 MPa (Figure 1E), remained constant up to 4 dS.m^{-1}, and decreased with the increase in the concentration of the NaCl solution to 8 dS.m^{-1} (Figure 1F). Considering that APX is an alternative route in removal of hydrogen peroxide from the medium, it may be supposed that the production of ROSs was low in the *E. velutina* seeds subjected to these water potentials and saline concentrations during the germination process due to the water restriction caused by these treatments, which maintained the activity of this enzyme at extremely low levels, or even that catalase has sufficient activity to put the

peroxide levels at an acceptable concentration.

The level and the type of ROSs are determining factors for the type of response. Hydrogen peroxide (H_2O_2) and superoxide (O_2^-) may induce different genes, together or separately, giving more flexibility to the signaling of ROS. However, the biological effects of H_2O_2 that benefit the plants, mediating acclimatization and cross-tolerance to biotic and abiotic stresses, prove to be dependent not only on their concentration but also on their production site, on the stage of plant development, and on previous exposure of the plant to other types of stress (Petrov and Breusegem, 2012). In this context, peroxidases play a critical role in metabolism of the seeds through using peroxides as a hydrogen acceptor, regulating the formation of H_2O_2 in the plants (Kim et al., 2008), and they are able to contribute to the increase in defense mechanisms and prevention of loss in quality.

A certain similarity may be seen in the behavior of the APX enzymes (Figure 1) and that of guaiacol peroxidase (GOPX) (Figure 2) in the embryonic axis of the *E. velutina* seeds in response to stresses, except for water stress. Nevertheless, little activity of the enzyme GOPX was seen in the cotyledons of the seeds subjected to the different treatments. The activity of GOPX in the axis remained constant at the three temperatures evaluated (Figure 2A). In the axis of the seeds subjected to water stress, it was seen that GOPX activity was inhibited with the decrease in osmotic potential of the solution from 0.0 to -0.6 MPa (Figure 2B). With the increase in the concentration of the NaCl solution from 0 to 4 dS.m^{-1}, it remained practically constant, and decreased with the increase in the concentration to 8 dS.m^{-1} (Figure 2C). From these results, it may be inferred that under the aspect of the detoxification process, the mechanism is more efficient in seeds subjected to different temperatures, followed by salt stress, and finally water stress.

Under normal physiological conditions, there is a balance between the ROSs and the antioxidants; when the plants are subjected to environmental stresses, the balance between production of ROSs and antioxidant activity increases, resulting in oxidative damage. Although this increase may be a threat to cells, these species may also act as signalers and regulators key to many biological processes of stress response and means of defense of the plant (Fujita et al., 2006; Mittler et al., 2011).

The increase in enzymatic and non-enzymatic antioxidant activity may be an adaptive response of the cells to the increase in ROSs. According to Hernandez et al. (2010), plants that show greater activity of the antioxidant system are more resistant to oxidative damages.

There was variability in the activity of glutathione-S-transferase (GST) among the treatments applied (Figures 2D, E). Greater activity of the enzyme was seen in the embryonic axis

subjected to the treatment of 35 °C (5.85 µmol CNDB.min⁻¹.mg of protein⁻¹) (Figure 2D). In contrast, the embryonic axis subjected to -0.6 MPa was that which showed least activity (1.80 µmol CNDB.min⁻¹.mg of protein⁻¹) (Figure 2E). Seeds subjected to the different concentrations of NaCl, despite having obtained greater activity of GST when compared to seeds subjected to the different osmotic potentials, showed the same tendency (Figure 2F).
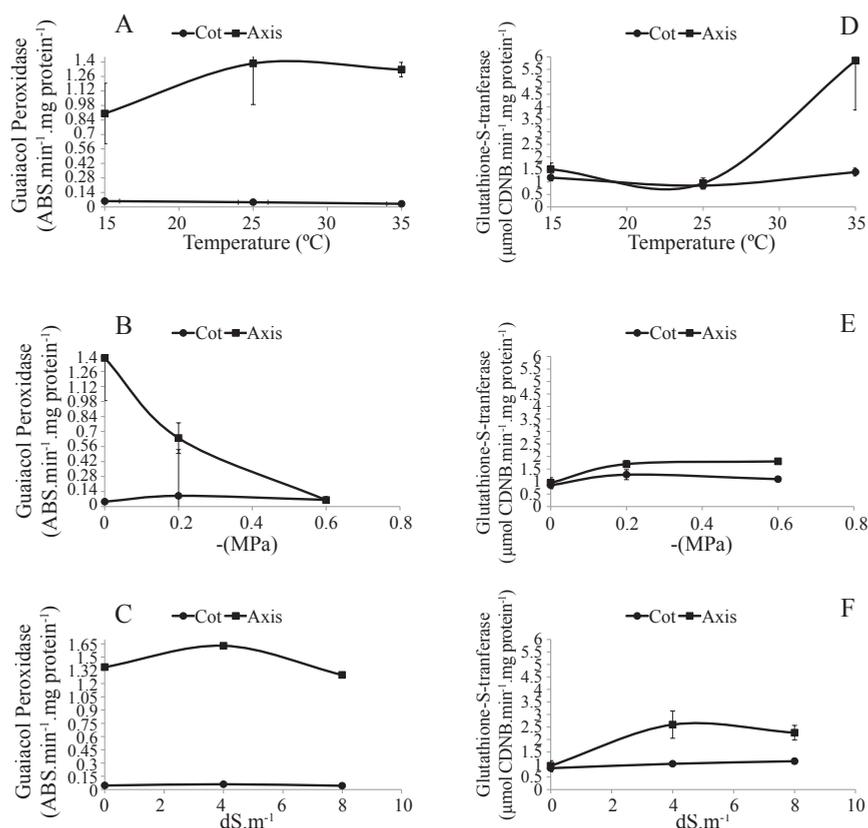

