Research Article

Soybean plant osmotic and oxidative stress as affected by herbicide and salinity levels in soil

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Conflict of Interest:
The authors declare that there is no conflict of interest regarding the publication of this manuscript.

HIGHLIGHTS

- Sulfentrazone and S-metolachlor herbicides in combination with salt stress, can alter physiological and biochemical processes in soybean.
- Soybean submitted to salt and herbicides has reduced osmotic potential and content of hydrogen peroxide.
- Action of antioxidant enzymes minimize the effects of ROS, to maintain cellular metabolism and allow plants to adapt to the environment.

ABSTRACT

Background: Soybean cultivation has been an option used to diversify the production system and perform herbicide rotation in irrigated rice crops in the lowland soils of the Rio Grande do Sul State, southern Brazil. However, elevated salinity levels have been detected in these soils that can inhibit plant growth due to the reduction of the osmotic potential of the soil solution and can causes toxicity. The combination of stress factors in the plantation areas can intensify deleterious effects, such as changes in salinity associated with herbicides that trigger oxidative stress in crops.
Objective: This study aimed to evaluate osmotic potential, cell damage, and enzyme activities of the antioxidant metabolism on soybean after treatment with herbicides and salinity stress conditions.
Methods: For this purpose, completely randomized design was used in a factorial scheme with three replicates. The A factor included four herbicide treatments, as follows: control (no herbicide), sulfentrazone, S-metolachlor, and sulfentrazone + S-metolachlor. The B factor was represented by the following three salinity levels: 0 (control), 60, and 120 mM NaCl, which were applied 24 hours after soybean sowing.
Results: The results showed a significant alteration in the osmotic potential of soybean plants, mainly at higher salt concentrations. Although an increase in the lipid peroxidation has been detected in some treatments, antioxidant enzyme action combined with osmotic adjustment to reduce oxidative damage were mechanisms found to be employed by plants to reduce hydrogen peroxide levels.
Conclusions: We concluded that herbicide treatment, in combination with saline stress, can alter physiological and biochemical processes of soybean plants.

1 INTRODUCTION

In recent years, many studies have been conducted to evaluate impacts of climate change on agriculture and analyze response trends, as well as to propose management strategies for different crops (Thornton et al., 2014). One of the most critical challenges of the 21st century is to provide enough

food for the growing population, while climate change threatens world food security (Lal, 2013). Thus, it is imperative to understand the impact of climate change on crops to be able to implement methodologies to mitigate its adverse effects.

Climate change is rapidly contributing to the observed change in the climate profile and rise of sea levels, which in turn leads to flooding and saline contamination of soils (Chen and Mueller, 2018). Salinity can inhibit plant growth by reducing the osmotic potential (Ψs) of the soil solution, thereby restricting water availability, which hampers water uptake by the roots and leads to physiological drought (Prisco and O'Learv 1970; Amorim et al., 2002). In response to water restriction, stomatal closure occurs to reduce transpiration, which consequently reduces the photosynthetic rate and biomass production, and decreases plant growth (Flowers, 2004; Munns and Tester, 2008). Another effect of changes in soil salinity levels is ion accumulation. Excess salt concentration in plant tissues causes ion toxicity, nutritional imbalance, and degradation of the chlorophyll content (Tester and Davenport, 2003; Kaya et al., 2015).

In the Rio Grande do Sul (RS) State, southern Brazil, lowland soils are commonly cultivated with irrigated rice. However, the no-tillage system has been gaining ground in this region as an alternative to rice cultivation, which is associated with the expansion of rotation/succession of rainfed crops (Ribeiro et al., 2016). Adopting sustainable and conservative production systems, in combination with alternative crops with commercial value and liquidity, such as soybeans, can contribute to the maintenance of productive competitiveness in the lowlands areas of this state (Vernetti Jr et al., 2009). Besides, this strategy can reduce the problems associated with the presence of weeds resistant to imidazolinone herbicides, such as weedy rice (*Oryza sativa*), providing more options for chemical control with different action mechanisms (Zemolin et al., 2014).

