

Physiological and biochemical performance of sunflower seeds subjected to different osmotic potentials

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ABSTRACT

The aim of this study was to evaluate the performance of sunflower seed lots under different osmotic potentials. Four lots of sunflower seeds, cv. Paraíso 33 were used, submitted to different water restrictions 0 (control), -0.10 MPa (1.310 g L⁻¹ of NaCl), -0.20 MPa (2.620 g L⁻¹ of NaCl), -0.30 MPa (3.930 g L⁻¹ of NaCl) and -0.40 MPa (5.240 g L⁻¹ of NaCl), through the use of saline solutions in conducting the tests. The physiological quality was evaluated by: germination, emergence rate index, cold, emergence, accelerated aging, seedling length and seedling dry weight, as well as the enzymatic activity. The results obtained showed that the physiological potential of sunflower seeds is negatively affected in water restrictions -0.30 and -0.40 MPa, and the enzymes superoxide dismutase (SOD) and peroxidase (PO) are activated when sunflower seeds are submitted to saline stress.

Key words: *Helianthus annuus* L., salinity, stress

Desempenho fisiológico e bioquímico em sementes de girassol submetidas a diferentes potenciais osmóticos

RESUMO

O objetivo neste estudo foi avaliar o desempenho de lotes de sementes de girassol submetidas a diferentes potenciais osmóticos. Para isto foram utilizados quatro lotes de sementes de girassol, cv. Paraíso 33, submetidos a diferentes restrições de água 0 (controle); -0,10 MPa (1,310g L⁻¹ de NaCl); -0,20 MPa (2,620 g L⁻¹ de NaCl); -0,30 MPa (3,930 g L⁻¹ de NaCl) e -0,40 MPa (5,240 g L⁻¹ de NaCl) através da utilização de soluções salinas na condução dos testes. A qualidade fisiológica foi avaliada pelos testes: germinação, índice de velocidade de emergência, frio, emergência, envelhecimento acelerado, comprimento e massa seca de plântulas, como também a atividade enzimática. O potencial fisiológico de sementes de girassol é afetado negativamente em restrições de água -0,30 e -0,40 MPa e as enzimas superóxido dismutase (SOD) e peroxidase (PO) são ativadas quando as sementes de girassol são submetidas ao estresse salino.

Palavras-chave: *Helianthus annuus* L., salinidade, estresse

Introduction

The conditions that the seeds are in the soil for germination, are often adverse, such as saline and sodic soils. The sunflower (*Helianthus annuus* L.) plant originated from the North America, is a herbaceous annual species of summer growth, magnoliopsida, belonged to the Asteraceae family, cultivated in many parts of the world. It is an oilseed crop that has important agronomic features, such as increased resistance to drought, cold and heat, than the most commonly cultivated species in Brazil (Leite et al., 2007).

In the field, plants are subjected to multiple stresses that can limit their development and their chances of survival, being the stress by salinity and/or sodicity, one of the most observed in the production areas. One of the most widespread methods for the determination of plant tolerance to the salts in excess is the observation of the germination in saline substrates (Lima & Torres, 2009). According to Góis et al. (2008) the reduction of the germination percentage, in comparison with the control, serves as an indicator of the tolerance level of species to salinity. In this method, the ability to germinate also indicates the plant tolerance to salts in subsequent stages of development (Taiz & Zeiger, 2006).

The high concentration of salts is a stress factor for plants, because it reduces the osmotic potential and provides the action of ions on the protoplasm, thus, with the salinity increase occurs a reduction of the soil osmotic potential, making difficult the absorption of water, especially for the seeds during the germination process, that also suffer significant influence of the soil salinity condition (Lopes & Macedo, 2008). The high content of salts, especially sodium chloride (NaCl), can inhibit the germination due to the osmotic potential decrease, causing damage to the other process stages (Lee et al., 2005). On the other hand, under conditions of full water availability in the soil, seeds, especially the drier ones, can absorb water quickly, causing imbibition damage with consequent loss of germination.

Among the many enzymes that participate in plants metabolic pathways, stand out the peroxidase and superoxide dismutase, because they act in the protection against biotic and abiotic stresses. Peroxidase is an enzyme routinely found in plants, which exerts an antioxidative protection and several functions since growth, differentiation and cell development (Aouad et al., 1998). The enzyme activity may increase in plants subjected to several types of stress, being the first enzyme to have the activity altered, regardless of the substrate used or the stress applied (Siegel, 1993).