Figure 2. Activity of Guaiacol peroxidase (A, B, C) and Glutathione-S-transferase (D, E, F) in the cotyledon (COT) and embryonic axis (AXIS) of *Erythrina velutina* seeds subjected to different temperatures (A, D), osmotic potentials (B, E), and concentrations of NaCl solution (C, F) during germination. Mean value of four replications ± Standard Error of the Mean (SEM).

The GSTs are considered important enzymes which have the capacity of metabolizing various xenobiotics, thus providing for detoxification of plants, as seen in various studies in wheat, pea, corn, and soybean crops (Uotila et al., 1995, Cataneo et al., 2003, Moldes et al., 2008). The GSTs promote the conjugation of GSH (reduced glutathione) with endogenous cytotoxic products and agents of oxidative damages, like hydroxyl radicals, membrane lipid peroxides, and products of oxidative degradation of DNA, aiming at their detoxification. The GSTs also function like glutathione peroxidases by acting directly on such products (Dixon et al., 2008). Although knowledge of the mechanisms of action of the GSTs in increasing tolerance of plants to herbicides is well clarified, in this study, the activity of this enzyme was also an indication of sensitivity in enzymatic response of *E. velutina* seeds under abiotic stresses (Figure 2).

In general, variability of accumulation of proline among the treatments applied was observed. An increase in the proline contents in the embryonic axis subjected to temperature treatment as of 15 °C was seen, which was more accentuated than in the cotyledons (Figure 3A). In the seeds subjected to water treatment, it was observed that the levels of proline in the cotyledons remained constant, and in the embryonic axis there was a slight increase in the contents from 0.0 to -0.2 MPa (Figure 3B). As of this potential, there was reduction, returning practically to the initial levels found.

In relation to the salt treatment, the proline levels remained practically at constant levels (Figure 3C), and the highest levels were found in the embryonic axis.
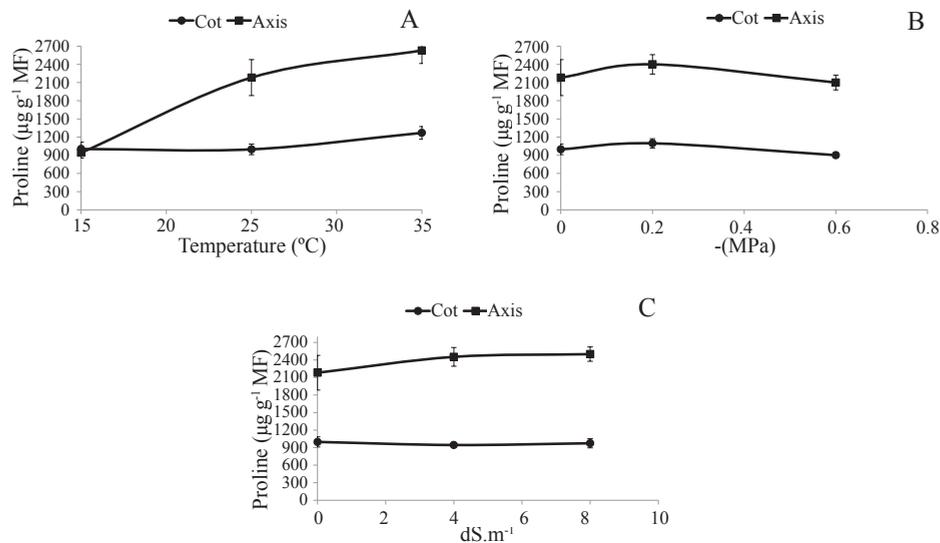
Figure 3. Proline Content in the cotyledon (COT) and embryonic axis (AXIS) of *Erythrina velutina* seeds subjected to different temperatures (A), osmotic potentials (B), and concentrations of NaCl solution (C) during germination. Mean value of four replications ± Standard Error of the Mean (SEM).

