THE EFFECT OF SALINE STRESS ON ACTIVITY OF SOME ANTIOXIDATIVE ENZYMES DURING WHEAT SEED GERMINATION

LĂCRĂMIOARA OPRICĂ[1*]

Keywords: catalase, peroxidase, saline stress, wheat.

Abstract: Salinity is one of major environmental factors limiting agricultural production and has a considerable effect on world agriculture. Wheat is one of the main crops occupying a large area in the world. For these reasons, we studied the effect of the saline stress on the activity of some antioxidative enzymes at this graminaceous plant. As biological material, we used seeds of three wheat cultivars (Faur, Fundulea and Iaşi 2) which were treated with different NaCl concentrations. Simultaneously, three controls not exposed at treatment were prepared. The dynamics of catalase and peroxidase activities was determined at 96h, 168h and 240h, namely in early stages of ontogeny. The results evidenced that the saline stress determined modifications of seedlings enzyme activities, at the three moments of analysis, depending on wheat variety and tested concentration of NaCl solutions.

INTRODUCTION

Environmental factors influence the characters, composition, growth and development of individual plants and plant communities. When any of these environmental factors exceeds the optimum tolerance of a plant, the result is stress to the plant, which is turn influences the developmental, structural, physiological and biochemical processes. Soil salinity is one of these environmental stresses (Lawlor D. W., 2002). An important consequence of saline stress in plants is the generation of reactive oxygen species (ROS) such as superoxide anion (O_2^-), H_2O_2 and the hydroxyl radical, particularly in chloroplasts and mitochondria (Mittler, 2002, Neill et al., 2002). The abiotic factors such as salinity and drought induce osmotic stress and have similar biochemical responses that include the increase of reactive oxygen species level (Zhu et al., 1997). Cereals are one of the most important crops in our country and play a special role in nutrition of peoples. But, unfortunately, the abiotic stress, such as salinity, decreases wheat growth and productivity (Esfandiari et al., 2007).

In this paper, we assessed the antioxidative potential of three wheat cultivars (Fundulea, Iaşi 2, Faur) by evaluating the catalase and peroxidase activities as reponse to saline stress.

MATERIAL AND METHODS

The research, accomplished in the laboratories of Department of Molecular and Experimental Biology of Faculty of Biology from Iasi, was performed on seeds of Fundulea, Iasi 2, and Faur wheat cultivars. Firstly, 100 seeds from each wheat cultivar were sterilized in 3% H_2O_2 and then washed to remove H_2O_2. The seeds were subjected to the treatment with 50mM, 100mM, 150mM, and 200mM NaCl, for 4 hours. Control seeds were stored for 4 hours in distilled water. The seeds were then transferred into sterile Petri dishes, containing 2 layers of Whatman 1 paper, imbued with 10 ml distilled water. Each Petri dish contained 100 seeds, and three rehearsals for each variant were carried out. The Petri dishes were kept in dark, at 25^0 C to promote the germination. After that, the Petri plates were transferred in a room assuring the normal conditions for plantlets growth. For enzymatic determinations, the seedlings were randomly harvested at 96h, 168h and 240h.

Enzyme extract (for both enzymes) was obtained from seedlings with disodium phosphate buffer, pH 7. Determination of catalase activity was done by titration method with sodium thiosulphate, while peroxidase activity was determined by spectrophotometric method with o-dianisidine, according to standard method (Artenie, 2008).

RESULTS AND DISCUSSIONS

In Fundulea wheat cultivar, at 96h, the catalase activity presented lower values at 100mM and 200mM NaCl concentrations (614.37 UC/g, respectively 706.04 UC/g), comparatively with control. Enzyme activity is higher at 50mM (1069.51 UC/g) and 150mM (924.21 UC/g), compared to control, fact indicating an increased sensitivity of 96h old seedlings to the treatment with respective NaCl concentrations. Therefore, the maximum enzyme activity was registered for seedlings treated with 50mM NaCl, while the minimum value was noted in 100mM NaCl treated variant (Figure 1). At 168h, the enzyme activity shows a very close value with control for the treatment with 50mM NaCl; as NaCl concentrations increase, the trend of catalase activity is to decrease.