On the other hand, the enzyme superoxide dismutase (SOD) catalyzes the superoxide dismutation into oxygen and hydrogen peroxide; for this reason, it constitutes an important antioxidant defense mechanism in most cells exposed to oxygen. Inside the cell, the enzyme superoxide dismutase (SOD) is the first to act against oxidative stress (Alscher et al., 2002).

Being grown in the second season or in winter, the sunflower crop is subject to possible weather conditions less favorable for sowing and emergence, thus, the study of seed germination performance of this species under different osmotic potentials

may generate information about its behavior in unfavorable environmental conditions for germination.

The aim of this study was to verify the behavior of sunflower seed lots under different osmotic potentials.

Material and Methods

The study was conducted at the Didactic Laboratory of Seed Analysis (DLSA), Faculty of Agronomy Eliseu Maciel (FAEM), Federal University of Pelotas (UFPel). Four lots of sunflower seeds, cv. 'Paraíso' 33 were used, submitted to different water restrictions 0 (control), -0.10 MPa (1.310 g L⁻¹ of NaCl), -0.20 MPa (2.620 g L⁻¹ of NaCl), -0.30 MPa (3.930 g L⁻¹ of NaCl) and -0.40 MPa (5.240 g L⁻¹ of NaCl). The substrate used in the tests conduction for evaluating the quality of seeds was soaked with saline solutions with different osmotic potentials. The physiological quality of seeds was evaluated by the following tests:

Germination - conducted with four subsamples of 50 seeds in germitest paper rolls, moistened with solutions in the ratio of 2.5 times its dry weight, transferred to a germinator at 25 °C; the evaluations were performed on the fourth and tenth day after sowing, counting the number of normal seedlings (Brasil, 2009).

Emergence - determined in four subsamples of 50 seeds distributed in expanded polystyrene trays with individual cells filled with commercial substrate for vegetables - Plantmax®. The trays were placed in a greenhouse heated with an average temperature of 25 °C and the count held on the 14th day after sowing, being tallied up the normal seedlings larger than 50 mm.

Emergence speed index - determined by evaluations performed daily, in the morning, by counting the number of seedlings emerged until the stabilization of the number of seedlings and the calculation of speed index performed according to Maguire (1962).

Cold test - conducted with four subsamples of 50 seeds in germitest paper rolls, moistened with solutions in the ratio of 2.5 times its dry weight, placed in plastic bags and placed in a refrigerator set at a temperature of 8 °C for seven days. After this period, they were transferred to the germinator at 25 °C and the conditions of the germination test were maintained, according to Cicero & Vieira (1994).

Accelerated aging with saline solution - conducted with the use of gerbox acrylic boxes (11 x 11 x 3,5 cm), with individual compartment (mini cameras) containing 40 mL of NaCl saturated solution (40 g NaCl in 100 mL of water), a tray of aluminum screen, where seeds were distributed forming a uniform layer on the screen surface. The boxes were kept for 96 hours at 42 °C, after the aging period, four subsamples of 50 seeds were subjected to germination test, according to the methodology suggested by Braz et al. (2008).

Seedling length - conducted with four subsamples of 20 seeds in germitest paper rolls, moistened with solutions in the ratio of 2.5 times its dry weight, placed in plastic bags and placed in a germinator at 25 °C. The measurement was performed on the tenth day after sowing, with the aid of a millimetered ruler, measuring only normal seedlings.

Dry biomass - evaluated together with the seedling length, using four subsamples of 20 seedlings, on the tenth day after sowing, the seedlings were dried in an oven with forced air circulation at a temperature of 72 °C, for 72 hours later each subsample was weighed on an analytical balance with an accuracy of 0.0001 g.

Isoenzymes - evaluations of the enzymes activity superoxide dismutase (SOD) and peroxidase (PO) were performed using the system of vertical electrophoresis in polyacrylamide gel. The plant material composed of 10 seedlings with five days, from each treatment, was macerated in a porcelain mortar on ice cubes. Approximately 200 mg of each plant extract were placed in a microcentrifuge tube together with the extracting solution composed with the gel buffer (Lithium borate 0.2 M at pH 8.3 + Tris citrate + 0.2 M at pH 8.3) + 0.15% of 2-mercaptoethanol) in the ratio 1:2 (w/v). The electrophoresis was performed on polyacrylamide gels 7%, applying 20 µL of each sample, using the coloring systems described by Alfenas (1998). The interpretation of results were based on visual analysis of the electrophoresis gels, taking into account the presence and absence, and the expression intensity of each band.