Some studies associate the high levels of proline to tolerance to osmotic stresses, like drought and salinity (Azooz et al., 2004; Kavi Kishore et al., 2005). The accumulation of proline provides an environment compatible with the structure and function of macromolecules and contributes to tolerance to salinity in *Vigna radiata* (Misra and Gupta, 2005). In *E. velutina,* the constant presence of this osmoprotector may have provided seeds with tolerance to the thermal and saline treatments. Nevertheless, the osmotic adjustment was not sufficient with the proline contents present in the mulungu seeds subjected to -0.6 MPa which would allow protection of the seeds to water restriction.

Conclusions

Activities of the antioxidant enzymes are detected in the cotyledons and embryonic axis of mulungu seeds;

The accumulation of proline is detected in the cotyledons and embryonic axis of mulungu seeds;

The greater activity of APX and GOPX in the temperature treatment indicates greater tolerance to this stress;

The constant presence of proline in the mulungu seeds subjected to salinity contributed to osmotic adjustment, conferring tolerance to this abiotic stress.

The lower activities of the antioxidant enzymes and lower accumulation of proline in the *E. velutina* seeds subjected to osmotic stress during the germination process may be indicative of low tolerance to this condition.

References

ANDREO-SOUZA, Y.; PEREIRA, A.L.; SILVA, F.F.S.; RIBEIRO-REIS, R.C.; EVANGELISTA, M.R.V.; CASTRO, R.D.; DANTAS, B.F. Efeito da salinidade na germinação de sementes e no crescimento inicial de mudas de Pinhão-manso. *Revista Brasileira de Sementes*, v.32, n.2, p.83-92, 2010. http://www.scielo.br/pdf/rbs/v32n2/v32n2a10

APEL, K.; HIRT, H. Reactive oxygen species: metabolism, oxidative stress, and signal transduction. *Annual Review of Plant Biology*, v.55, p.373-399, 2004. http://www.annualreviews.org/doi/abs/10.1146/annurev.arplant.55.031903.141701

AZEVEDO, R.A.; ALAS, R.J.; SMITH, R.J.; LEA, P.J. Response of antioxidant enzymes to transfer from elevated carbon dioxide to air ozone fumigation, in the leaves and roots of wild-type and a catalase-deficient mutant of barley. *Physiologia Plantarum*, v.104, p.280-292, 1998. http://onlinelibrary.wiley.com/doi/10.1034/j.1399-3054.1998.1040217.x/pdf

AZOOZ, M.M.; SHADDAD, M.A.; ABDEL-LATEF, A.A. The accumulation and compartmentation of proline in relation to salt tolerance of three sorghum cultivars. *Indian Journal of Plant Physiology*, v.9, p.1-8, 2004. http://cat.inist.fr/?aModele=afficheN&cpsidt=15979567

BAILEY-SERRES, J.; MITTLER, R. The roles of reactive oxygen species in plant cells. *Plant Physiology,* v.141, n.2, p.311, 2006. http://dx.doi.org/10.1104/pp.104.900191

BAILLY, C. Active oxygen species and antioxidants in seed biology. *Seed Science Research*, v.14, n.2, p.93-107, 2004. http://dx.doi.org/10.1079/SSR2004159

BAILLY, C.; LEYMARIE, J.; LEHNER, A.; ROUSSEAU, S.; COÃME, D.; CORBINEAU, F. Catalase activity and expression in developing sunflower seeds as related to drying. *Journal of Experimental Botany*, v.55, n.396, p.475-483, 2004. http://jxb.oxfordjournals.org/content/55/396/475.full.pdf+html

BATES, L.S. Rapid determination of free proline for water stress studies. *Plant Soil,* v.39, p.205-207, 1973. http://link.springer.com/article/10.1007%2FBF00018060#page-1

BRADFORD, M.M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry***,** v.72, n.1/2, p.248-254, 1976. http://www.plantstress.com/methods/Proline%20analysis.pdf

CAI, F.; MEI, L.; AN, X.; GAO, S.; TANG, L.; CHEN, F. Lipid peroxidation and antioxidant responses during seed germination of *Jatropha curcas*. *Journal of Agriculture and Biology*, v.13, p.25-30, 2011. . http://dx.doi.org/10-423/DCX/2011/13-1-25-30.