Sulfentrazone, which belongs to the aryl triazolinone chemical group, can be used in the weed control as a pre-emergent herbicide for soybean (Senseman, 2007). When applied to the soil, this herbicide is absorbed by the roots and translocated by the xylem to the point of action, where, in the presence of light, it inhibits the protoporphyrinogen oxidase (PPO). This enzyme acts on the chlorophyll biosynthesis, accumulating protoporphyrin IX and forming free oxygen. This process can lead to lipid peroxidation of the cell membrane and, consequently,

its rupture, causing the death of susceptible plants (Oliveira Júnior et al., 2011). S-metolachlor, which belongs to the chloroacetamide chemical group, is another recommended pre-emergent herbicide for soybean. This herbicide is absorbed through the coleoptile and broadleaf hypocotyl of grasses, acting on the terminal bud and inhibiting plant growth (Oliveira Júnior et al., 2011).

The joint action of herbicides and increased soil salinity can cause oxidative stress in soybean plants, leading to the increase of reactive oxygen species (ROS) and promoting the action of antioxidant enzymes, like the superoxide dismutase (SOD), ascorbate peroxidase (APX), and catalase (CAT). Isolated factors of abiotic stress can generate oxidative damage, as previously evidenced by Rivero et al. (2014) when analyzing tomato plants growing at different salinity conditions. In addition, morphological and biochemical changes in response to herbicide stress and total submersion in water were observed in the study by Marchezan et al. (2017) on irrigated rice.

This work aimed to evaluate Ψs, cell damage, and enzyme activities of the antioxidant metabolism of soybean after treatment with different herbicides recommended for this crop and irrigation with water at three saline conditions. We also determine whether the interaction between saline stress and herbicide has synergistic or antagonistic effects on the physiological and biochemical processes of soybean plants.

2 MATERIALS AND METHODS

The experiment was conducted in a greenhouse and laboratory at the Universidade Federal de Pelotas – UFPel (31°52'S, 52°21'W), RS, Brazil, in completely randomized design, distributed in a 4x3 factorial scheme (herbicide treatments x salinity levels) and with three replicates. Three replicates were performed in the laboratory (technical replicates) from the same extract for each replicate from the greenhouse (biological replicates).

Factor A included four herbicide treatments, as follows: no herbicide (control), sulfentrazone (600 g a.i. ha^{-1}), S-metolachlor (1,200 g a.i. ha^{-1}), and sulfentrazone + S-metolachlor (600+1,200 g a.i. ha^{-1}, respectively). Factor B consisted of the following three salinity levels in irrigation water: 0 (control), 60, and 120 mM of sodium chloride (NaCl).

The experimental units were represented by 0.7 L plastic pots (10 cm in diameter x 15 cm in height)

filled with properly sifted and fertilized soil, as recommended for this crop. The soil used was a typical eutrophic hydromorphic planosol with the following characteristics: 5.6 pH water (1:1); 5.7 $cmol_c$ dm^{-3} effective CTC; 1.93% organic matter; 13% clay; clay class 4; 3.6 $cmol_c$ dm^{-3} Ca; 1.8 $cmol_c$ dm^{-3} Mg; 0.1 $cmol_c$ dm^{-3} exchangeable Al; 51.9 mg dm^{-3} P-Mehlich; 51.9 mg dm^{-3} K. Two soybean seeds of the 'BMX Potência RR' cultivar was sown per pot, and one plant was kept in each pot after plant emergence.

Irrigation with the different saline conditions was started 24 hours after sowing and repeated within two days until the end of the experiment (50 mL per pot). After sowing and irrigation, herbicides were applied in pre-emergence with a CO_2 pressurized costal sprayer equipped with four series 110-02 flat fan type spray nozzles, which were spaced 50 cm and calibrated to spray 150 L ha^{-1}. On the 28th day after treatment (DAT), when the plants were at the V2 stage, all the fully developed trifoliate leaves were collected for further analysis.

