EARLY BIOCHEMICAL RESPONSES OF *Brassica napus var Exagone* SEED GERMINATION AT SALT TREATMENT

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Abstract: The rapeseed is the third most important edible oil source in the world, after soybean and palm. This plant is very sensitive to salt stress throughout the growth and development cycle. Salt stress reduces plant growth and productivity and can affected several physiological and biochemical process. For this reason, this study was carried out to determine early the effects of NaCl treatment on the protein content, amylase (EC 3.2.1.1), acide phosphatase (E.C. 3.1.3.2), peroxidase (EC 1.11.1.7) and catalase (EC 1.11.1.6) in young rape plant (*Brassica napus* L. var Exagone). The rape seed were treated four hour with three NaCl solutions (50mM, 100mM, 150mM) comparatively with a control who wasn't exposed at treatment. Amylase activity was inhibited by all used salts concentrations at both time intervals studied. Salinity stimulated the acid phosphatase activity, more intense at 4 days and moderately at 7 days after treatment. The activity of the two assayed antioxidant enzymes (except peroxidase in the second period) increase in salinity conditions with increasing salt concentration. Soluble protein content is reduced compared to the control at both studied intervals.

INTRODUCTION

Cultivated soils worldwide are becoming more saline from excessive fertilization, marginal irrigation water and desertification processes. Salinity is a major and common abiotic stress reducing the yield of a wide variety of crops all over the world (Ashraf and Foolad, 2007). Excess amount of salt in the soil adversely affects plant growth and development.

Brassica napus plants belong to the Brassicaceae (Cruciferae) family that comprises about 350 genera and 3000 species. The rapeseed (*Brassica napus*) is the third most important edible vegetable oil source in the world, after soybean and palm (Ashraf and McNeilly 2004). This plant has considerable potential to grow in salt-affected areas. Plant responses to stress are complex (Chaves et al. 2003), reduces plant growth and productivity by affecting morphological, anatomical, biochemical and physiological characteristics.

One of the biochemical changes occurring when plants are subjected to stress and salt stress particularly is the production of reactive oxygen species (ROS), such as superoxide ($O2^-$), hydrogen peroxide (H_2O_2) and hydroxyl radicals (-OH) (Foyer et al. 1994; Mittler 2002; Zhu 2000, Neill et al. 2002; Foyer and Noctor 2005). These ROS are highly reactive and attack the most sensitive biological macromolecules and membranes to impair their functions (Nayyar and Gupta, 2006). It has been generally accepted that an enhanced production of ROS during stress can disturb redox homeostasis, by enhancing oxidative processes such as membrane lipid peroxidation, protein oxidation, enzyme inhibition and DNA and RNA damage (Asada, 2006). To mitigate and repair damage initiated by ROS, plants have developed a complex antioxidant system (del Rio et al. 2002). In plant cells, one such protective mechanism is an antioxidant system, composed by both non-enzymatic and enzymatic antioxidants (Foyer et al. 1994). Antioxidative enzymes like superoxyde dismutase (SOD), catalase (CAT), peroxidase (PRX), ascorbate peroxidase (APX), and glutathione reductase (GR) are the most important components in the scavenging system of ROS (Mc Kersie and Leshem 1994; Noctor and Foyer 1998). The primary scavenger in plant cells is superoxide dismutase (SOD) which converts O_2^- to H_2O_2. The peroxidase (PRX) has an important role in eliminating this toxic product, H_2O_2 who is also scavenged by catalase (CAT).

Salinity stress can affect other metabolic processes such as photosynthesis (Borsani et al. 2001; Chattopadhayay et al. 2002), protein synthesis, respiration, nitrogen assimilation and phytohormone turnover (Arshi et al. 2002). In plant, acid phosphatase and alkaline phosphatase are widely distributed. Acid phosphatase is known to act under stress by maintaining a certain level of inorganic phosphate in plant cells (Lefebvre et al., 1990, Chiung-Yueh et al., 1998, Olmos and Hellin, 1997). The salinity stress on the canola seeds caused an increase in both acid phosphatase and alkaline phosphatase activity (Bybordi Ahmad and Elnaz Ebrahimian, 2011).