CAKMAK, T.; ATICI, O.; AGAR, G.; SUNAR, S. Natural aging-related biochemical changes in alfafa (*Medicago sativa* L.) seeds stored for 42 years. *International Research Journal of Plant Science*, v.1, n.1, p.1-6, 2010. http://interesjournal.org/IRJPS/Pdf/2010/July%202010/CAKMAK%20et%20al%20.pdf

CARVALHO, P.E.R. *Mulungu (Erythrina velutina)*. Embrapa Florestas, Colombo, PR, 2008. 8p. (Circular Técnica, 160). http://www.bibliotecaflorestal.ufv.br/bitstream/handle/123456789/4204/circtec160.pdf?sequence=1

CARVALHO, N.M.; NAKAGAWA, J. *Sementes:* ciência, tecnologia e produção. Jaboticabal: FUNEP, 2012. 590p.

CATANEO, A.C.; DESTRO, G.F.G.; FERREIRA, L.C.; CHAMMA, K.L.; SOUZA, D.C.F. Atividade de glutationa S-transferase na degradação do herbicida glyphosate em plantas de milho (*Zea mays*). *Planta Daninha,* v.21, n.2, p.307-312, 2003. http://dx.doi.org/10.1590/S0100-83582003000200017

CHEN, C.T.; CHEN, L.M.; LIN, C.C.; KAO, C.H. Regulation of proline accumulation in detached rice leaves exposed to excess copper. *Plant Science*, v.160, n.2, p.283-290, 2001. http://www.sciencedirect.com/science/article/pii/S0168945200003939#

CUSTÓDIO, C.C.; MACHADO-NETO, N.B.; MORENO, E.L.C. Water submersion of bean seeds in the vigour evaluation. *Revista Brasileira de Ciências Agrárias*, v.4, n.3, p.261-266, 2009. http://dx.doi.org/ 10.5039/agraria.v4i3a5

DANTAS, C.V.S.; SILVA, I.B.; PEREIRA, G.M.; MAIA, J.M.; LIMA, J.P.M.S.; MACEDO, C.E.C. Influência da salinidade e déficit hídrico na germinação de sementes de *Carthamus tinctorius* L. *Revista Brasileira de Sementes*, v.33, n.3, p.574-582, 2011. http://dx.doi.org/10.1590/S0101-31222011000300020

DEMIRAL, T.; TURKAN, I. Exogenous glycine betaine affects growth and proline accumulation and retards senescence in two rice cultivars under NaCl stress. *Environmental and Experimental Botany,* v.56, p.72-79, 2006. http://dx.doi.org/10.1016/j.envexpbot.2005.01.005

DIXON, D.P.; LAPTHORN, A.; MADESIS, P.; MUDD, E.A.; DAY, A.; EDWARDS, R. Binding and glutathione conjugation of porphyrinogens by plant glutathione transferases. *Journal of Biological Chemistry,* v.283, p.20268-20276, 2008. http://www.jbc.org/content/283/29/20268.full.pdf+html

FANTI, S.C.; PEREZ, S.C.J.G.A. Processo germinativo de sementes de painera sob estresses hídrico e salino. *Pesquisa Agropecuária Brasileira*, v.39, n.9, p.903-909, 2004. http://seer.sct.embrapa.br/index.php/pab/article/view/6855/3911

FOYER, C.H.; NOCTOR, G. Redox homeostasis and antioxidant signaling: a metabolic interface between stress perception and physiological responses. *Plant Cell,* v.17, p.1866-1875, 2005. http://dx.doi.org/10.1105/tpc.105.033589

FUJITA, M.; FUJITA, Y.; NOUTOSHI, Y.; TAKAHASHI, F.; NARUSAKA, Y.; YAMAGUCHI-SHINOZAKI, K.; SHINOZAKI, K. Crosstalk between abiotic and biotic stress responses: a current view from the points of convergence in the stress signaling networks. *Current Opinion Plant Biology*, v.9, p.436-442, 2006. http://dx.doi.org/10.1016/j.pbi.2006.05.014