To determine Ψs, a vapor pressure osmometer model 5600 VAPRO (Wescor, Logan, Utah, USA) was used. Leaf samples of approximately 700 mg were macerated in 2 mL polypropylene tubes with a glass rod. Subsequently, the resulting extract was centrifuged at 12000 rpm at a temperature of 4 ºC for 20 minutes. A 10 µL aliquot was used to perform the readings. The Van't Hoff equation was used to convert the values obtained from mmol kg^{-1}Ψs, where Ψs = - C × 2.58 × 10^{-3} and C is the osmolality value obtained in 10 µL of cell extract at each reading.
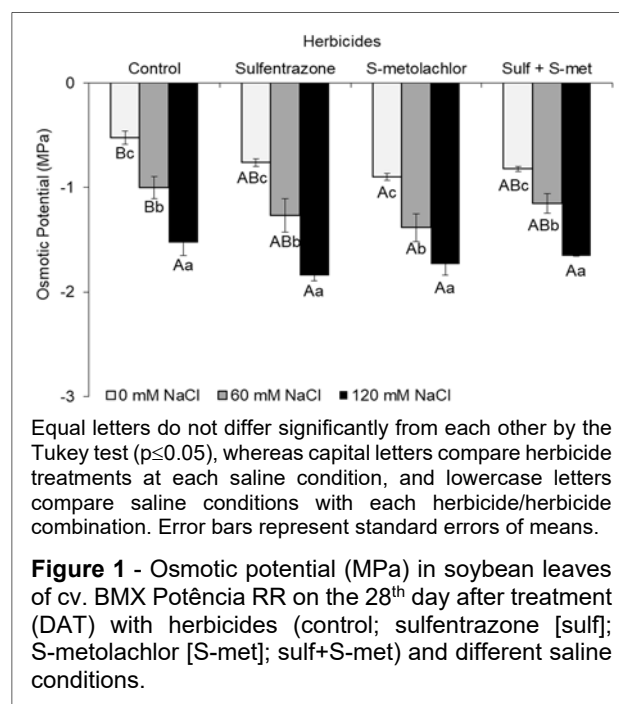
Cell membrane damage was determined by quantifying thiobarbituric acid reactive species (TBARS) via malondialdehyde accumulation (MDA), also known as lipid peroxidation, as described by Heath and Packer (1968), and by the content of hydrogen peroxide (H_2O_2) according to Loreto and Velikova (2001). The SOD activity was determined according to Giannopolitis and Ries (1977), and the results were expressed in U mg^{-1} protein. The CAT activity was determined according to Azevedo et al. (1998) based on the H_2O_2 consumption (39.4 mM cm^{-1} molar extinction coefficient) with results presented in µmol H_2O_2 min^{-1} mg^{-1} protein. The APX activity was determined according to Nakano and Asada (1981) based on the ascorbate oxidation rate (2.8 mM cm^{-1} molar extinction coefficient), and the results were expressed in µmol ASA min^{-1} mg^{-1} protein.

The findings were submitted to analysis of variance and, when there was a significant difference, the means were compared by the Tukey test ($p \leq 0.05$) on WinStat (Winstat, 2003).
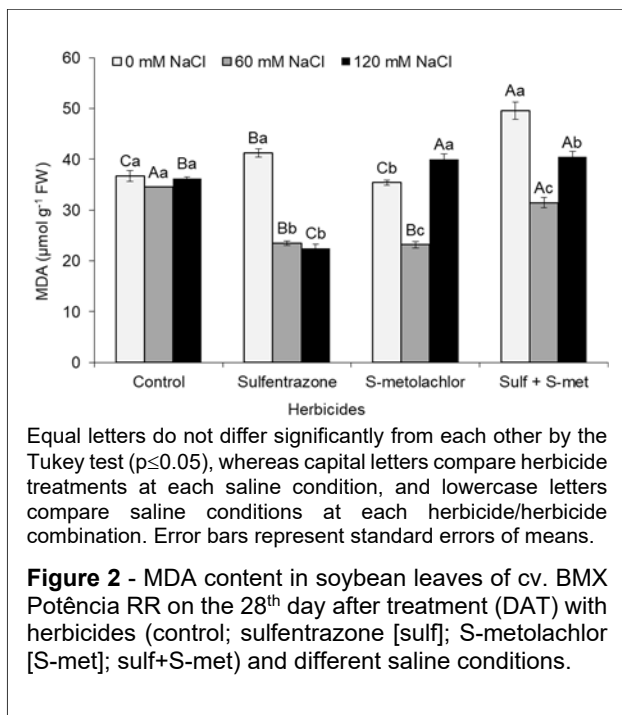
3 RESULTS AND DISCUSSION