The aims of the present work was to determine effects of treatment of different NaCl concentrations on some metabolic (phosphatase, α-amilase, catalase, peroxidase activity and soluble protein content) and physiologic parameters (root size) during seed germination of *Brassica napus* var Exagone.

MATERIAL AND METHODS

The researches, accomplished in the laboratories of Department of Molecular and Experimental Biology of Faculty of Biology from Iasi, was performed on *Brassica napus* var. Exagone seeds. This seeds were obtained from the Territorial Institute for Quality Seeds and Planting Material Iasi. Firstly, 100 rape seeds 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 and 150mM NaCl, for 4 hours. Control seeds were stored for 4 hours in distilled water. Then these were transferred into sterile Petri dishes, containing 2 layers of Whatman 1 paper, moistened with 10 cm^3 50, 100, 100 mM NaCl and distilled water for control sample. Each Petri dish contained 100 seeds and three rehearsals for each variant were carried out. The Petri dishes were kept in dark, at 25^0C to promote the germination. After that, the Petri plates were transferred in a room assuring the normal conditions for seedlings growth and watered every 2 days. For enzymatic determinations, the fresh samples were randomly harvested from each treatment at four and, respectively, seven days after exposure to salinity stress the NaCl treatment.

Enzyme extract (for both antioxidants enzymes) was obtained from seedlings with disodium phosphate buffer, pH 7. Determination of catalase (EC 1.11.1.6) activity was done by spectrophotometric method using potassium dichromate-acetic acid mixture, while peroxidase (EC 1.11.1.7) activity was determined by spectrophotometric method with o-dianisidine. (Artenie et al., 2008). Both enzymes enzymes concentration was reported as units/mg protein.

The acid phosphatase (EC 3.1.3.2) activity was measured in reaction mixtures whith citric acid, citrate, disodic p-nitrophenil phosphate buffer (pH 4.8) and the activity of this enzymes is expressed in micromoli/g vegetal matter/min. (Artenie et al. 2008). The α-amylase was extracted in bidistillated water and the activity was determined using the Noelting-Bernfeld method partial modified by Artenie (Artenie at al., 2008). The quantitative determination of proteins was made with 50mM Tris-HCl buffer, pH – 7.0 according Bradford method (Bradford, 1976), using serum albumine (Sigma-Aldrich) as standard. The results are expressed in mg% fresh matter.

RESULTS AND DISCUSSIONS

In plants, acid phosphatase activity is increased by drought, salt, water and osmotic stress (Shu-Mei Pan and Yung-Reui Chen, 1988, Ehsanpour and Amini, 2003). Also, the phosphorous deficiency increase acid phosphatase activity in various plants (Dracup et al., 1984).

The effects of different NaCl concentration on phosphatase activity in plants of *Brassica napus* var. Exagone are shown in Figure 1. In both intervals used for determining it is observed that the acid phosphatase activity of seedlings is stimulated a fact confirmed by other authors at the same species (Ahmad and Elnaz Bybordi Ebrahimian, 2011). This increase of enzyme activity is intense at 4 days old and lower at 7 days old. Thus, for the first interval, the enzyme activity is maxim at 100 mM variant compared to the control and the other two 50 mM and 150 mM variants values are very close. At 7 days after saline treatment the acid phosphatase activity increases with increasing of NaCl concentration, the maximum being reached at 150 mM variant.