Statistical design - The experiment was conducted as a 4x5 factorial (4 lots and 5 osmotic potentials), in a completely randomized design with three replications. For the statistical analysis the statistical program Winstat (Machado, 2002) was used, being the means subjected to the variance analysis. As for the lot factor, comparison of means was performed by Tukey test at 5% level of probability, and for the osmotic potential factor, a regression analysis was performed.

Results and Discussion

The initial quality of batches of sunflower seeds are presented in Table 1, where data obtained by the emergence speed index - IVE, indicate that there were differences in behavior among batches. The Lot 2 did not defer in comparison to Lot 3 but showed superiority for Lots 1 and 4.

The behavior of each lot depending on the applied osmotic potential (Figure 1A) shows that three of the four lots analysed responses obtained were cubic decline occurring in the IVE from potential -0.30 MPa. Working with barley seeds Silva et al. (2007) and rice seeds Lima et al. (2005) found that increased salinity caused a decrease in the germination rate.

The reduction in the emergence speed index with the increase in saline stress may be related to the physiological drought produced, because when there is an increase in salt concentration in the germinative medium, there is a reduction

of the water potential. And according to Tôrres et al. (2004), occurs a nutritional imbalance, caused by the uptake inhibition and transport of nutrients, as well as toxic effects of ions, particularly chloride and sodium.

For the variable emergence (Table 1), there were no differences between the lots, probably the similarity between the results occurred because the conditions used favor the emergence in field. Given that the sowing was performed under favorable conditions at the beginning of the recommended time for sunflower crop, therefore, not finding weather problems, the emergence observed were high. Lots L1 and L2 showed no significant differences (Figure 1B). However, the lot L3 responds well to salinity stress, on the other hand, the lot L4 remains itself at the dose of -0.10; -0.20 and -0.30 MPa and drops sharply at a dose -0.40 MPa.

According to the data of the germination test (Table 1), there was a difference between the lots, and, as observed in the Figure 1C, there is a strong tendency in the germination reduction with the increase in saline concentration, regardless of the lot. However, in the lots L1 and L4, there were a more pronounced decrease with the osmotic potentials decrease. In agreement with the results obtained by Silva et al. (2007), who working with barley seeds, observed lower germination percentage with the salt concentration increase, suggesting that NaCl reduced the viability of seeds. Such behavior was also observed in watermelon seeds, which suffered a reduction in germination with the salinity increase (Torres, 2007).

Disagreeing with the results obtained by Andréo-Souza et al. (2010), where the authors working with lots of physic nuts seeds observed differences between the behavior of lots in germination tests submitted to saline stress. Working with sunflower seeds, Dickmann et al. (2005) observed different behavior between the materials used, in which the cultivar MG2 shows to be more sensitive to the saline stress of NaCl and the cultivars MG50 and M734 to the stress of CaCl₂, however, in rice, Lima et al. (2005), found that the effect of NaCl on the seed quality of rice seeds varies according to the cultivar used.

Through the accelerated aging test results (Table 1), it was possible to stratify the lots at different vigor levels, being the L1 superior to the others. All lots responded to the accelerated aging test (Figure 2A) in a similar manner, however the L2 suffered lower reduction at the accelerated aging in the highest potential, proving to be more vigorous than the other lots.

In the cold test (Table 1) it was possible to differentiate the lots, the L1 showed superior to lots 3 and 4, while the L2 has intermediate quality, with no significant difference between the osmotic potential for this lot (Figure 2), however the others

Table 1. Physiological quality of four sunflower seed lots, cv Paraiso 33, evaluated by the tests of germination (G), accelerated aging (AA), cold (C), seedling length (SL), dry biomass (DB), emergency speed index (ESI) and emergence in field (EF)

LOTS	G			SL (cm)	DB (mg)	ESI	EF (%)
	AA (%)	C	SL (cm)				
L1	73 A	65 A	69 A	19,26 A	43,3 A	3,593 C	98 A
L2	48 B	48 B	61 AB	12,73 B	36,2 B	4,575 A	98 A
L3	50 B	51 B	56 B	12,5 B	37,3 B	4,375 AB	98 A
L4	66 A	42 B	55 B	12,33 B	36,2 B	4,029 BC	95 A
CV(%)	12,9	12,3	6,96	5,56	4,68	5,99	2,91

Means followed by same letter in the column, for each evaluated parameter, do not differ between themselves by Tukey test at 5% level of significance.