Based on the analysis of variance, all variables had a significant effect between the studied factors (salinity levels and herbicide treatments). The increase in salt concentrations in the soil solution induced a significant reduction of Ψs in soybean plants, with more negative values in the treatment with 120 mM NaCl, regardless of the herbicide treatment (Figure 1).

Equal letters do not differ significantly from each other by the Tukey test ($p \leq 0.05$), whereas capital letters compare herbicide treatments at each saline condition, and lowercase letters compare saline conditions with each herbicide/herbicide combination. Error bars represent standard errors of means.

Figure 1 - Osmotic potential (MPa) in soybean leaves of cv. BMX Potência RR on the 28th day after treatment (DAT) with herbicides (control; sulfentrazone [sulf]; S-metolachlor [S-met]; sulf+S-met) and different saline conditions.

These results are in line with the study by Coelho et al. (2014), in which a linear and decreasing response of Ψs in leaves of cowpea (*Vigna unguiculata*) was found due to higher levels of soil salinity. Regarding the herbicide treatments, only isolated S-metolachlor differed from the control at 0 and 60 mM NaCl. These responses could have been caused by the contact of the herbicide molecule with the plant roots, reducing its absorption capacity, promoting the decrease of Ψs in leaves to normalize stress, and resuming assimilation through root tissues (Santos et al., 2012).

Regarding MDA, no significant differences were observed between salinity levels for treatments without herbicide. For those not submitted to salinity increases, the highest MDA values were observed in the treatments with combined sulfentrazone+ S-metolachlor, followed by sulfentrazone alone (Figure 2). Sulfentrazone inhibits the PPO enzyme in

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Benedetti L, et al. Soybeans stress by herbicide and salt

Equal letters do not differ significantly from each other by the Tukey test (p≤0.05), whereas capital letters compare herbicide treatments at each saline condition, and lowercase letters compare saline conditions at each herbicide/herbicide combination. Error bars represent standard errors of means.