There are many reports which indicate that salinity reduced the α-amylase activities at different plants like sorgum, mays, *Vigna radiata*, *Suaeda salsa* (Khan et al. 1989, Promila and Kumar, 2000, Shi Li-ran, 2007, Li Cunzhen et al., 2005). NaCl treatment at *Brassica napus* (var. Exagone) seed reveals an inhibition of α-amylase activity at both time intervals. In general, the enzymatic activity of seedlings decreases with increasing concentration of salt (Figure 2).

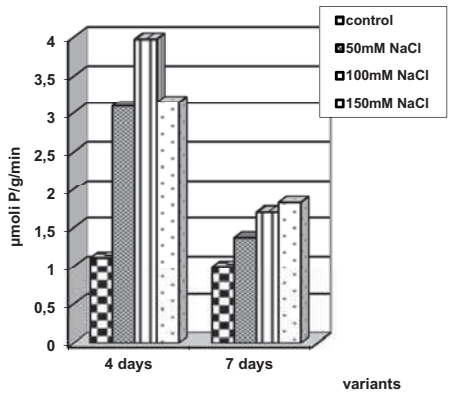


Fig. 1. The variation of phosphatase activity at *Brassica napus* (var. Exagone) seedling after NaCl treatment

In many cases, it has been proposed that salt stress tolerance is related to a higher activity of antioxidant enzymes and that lower activity is found in sensitive species (Shalata and Neumann 2001). However, a direct correlation cannot always be found between salt stress tolerance and the induction of antioxidant enzymes. Recent works showed that salt tolerance is closely related to the efficiency of antioxidant enzymes (Nawaz and Ashraf, 2010; Joseph and Jini, 2011). Among the different ROS, H_2O_2 is of great significance owing to its enormous stability and the role as a secondary messenger to regulate the expressions of defense genes (Yang *et al.,* 2007 Twiner and Trick. 2000). In stress conditions catalase and peroxidase, two major scavenging enzymes, reduced and detoxified the increased production of H_2O_2 by a combined effort (Garratt et al., 2002).

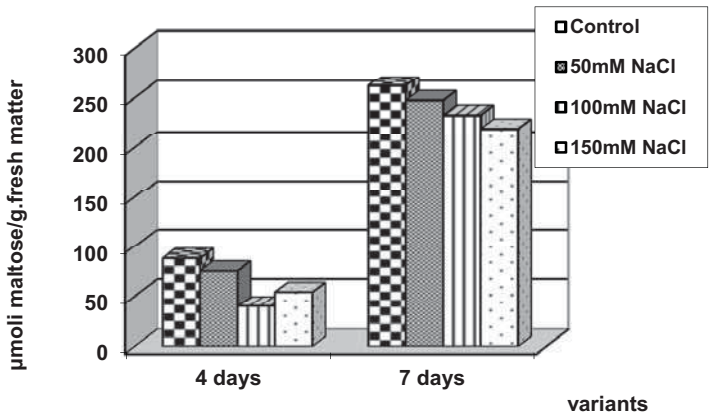


Fig. 2. The variation of α-amilase activity at *Brassica napus* (var. Exagone) seedling after NaCl treatment

At both time intervals after NaCl treatment application, the catalase activity of *Brassica napus* seedling (var. Exagone) have higher values witness, less stressed and more intense 4 days to 7 days, suggesting sensitivity of seedlings to different salt concentrations. The four and seven days after treatment, maximum enzyme activity was obtained in 100 mM variant. Thus, it was 196,33% higher than the control (at 4 days old), while in seven days old, the same variant shows an increase of 506,45% (Figure 3).