At 240h, the catalase activity has smaller values than control at 50mM and 100mM NaCl concentrations, and higher values at the highest NaCl concentrations (150mM and 200mM NaCl). The catalase activity recorded the maximum value in Fundulea wheat cultivar (2837.46

UC/g), at 200mM NaCl, and the minimum value of enzyme activity was registered at 50mM NaCl concentration - 213.61 UC/g.

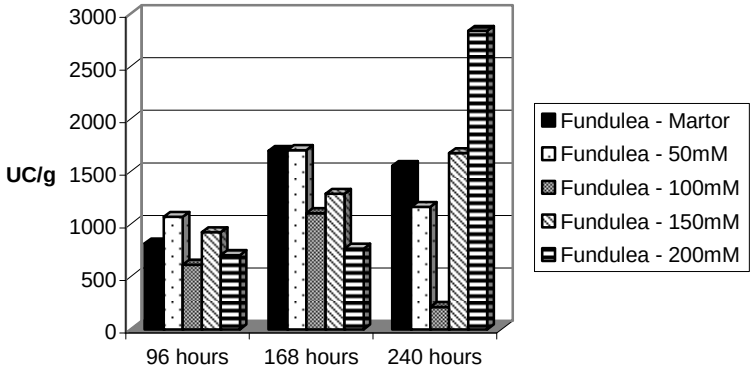

Fig. 1. The catalase activity variation at Fundulea wheat cultivar, under saline stress

Our data on the influence of different concentrations of NaCl on catalase activity in Iasi 2 wheat cultivar showed, at 96h, a lower enzyme activity enzyme face to control for all treated variants, aspect that may suggest that seedlings have not suffered at saline stress. At 96h, catalase has relatively similar activities in the first three concentration variants, while the variant treated with 200mM NaCl was registered with a lower enzyme activity (562 UC/g) (Figure 2). At the next moment - 168h - catalase activity is higher comparative to control only in seedlings treated with 50mM NaCl, the other NaCl concentrations induced decreases of catalase activities. Enzyme activity decreased, at 240h, as the NaCl concentration increased, as follows: 1325.39 UC/g (at 50mM), 992.82 UC/g (at 100mM) and 374.43 UC/g (at 150 mM). On the contrary, the 200 mM NaCl concentration induced a significant increase of catalase activity (2086.81 UC/g), as protective response of plantlets to the oxidative stress induced at this moment by this high concentration of the tested saline solution.

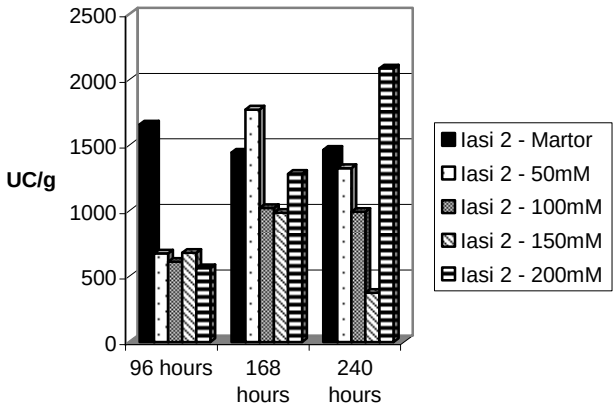

Fig. 2. The catalase activity variation at Iaşi 2 wheat cultivar, under saline stress

In Faur wheat cultivar, at 96h, catalase activity decreased under the influence of salt stress and followed a similar dynamics as in Iasi 2 wheat cultivar; all NaCl concentration variants have close levels of enzyme activity, but they were smaller than control (Figure 3). At 168h, in plantlets obtained by the germination of 50mM NaCl treated seeds, the catalase activity is slightly increased to the control (1516.26 UC/g comparatively to 1467.79 UC/g). As NaCl concentration increases, the enzyme activity also registered increases, as follows: 933.4 UC/g at 100mM, 1294.21 UC/g at 150mM, 1593.93 UC/g at 200mM.