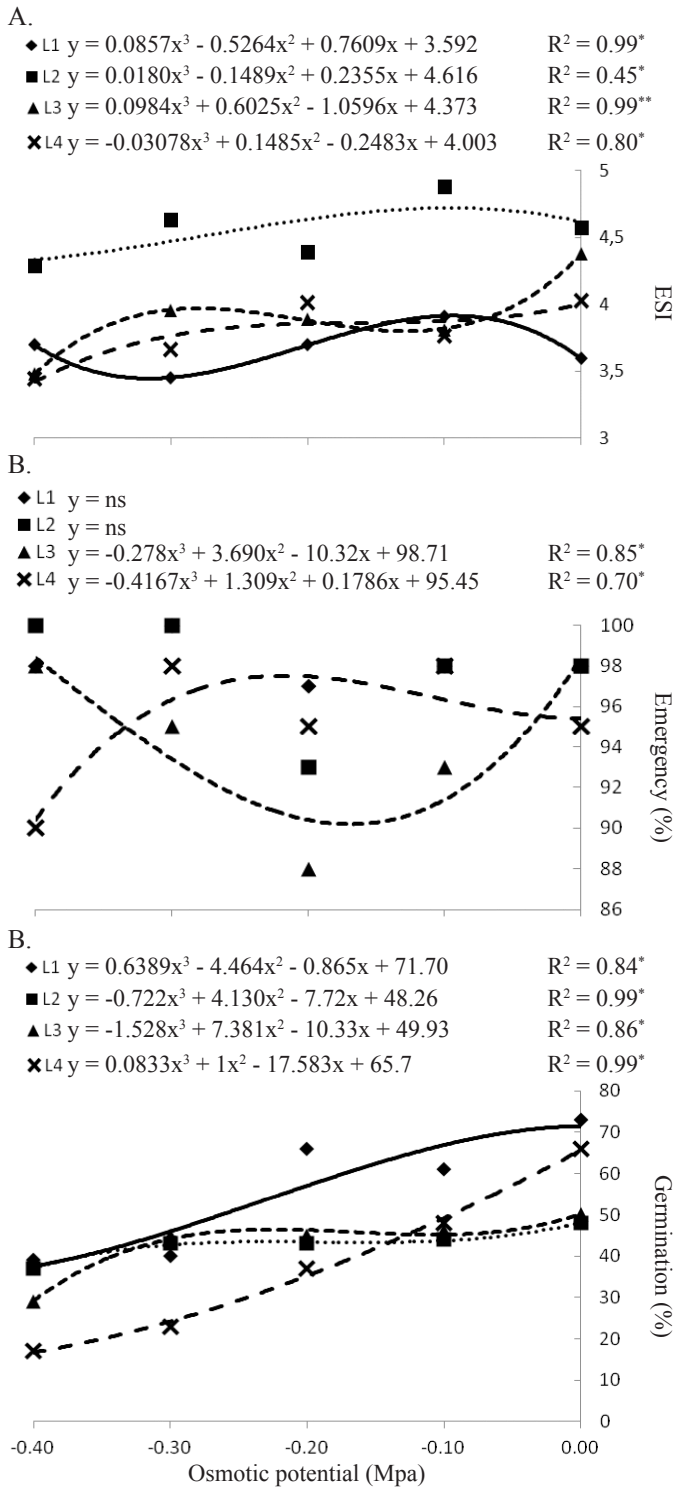


Figure 1. (A) Emergence speed index (ESI), (B) Percentage of emergence, (C) Percentage of germination of four sunflower seed lots subjected to different water restrictions T0 (control), T₁ (-0.10 Mpa), T₂ (-0.20 Mpa), T₃ (-0.30 Mpa) and T₄ (-0.40 Mpa)

lots reduce the quality with the decrease of osmotic potential. For all other lots there was a decrease in the values observed at lower potentials, stands out the L1 was more sensitive than others, because it presents a greater reduction between dose zero and other doses, and the L4 that show stability in the results from the dose -0.20 Mpa.