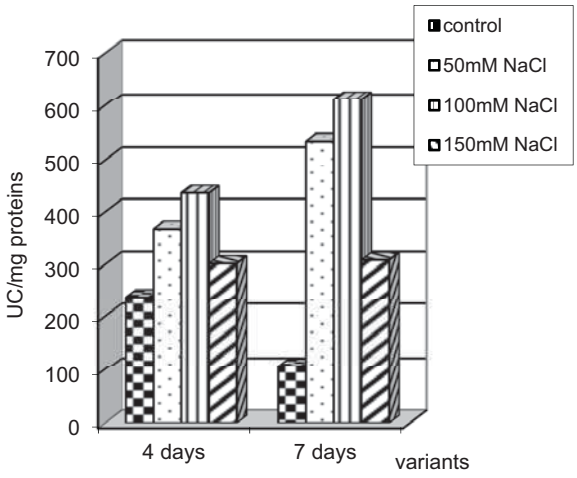


Fig. 3. The variation of catalase activity at *Brassica napus* (var. Exagone) seedling after NaCl treatment

The variation of peroxidase activity in *Brassica napus* (var. Exagone) seedlings is more pronounced at 4 days after treatment with NaCl compared with the next interval. The effect of NaCl salt concentration on peroxidase activity is stimulated in the first interval tested, sowing probably that the H_2O_2 detoxification effect started by catalase is more intense implemented by peroxidase. The next period shows a gradual decrease of enzyme activity with NaCl concentration increasing (Figure 4).

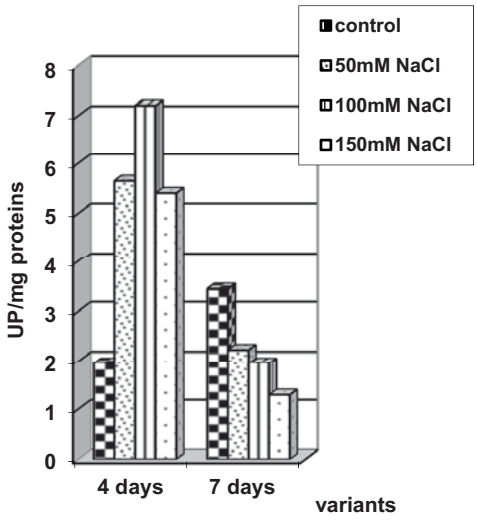


Fig. 4. The variation of peroxidase activity at *Brassica napus* (var. Exagone) seedling after NaCl treatment

Another mechanism affected by salt stress in plants was protein synthesis. It is known that soluble protein content is an important indicator of physiological status of plants (Doganlar et al., 2010). Compared to control, salt treatment caused at 4 days a decrease of soluble protein content at all *Brassica napus* seedlings (var. Exagone) variants and maximum decrease was determined at 100 mM NaCl concentrations.

The protein content in *Brassica napus* seedlings (var. Exagone) after treatment with NaCl have a tendency to decrease compared with the control at the first time interval. The same downward trend is observed at 7 days after treatment. The minimum content of the variant protein content is 100 mM, at both intervals (Figure 5). The same results were obtained Porgali and Yurekli, 2005, who reported that compared with control plants, protein amount in salt sensitive *L. esculentum* plants decreased with the salt application.

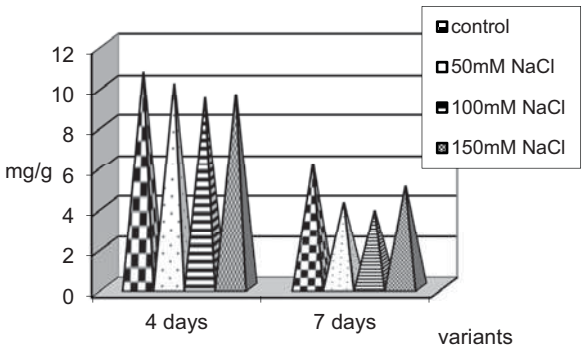


Fig. 5. The variation of protein content at *Brassica napus* (var. Exagone) seedling after NaCl treatment

Data regarding the changes in dry matter and water content in *Brassica napus* (var. Exagone) seedlings after treatment with NaCl are shown in Figure 6. The saline treatment cause after 4 days a dry matter content stimulation compared with the control, ranging from 18,53 g% (control) and 21.47 g% (range 100 mm). At 7 days after treatment a decrease salt content of dry matter compared to the first interval, which is between 9,39 g% (100 mM variant) and 14,02 g% (150 mM variant). The water content in both intervals, presents an inverse variation obtained by difference to 100%.