GILL, S.S.; TUTEJA, N. Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. *Plant Physiology and Biochemistry*, v.48, p.909-930, 2010. http://dx.doi.org/10.1016/j.plaphy.2010.08.016

GUEDES, R.S.; ALVES, E.U.; GONÇALVES, E.P.; COLARES, P.N.Q.; MEDEIROS, M.S.; VIANA, J.S. Germinação e vigor de sementes de *Myracrodruon urundeuva* Allemão em diferentes substratos e temperaturas. *Revista Árvore*, v.35, n.5, p.975-982, 2011. http://dx.doi.org/10.1590/S0100-67622011000600003

GOMES-JUNIOR, R.A.; MOLDES, C.A.; DELITE, F.S.; POMPEU, G.B.; GRATÃO, P.L.; MAZZAFERA, P.; LEA, P.J.; AZEVEDO, R.A. Antioxidant metabolism of coffee cell suspension cultures in response to cadmium. *Chemosphere,* v.65, p.1330-1337, 2006. http://dx.doi.org/10.1016/j.chemosphere.2006.04.056

HENDRY, G.A.F. Oxygen, free radical processes and seed longevity. *Seed Science Research*, v.3, p.141-153, 1993. http://journals.cambridge.org/abstract_S0960258500001720

HERNANDEZ, M.; FERNANDEZ-GARCIA, N.; DIAZ-VIVANCOS, P.; OLMOS, E. A different role for hydrogen peroxide and the antioxidative system under short and long salt stress in *Brassica oleracea* roots. *Journal of Experimental Botany*, v.61, n.2, p.521-535, 2010. http://jxb.oxfordjournals.org/content/61/2/521.full.pdf+html

KAVI KISHORE, P.B.; SANGAM, S.; AMRUTHA, R.N.; SRILAXMI, P.; NAIDU, K.R.; RAO, K.R.S.S.; RAO, S.; REDDY, K.J.; THERIAPPAN, P.; SREENIVASULU, N. Regulation of proline biosynthesis, degradation, uptake and transport in higher plants: its implications in plant growth and abiotic stress tolerance. *Current Science,* v.88, p.424-438, 2005. http://tejas.serc.iisc.ernet.in/~currsci/feb102005/424.pdf

KIM, Y.H.; KIM, C.Y.; SONG, W.K.; PARK, D.S.; KWON, S.Y.; LEE, H.S.; BANG, J.W.; KWAK, S.S. Over expression of sweet potato swpa4 peroxidase results in increased hydrogen peroxide production and enhances stress tolerance in tobacco. *Planta,* v.227, p.867-881, 2008. http://link.springer.com/article/10.1007/s00425-007-0663-3

KOTOWSKI, F. Temperature relations to germination of vegetable seed. *Proceedings of the American Society for Horticultural Science*, v.23, p.176-184, 1926.

LABOURIAU, L.G. *A germinação das sementes*. Washington: Secretaria da OEA, 1983. 173p.

MAGUIRE, J.D. Speed of germination - aid in selection and evaluation for seedling emergence and vigor. *Crop Science*, v.2, p.176-177, 1962. DOI:10.2135/cropsci1962.0011183X000200020033x

MARCOS-FILHO, J. *Fisiologia de sementes de plantas cultivadas*. Piracicaba: FEALQ, 2005. 495p.

MATSUNO, H.; URITANI, I. Physiological behavior of peroxidase isozymes in sweet potato root tissue injured by cutting or with black rot. *Plant Cell Physiology,* v.13, p.1091-1101, 1972. http://pcp.oxfordjournals.org/content/13/6/1091.full.pdf+html

MEIADO, M.V.; ALBUQUERQUE, L.S.C.; ROCHA, E.A.; ROJAS-ARÉCHIGA, M.; LEAL, I.R. Seed germination responses of *Cereus jamacaru* DC. ssp. *jamacaru* (*Cactaceae*) to environmental factors. *Plant Species Biology*, v.25, p.120-128, 2010. DOI: 10.1111/j.1442-1984.2010.00274.x

MIRANDA, R.Q.; CORREIA, R.M.; ALMEIDA-CORTEZ, J.S.; POMPELLI, M.F. Germination of *Prosopis juliflora* (Sw.) D.C. seeds at different osmotic potentials and temperatures. *Plant Species Biology,* v.26, n.2, p.186-192, 2013. http://doi/10.1111/1442-1984.12025/