As for seedling length, L1 was superior to the other lots, such behavior was also observed in dry biomass (Table 1),

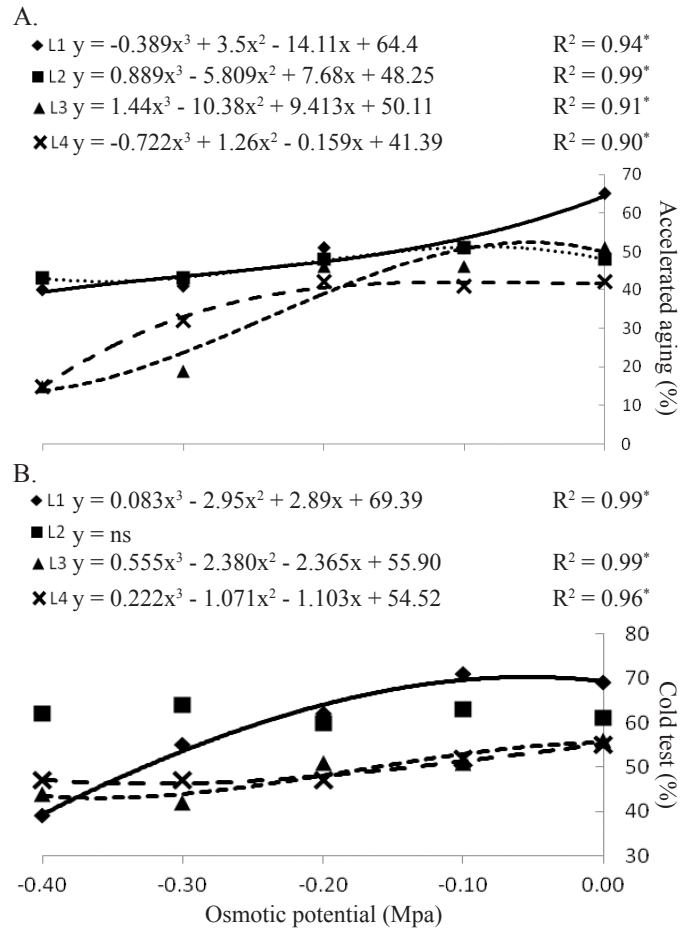


Figure 2. (A) Accelerated aging (%), (B) cold test (%) of four sunflower seed lots subjected to different water restrictions T0 (control), T₁ (-0.10 Mpa), T₂ (-0.20 Mpa), T₃ (-0.30 Mpa) and T₄ (-0.40 Mpa)

these results are expected considering the dependence between these parameters. All lots presented similar behavior (Figure 3A), in other words, there was a reduction in the average length of seedlings with the increase in saline concentration, however, for lot L1 this reduction was more pronounced, reaching to 52% in the potential dose -0.40 Mpa in relation to control. Agreeing with Dickmann et al. (2005), who concluded that the osmotic potential increase of the saline solutions produces a decrease in the vigor of sunflower seeds, this species may be included in the category of glycophyte less tolerant to salinity. Similar results were observed in seeds of *Chorisia glaziovii* O. Kuntze, where the authors (Guedes et al., 2011) observed that the length of root and aerial part was affected as there was an increase in salinity levels.

For dry mass (Figure 3B) no variation between batches highlighted, although an increase was found in the weight of dry biomass with increasing salt concentrations, the results are in agreement with Secco et al. (2010) who, working with melon seeds, observed an increase in dry weight of seedlings from seeds subjected to salinity stress, the same authors state that this fact is due to the accumulation of nutrients in the root. However such results differ from those obtained by Oliveira et al. (2010), where the authors found that increased salt concentrations linearly decreased dry matter in the early development stage of sunflower plants. In plants popcorn (Oliveira et al., 2009) also observed a decrease in dry matter