Figure 2 - MDA content in soybean leaves of cv. BMX Potência RR on the 28th day after treatment (DAT) with herbicides (control; sulfentrazone [sulf]; S-metolachlor [S-met]; sulf+S-met) and different saline conditions.
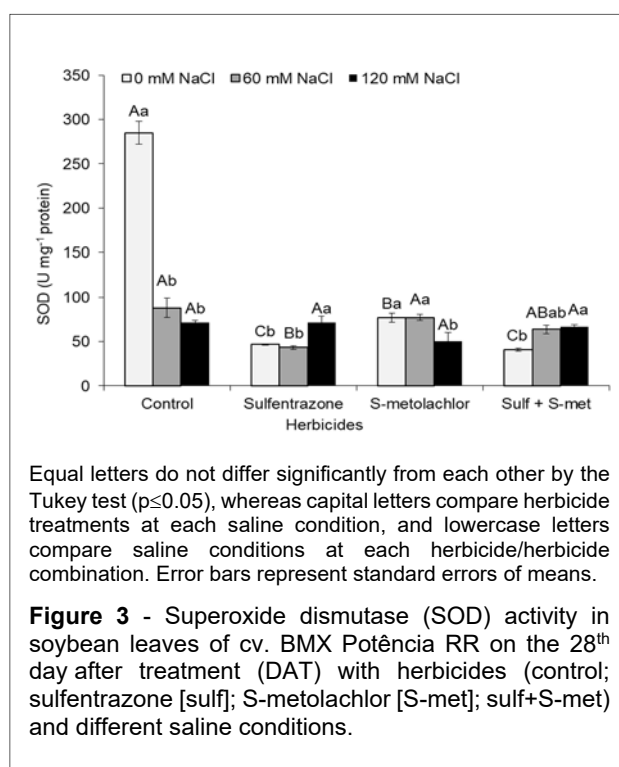
plants, thus forming ROS within cells, promoting lipid peroxidation, and causing cell death (Carvalho and López-Ovejero, 2008).
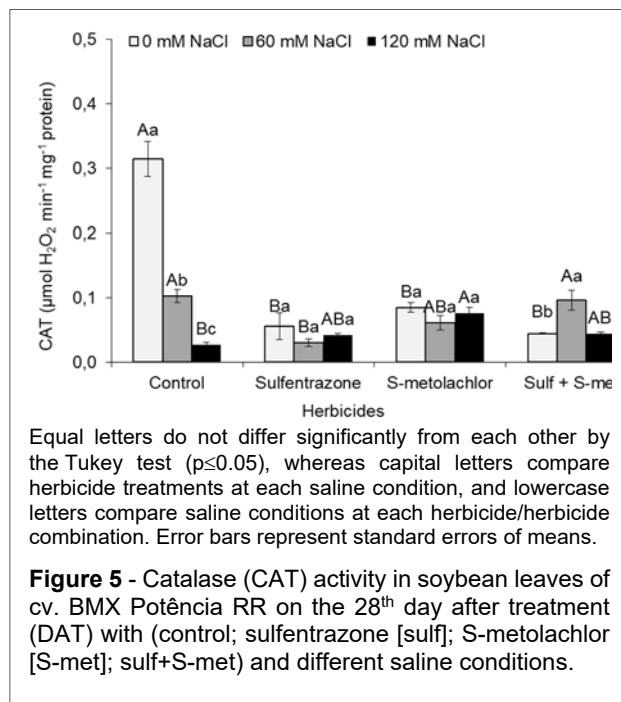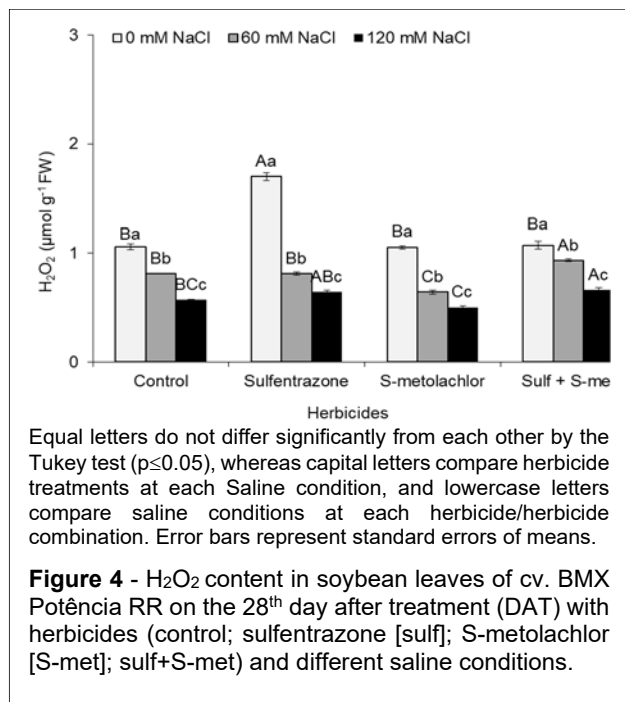
Treatments with herbicides associated with salinity levels can lead to MDA values below control and can be related to the tolerance level of the cultivar to saline stress (Turan and Tripathy, 2013). In this study, MDA accumulation was significantly high in the 120 mM NaCl treatment with S-metolachlor (Figure 2). This finding was possibly related to the production of oxygen radicals, resulting in increased lipid peroxidation and oxidative stress in the roots (Fadzilla et al., 1997; Gomez et al., 1999; Hernandez et al., 2000). Also, concerning the higher salinity level tested in this study, the combination of sulfentrazone+ S-metolachlor increased lipid peroxidation compared to sulfentrazone alone, probably because S-metolachlor acts on cell walls (Nagai et al., 2011). This result could also be associated with the action mode of S-metolachlor, which inhibits the synthesis of very long chain fatty acids, unbalancing the composition of the cell membrane (Dayan et al., 2015).