MISRA, N.; GUPTA, A.K. Effect of salt stress on proline metabolism in two high yielding genotypes of green gram. *Plant Science*, v.169, p.331-339, 2005. http://dx.doi.org/10.1016/j.plantsci.2005.02.013

MITTLER, R.; VANDERAUWERA, S.; SUZUKI, N.; MILLER, G.; TOGNETTI, V.B.; VANDEPOELE, K.; GOLLERY, M.; SHULAAEV, V; VAN BREUSEGEM, F. ROS signaling: the new wave? *Trends in Plant Science,* v.16, p.300-309, 2011. http://dx.doi.org/10.1016/j.tplants.2011.03.007

MOLDES, C.A.; MEDICI, L.O.; ABRAHÃO, O.S.; TSAI, S.M.; AZEVEDO, R.A. Biochemical responses of glyphosate resistant and susceptible soybean plants exposed to glyphosate. *Acta Physiologiae Plantarum*, v.30, n.4, p.469-479, 2008. http://link.springer.com/article/10.1007/s11738-008-0144-8

NAKANO, Y.; ASADA, K. Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplast. *Plant and Cell Physiology,* v.22, n.5, p.867-880, 1981. http://pcp.oxfordjournals.org/content/22/5/867.full.pdf+html

PETROV, V.D.; VAN BREUSEGEM, F. Hydrogen peroxide: a central hub for information flow in plant cells. *AoB Plants*, v.2012, p.1-13, 2012. http://dx.doi.org/10.1093%2Faobpla%2Fpls014

PRISCO, J.T.; GOMES-FILHO, E. Fisiologia e bioquímica do estresse salino em plantas. In: GHEYI, H.R.; DIAS, N.S.E; LACERDA, C.F. (Eds). Manejo da salinidade na agricultura: estudos básicos e aplicados. Fortaleza, Instituto Nacional de Ciência e Tecnologia em Salinidade, p.143-159, 2010. http://200.7.175.130/agroumsa/sites/default/files/repositorio/cap_10_Fisiologia%20e%20bioqu%C3%83%C2%ADmica%20do%20estresse.pdf

PRODANOVIC, O.; PRODANOVIC, R.; BOGDANOVIĆ, J.; MITROVIĆ, A.;MILOSAVIĆ,N.;RADOTIĆ,K.Antioxidativeenzymesduringgermination of two lines of serbian spruce [*Picea omorika* (Panč.) Purkyně]. *Archives Biological Science*, v.59, n.3, p.209-216, 2007. http://www.doiserbia.nb.rs/Article.aspx?id=035446640703209P&AspxAutoDetectCookieSupport=1#.UzFztBCmV1w

RIBEIRO-REIS, R.C. Tolerância a estresses abióticos em sementes de *Erythrina velutina* Willd. (leguminosae - Papilionoideae) nativa da caatinga. *Informativo Abrates*, v.22, n.3, p.28-31, 2012. http://www.abrates.org.br/portal/images/Informativo/v22_n3/Palestras_compactado.pdf

SHARMA, P.; JHA, A.B.; DUBEY, R.S.; PESSARAKLI, M. Reactive oxygen species, oxidative damage, and antioxidative defense mechanism in plants under stressful conditions. *Journal of Botany*, v.2012, p.1-26, 2012. http://dx.doi.org/10.1155/2012/217037

SILVA, F.A.S.; AZEVEDO, C.A.V. Principal Components Analysis in the Software Assistat-Statistical Attendance. In: WORLD CONGRESS ON COMPUTERS IN AGRICULTURE, 7, Reno-NV-USA: American Society of Agricultural and Biological Engineers, 2009.

UOTILA, M.; GULLNER, G.; KOMIVES, T. Induction of glutathione S-transferase activity and glutathione level in plants exposed to glyphosate. *Physiologia Plantarum*, v.93, n.4, p.689-694, 1995. DOI:10.1111/j.1399-3054.1995.tb05118.x

VILLELA, F.A.; DONI FILHO, L.; SEQUEIRA, E.L. Tabela de potencial osmótico em função da concentração de polietilenoglicol 6000 e a temperatura. *Pesquisa Agropecuária Brasileira*, v.26, n.11/12, p.1957-1968, 1991. https://seer.sct.embrapa.br/index.php/pab/article/view/3549/882