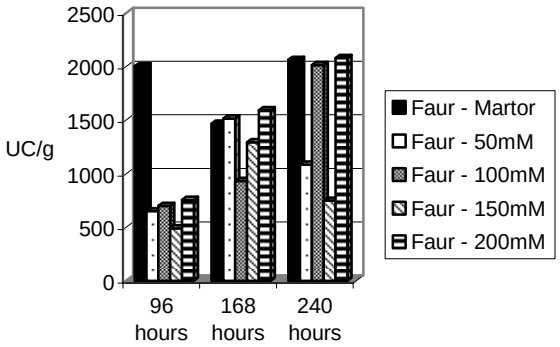

Fig. 3. The catalase activity variation at Faur wheat cultivar, under saline stress

At 240h after NaCl treatment, relatively constant activities are observed at 100mM NaCl (2015.64 UC/g) and 200mM NaCl (2081.13 UC/g) in relation to control (2064.74 UC/g). The

concentrations of 50mM and 150mM NaCl reduced the catalase activity by half (1085.58 UC/g) or more (748.61 UC/g) compared with control.

Peroxidase activity in Fundulea wheat cultivar is growing at 96h under the influence of NaCl concentrations; the maximum enzyme activity is observed at 200mM (100.48 UP/g) (Figure 4). On the contrary, at 168h a trend of decreasing of peroxidase activity was noted in relation to the control, as NaCl concentration was higher. At 240h as the seedlings have been developed, peroxidase activity showed fluctuations in relation to the control, namely it was lower at the smallest NaCl concentrations (50mM and 100mM) or was higher at the bigger NaCl concentrations (150mM and 200mM). The maximum value of the peroxidase activity is 117.19 UP/g in the case of seedlings of Fundulea wheat cultivar, at 200mM, and the minimum value of enzyme concentration is in 50mM - 80.05 UP/g.

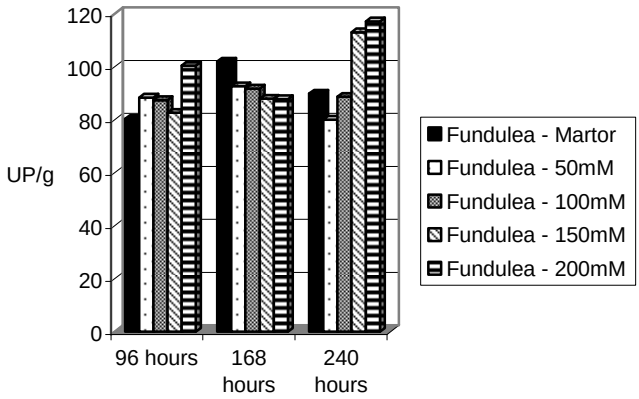

Fig. 4. The peroxidase activity variation at Fundulea wheat cultivar, under saline stress

Peroxidase activity in Faur wheat cultivar at 96h is fluctuate, the values being low at extreme concentrations 50mM and 200 mM (75 UP/g, respectively, 90.61 UP/g), while at the 100 mM and 150mM concentrations the values are comparable to control. At 96h, the maximum enzyme activity is 126.47 UP/g at 150mM and the minimum is 75.00 UP/g at 50mM. At 168h, enzyme activity shows values relatively comparable to the control (79.9 UP/g). They are lower in the seedlings of 100mM variant (72.30 UP/g) and 200mM variant (66.34 UP/g). At 240h, the enzyme activity registered lower levels than control at 50mM, 100mM, and 150mM, but the peroxidase had an almost double activity at 200mM NaCl.