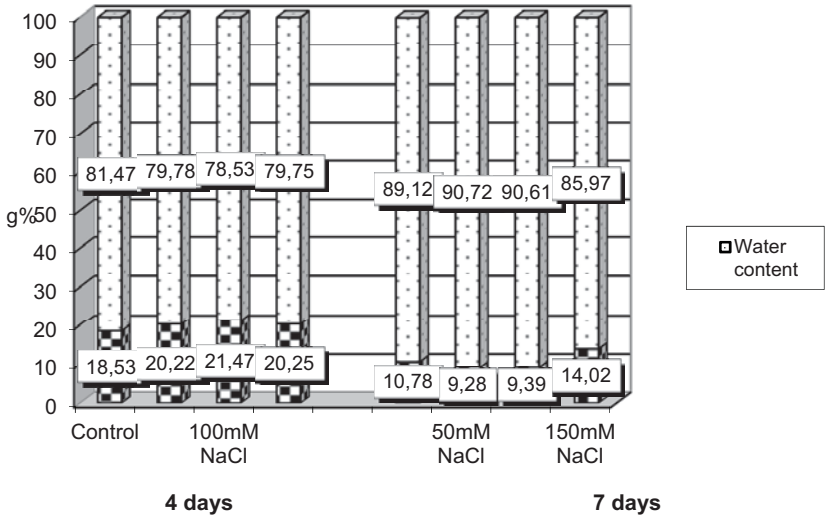
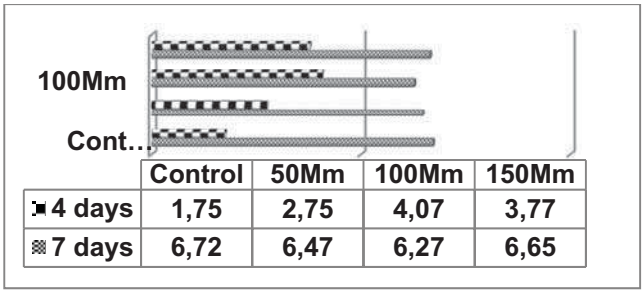


Fig. 6. The variation of dry matter and water content at *Brassica napus* (var. Exagone) seedling after NaCl treatment

There are very little data about the role of each part of plant in salinity tolerance (Khan et al. 2006). In fact, root, shoot and leaf have different responsibilities and operate particularly.

Normally, NaCl stress due to root growth inhibition (Rodríguez et al., 1997) because of direct contact with the saline agent but the metric measurements made at 4 days after saline treatment on *Brassica napus* (var. Exagone), seedlings marks a root growth stimulation comparative with control in all experimental variants, more pronounced at 100 mM variant (4.07 ± 0.33 to 1.75 ± 0.14). At 7 days after treatment the salt concentrations, plant roots have been a slow development compared to the control (Figure 7).



	Control	50Mm	100Mm	150Mm
4 days	1,75	2,75	4,07	3,77
7 days	6,72	6,47	6,27	6,65

Fig. 7. The dynamics of root development in *Brassica napus* (v. Exagone) seedlings under the influence of different concentrations of NaCl

CONCLUSIONS

In this study who evaluating the effect of NaCl stress treatment on *Brassica napus* (v. Exagone) rape seed it was found that salinity caused a stimulation of root growth at 4 days after treatment but slight inhibition at 7 days. Amylase activity and protein content were inhibited by all used salts concentrations at both time intervals studied. Salinity stimulated the acid phosphatase activity, more intense at 4 days and moderately at 7 days after treatment. The activity of two assayed antioxidant enzymes (except peroxidase in the second period) increase in salinity conditions with increasing salt concentration.

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