In plants submitted to 60 mM NaCl, the combination of sulfentrazone+S-metolachlor did not differ significantly from the control (no herbicide); however, when these herbicides were applied separately, there was a reduction in MDA values. This shows a possible elimination of ROS by the antioxidant defense system (Figure 2). This result could also be associated with the fine osmotic adjustment in the plants analyzed, which is the adaptive mechanism of many crops to several types of stress (Adolf et al., 2012).

There was a significant reduction in H_2O_2 as salinity levels increased in each herbicide treatment (Figure 3), a result inversely proportional to that observed for Ψs (Figure 1). In the absence of saline stress, the highest H_2O_2 values were observed in response to sulfentrazone, a behavior that is likely related to lipid peroxidation and the action mechanism of this herbicide (Oliveira Júnior et al., 2011). The photorespiratory process in photosynthetic tissues and the SOD action are among the main sources of H_2O_2 in plants. This enzyme catalyzes the dismutation of superoxide anion ($O_2^{\cdot-}$) in H_2O_2 (Gill and Tuteja, 2010; Sharma et al., 2012), and is characterized by the first antioxidant enzymatic defense line.

In the present study, the highest SOD activity was found in control plants and was not in response to herbicidal or saline conditions (Figure 4). However, comparing the salinity levels in each herbicide treatment for sulfentrazone and sulfentrazone+ S-metolachlor, there was a significant increase in SOD activity in the presence of 120 mM NaCl. For S-metolachlor, the response was the opposite since the lowest SOD activity was found at the highest salt concentration. The lower activity of this enzyme at a high salt concentration is possibly due to the induction of other mechanisms of cellular protection, as well as the synthesis of compatible osmolytes. Osmoprotectants are synthesized under adverse conditions and include a wide variety of amino acids; they can promote the integrity of membranes, proteins, and enzymes (Ashraf, 2010). This process

Equal letters do not differ significantly from each other by the Tukey test (p≤0.05), whereas capital letters compare herbicide treatments at each saline condition, and lowercase letters compare saline conditions at each herbicide/herbicide combination. Error bars represent standard errors of means.

Figure 3 - Superoxide dismutase (SOD) activity in soybean leaves of cv. BMX Potência RR on the 28th day after treatment (DAT) with herbicides (control; sulfentrazone [sulf]; S-metolachlor [S-met]; sulf+S-met) and different saline conditions.

Equal letters do not differ significantly from each other by the Tukey test (p≤0.05), whereas capital letters compare herbicide treatments at each Saline condition, and lowercase letters compare saline conditions at each herbicide/herbicide combination. Error bars represent standard errors of means.

Figure 4 - H_2O_2 content in soybean leaves of cv. BMX Potência RR on the 28th day after treatment (DAT) with herbicides (control; sulfentrazone [sulf]; S-metolachlor [S-met]; sulf+S-met) and different saline conditions.

Equal letters do not differ significantly from each other by the Tukey test (p≤0.05), whereas capital letters compare herbicide treatments at each saline condition, and lowercase letters compare saline conditions at each herbicide/herbicide combination. Error bars represent standard errors of means.