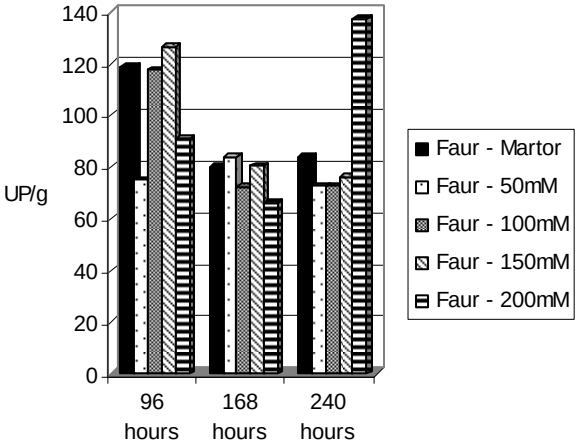

Fig. 5. The peroxidase activity variation at Faur wheat cultivar, under saline stress

At 96 h, the Iasi 2 wheat cultivar show values of peroxidase activity generally comparable to control at the concentrations of 50mM, 100mM, and 150mM NaCl, while at 200 mM the enzyme activity surpasses the control (Figure 6). The fact that seedlings of Iasi 2 wheat cultivar have been stressed by the highest concentration of 200 mM NaCl is evidenced by small sizes of roots and stems.

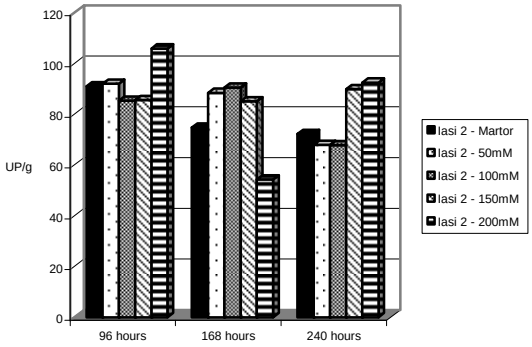

Fig. 6. The peroxidase activity variation at Iasi 2 wheat cultivar, under saline stress

At 168h, the peroxidase activity is superior to control at first three concentrations of NaCl and is inferior to control at 200mM. Behaviour of Iasi 2 wheat cultivar at 240h is similar to

the Fundulea cultivar in the sense that the peroxidase activity diminishes at lower concentrations of NaCl (50mM and 100mM) and increases at higher concentrations of NaCl (150mM and 200mM). The maximum of the peroxidase activity is 92.46 UP/g at 200mM and the minimum value was registered at 100 mM NaCl - 67.74 UP/g.

CONCLUSIONS

Our results evidenced that the saline stress, induced by different concentration of NaCl, determined modifications of seedlings enzyme activities, at the three moments of analysis, depending on wheat variety, age of seedlings and concentration of NaCl solutions.

At the last interval studied, 240h, we remark that, the highest concentration of NaCl (200 mM) induced generally, the stimulation of enzyme activity in the seedlings of all wheat cultivars.

REFERENCES

Artenie Vlad, Ungureanu Eugen, Anca Mihaela Negură, 2008. *Metode de investigare a metabolismului glucidic şi lipidic*, Editura Pim, pp. 94-97, 100-103.

Ezatollah Esfandiari, Fariborz Shekari, Farid Shekari, Manoucherhr Esfandiari, 2007. Not. Bot. Hort. Agrobot. Cluj, Vol. 35 (1), 48-56.

Lawlor D.W., 2002. Ann. Bot. 89: 1-15.

Mittler R. 2002. Trends Plant. Sci. 7: 405-410.

Neill S, Desikan R & Hancock J., 2002. Curr. Opin. Plant Biol. 5: 388-395.

Zhu J. K., Hasegawa P.M., Bressan R. A., 1997. Critical Reviews in Plant Science 16, 253-277.

1 Universitatea „Alexandru Ioan Cuza” Iaşi, Catedra de Biologie Moleculară şi Experimentală

* lacramioara.oprica@uaic.ro