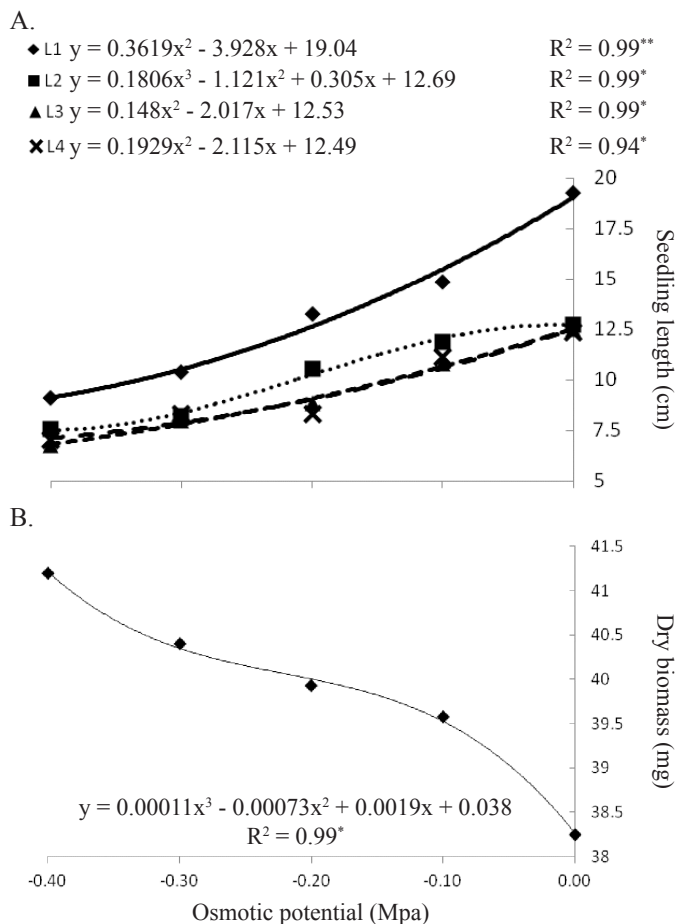


Figure 3. (A) Seedling length (cm), (B) dry biomass (mg) of four sunflower seed lots subjected to different water restrictions T0 (control), T1(-0,10 MPa), T2 (-0,20 MPa), T3 (-0,30 MPa) and T4(-0,40 MPa)

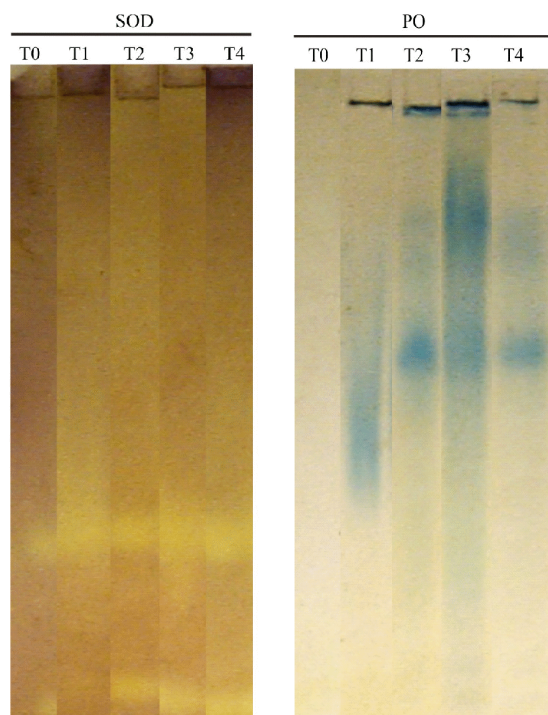


Figure 4. Electrophoretic pattern of superoxide dismutase (SOD) and peroxidase (PO) of sunflower seedlings under different water restrictions T0 (control), T₁ (-0,10 MPa), T₂ (-0,20 MPa), T₃ (-0,30 MPa) and T₄ (-0,40 MPa), collected five days after sowing

with increasing salinity, the same behavior was observed in cowpea seeds where the authors found that in different genotypes the dry matter was reduced with increasing salt concentrations (Deuner et al., 2011).

In the Figure 4 the electrophoretic pattern is presented of the isoenzymatic peroxidase systems (PO) and superoxide dismutase (SOD) in sunflower seedlings subjected to different water restrictions. The SOD formed bands more intense for the seeds that were subjected to saline stress, probably being a response of sunflower seedlings opposite to saline stress. Similar results were found by Alscher et al. (2002), when testing the expression of the SOD enzyme in *Arabidopsis thaliana* subjected to oxidative stress, they detected bands that characterized the expression of the isoenzyme at the period from 5 to 41 hours after the stress submission.

The banding pattern of the PO enzyme was similar to the SOD, in other words, with the formation of bands at all levels of saline stress. Similar results were found by Rossi & Lima (2001), who identified a higher peroxidase activity in bean seedlings as a function of exposure to heavy metals, and it can be used as a biochemical indicator of stress.

Conclusions

Water restrictions of -0.30 and -0.40 MPa cause reduction of the physiological potential of sunflower seeds.

The enzymes SOD and PO activation features if sunflower seeds are subjected to salt stress.

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