Figure 5 - Catalase (CAT) activity in soybean leaves of cv. BMX Potência RR on the 28th day after treatment (DAT) with (control; sulfentrazone [sulf]; S-metolachlor [S-met]; sulf+S-met) and different saline conditions.
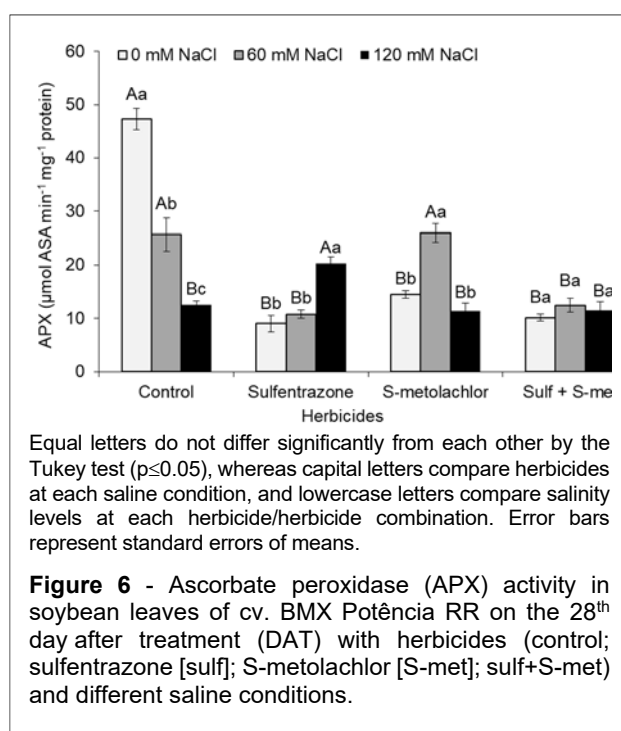
is usually accompanied by a reduction of Ψs in plants, which was observed in the present study for the condition of higher soil salinity.

The enzymes CAT and APX act in synchrony with SOD to eliminate H_2O_2. CAT acts mainly on peroxisomes due to the process of photorespiration and is effective at relatively high H_2O_2 concentrations, especially under severe stress conditions (Jaleel et al., 2009; Gupta, 2010). In soybean plants, CAT showed higher activity in the control treatment (absence of salinity), in agreement with that observed for SOD (Figure 5). The analysis of each herbicide treatment for the different salinity levels showed that only the combination sulfentrazone+S-metolachlorat 60 mM NaCl had a significant increase of CAT, indicating enzyme action as an alternative for reducing the H_2O_2 content and allowing adaptation to stress condition.

APX acts on chloroplasts and has a high affinity in eliminating H_2O_2 at low concentrations, (Jaleel et al., 2009). Our findings showed the joint action of the three antioxidant enzymes, where the highest APX activity was also observed in the control treatment (Figure 6). However, in the sulfentrazone treatment, the highest APX activity was observed with soil salinity at 120 mM NaCl, contrary to lipid peroxidation. This finding emphasizes the efficiency of this enzyme in mitigating the effects of H_2O_2. Although this herbicide is naturally capable of producing ROS and promoting lipid peroxidation, APX provided lower lipid peroxidation compared to other herbicide treatments at 120 mM NaCl. For S-metolachlor, a significant increase was observed in

Equal letters do not differ significantly from each other by the Tukey test (p≤0.05), whereas capital letters compare herbicides at each saline condition, and lowercase letters compare salinity levels at each herbicide/herbicide combination. Error bars represent standard errors of means.

Figure 6 - Ascorbate peroxidase (APX) activity in soybean leaves of cv. BMX Potência RR on the 28th day after treatment (DAT) with herbicides (control; sulfentrazone [sulf]; S-metolachlor [S-met]; sulf+S-met) and different saline conditions.

the treatment with 60 mM NaCl, not differing between saline conditions when herbicides were applied in combination.

4 CONCLUSIONS

The results of this study show that applying sulfentrazone and S-metolachlor herbicides on soybean crops subjected to saline stress conditions in reduced Ψs and membrane lipid peroxidation. However, the joint and effective action of antioxidant enzymes minimized the deleterious effects of ROS, which aim to maintain cellular metabolism and allow plants to adapt to the environment.

5 CONTRIBUTIONS

6 ACKNOWLEDGEMENTS

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Benedetti L, et al. Soybeans stress by herbicide and salt

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