INFLUENCE OF SALINITY STRESS ON SEVERAL BIOCHEMICALS ATTRIBUTES OF *BRASSICA NAPUS* cv. EXGOLD SEEDLING

INFLUENȚA STRESULUI SALIN ASUPRA UNOR PARAMETRI BIOCHIMICI LA PLANTULE DE *BRASSICA NAPUS* cv. EXGOLD

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Abstract: *The present work has been performed to study the NaCl treatment effect on growth and some biochemical indices in Brassica napus seedling. For this, the rapeseed seeds were treated four hour with NaCl concentrations (50, 100, 150mM) comparatively with a control free of salt exposure. The early response of seedling under salinity levels was different at 4-day-old and 7-day-old. Amylase and phosphatase activities were influenced by NaCl concentrations. Thus, at both studied intervals, a decreasing trend of amylase activity was observed, while the phosphatase activity showed an increasing trend. The antioxidant catalase and peroxidase activities were found to be influenced by NaCl concentration especially after 7 day of treatment application.*
Keyords: *saline stress, catalase, peroxidase, hydrolase*

Rezumat: *Prezenta lucrare a fost efectuată pentru a studia efectul tratamentului cu NaCl asupra creşterii şi a unor indici biochimici în plantule de rapiță. Astfel, semințele de rapiță au fost tratate timp de patru ore, cu concentrații de NaCl (50, 100, 150 mM). În paralel a fost realizat şi un control care nu a fost expus tratamentului salin. Răspunsul timpuriu la salinitate al plantulelor a fost diferit la cele două intervale de studiu (4 şi 7 zile). Activitățile α-amilazei și fosfatazei au fost influențate de concentrațiile de NaCl. Astfel, la ambele intervale studiate, există o tendință de diminuare a activității amilazei, în timp ce activitatea fosfatazei a indicat o tendință de creștere. Activitatea enzimelor antioxidante, catalaza și peroxidaza, a fost influențată, în special, la sapte zile, de la aplicarea tratamentului.*
Cuvinte cheie: *stres salin, catalază, peroxidază, hidrolaze*

INTRODUCTION

Brassica napus L. belonging to *Brassicaceae* family is one of the most cultivated plants in Middle Asia, North Africa and West Europe (Saeidnia and Gohari, 2012). Rapeseed is now the third most important source of edible oil in the world after soybean and palm oil (El-Beltagi and Mohamed, 2010). Beside that it is an important source of edible oil, the by-product provide from production of rapeseed oil is a high-protein animal feed. As one kind of the most important oilseed crops all over the world, *B. napus* L. are very sensitive to salt stress throughout the growth and development cycle. Thus, salinity stress affects plant growth, as well as, seed germination, seedling growth and vigor, flowering and fruit set (Sairam and Tyagi, 2004). Plants have evolved efficient antioxidant systems

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that can protect them from the damaging effects of biotic and abiotic stress. These protection mechanisms, enzymatic and non-enzymatic, remove reactive oxygen species (ROS) formed after stress because they are highly toxic. The most important enzymes to remove ROS are superoxide dismutase, peroxidase and catalase (Asada, 1999).

The aim of this study was to determine the early morphological and biochemical responses of *Brassica napus* seedling at NaCl treatment, to improve knowledge of rapeseed crop growth under conditions of salinity.

MATERIAL AND METHOD

Brassica napus cv Exgold seeds were provided by Territorial Institute for Quality Seeds and Planting Material, Iasi. The seeds were sterilized in 3% H_2O_2 solution for ten minutes followed by three rinses with distilled water and then treated with 50 mM, 100mM and 150mM NaCl for four hours. Control seeds were stored for four hours in distilled water. After sterilization, 100 seeds were transferred into Petri dishes on filter paper and then were wetted with 7 ml distilled water (control) or saline solutions. The Petri plates were transferred in a growth chamber and watered with saline solution (at variants) and water (at control) every two days.

Plant roots growth was evaluated as the elongation of root length young seedling at four and seven days old. Catalase activity was determined according to Sinha method based on reduction of dichromate, acetic acid mixture to chromic acetate when heated in the presence of hydrogen peroxide (Artenie et sal. 2008). Determination of peroxidase activity is based on the measurement of color intensity of o-dianisidine oxidation product with hydrogen peroxide in enzymes presence (Artenie et al., 2008). Protein content was determined according to Bradford method and all enzymes activities were reported as U/mg protein (Bradford, 1976). Acid phosphatase activity determination is based on ability of β-glycerophosphate disodium hydrolysis and dosing then phosphorus resulted (Artenie et al. 2008). The α-amylase activity was determined using the Noelting-Bernfeld method (Artenie at al., 2008).

RESULTS AND DISCUSSIONS

Plant growth and development are adversely affected by salinity - a major environmental stress that limits agricultural production. As different response of plant to salinity, Munns developed the concept “two-phase growth response to salinity”. Thus, the first phase of growth reduction happens quickly (within minutes) after exposure to salinity and is due to the osmotic changes outside the root. Several minutes after the initial decrease in leaf growth, there is a gradual recovery of the growth rate until a new steady state is reached, dependent upon the salt concentration outside the root. The second much slower effect, taking days, weeks or months is the result of salt accumulation in leaves, leading to salt toxicity in the plant, primarily in the older leaves (Munns, 2002).

In our experiment salinity affected the germination rate of rapeseed seedling at both interval studied. Thus, after four days of saline stress, the root length of seedling ranged between 1,97 cm (at 150 mM) and 4,77 cm in control (Table 1). At seven days it was observed the same effect of root inhibition with one exception, concentration 100 mM where the root length was greater then control (12,12 cm comparatively with 11,62cm). In general, salt stress affected negatively the plant root length, but the different effect depending on the concentration of NaCl, the age of seedling and the duration of treatment.

Table 1.

Effect of salt stress on growth root in rapeseed seedling. Means of ten replicates ± S.x. are shown

mM NaCl	Root length (cm)			
	4 days		7 days	
	x±Sx	STDEV	x±Sx	STDEV
0	4,77±0,13	0,26	11,62±1,79	3,58
50	3,47±0,33	0,67	8,1±0,05	0,11
100	4,05±0,36	0,71	12,12±0,71	1,43
150	1,97±0,24	0,49	9,9±0,9	1,80

Alpha amylase has an active role in the hydrolysis of starch just before a seed germinates (Ashraf et al., 2002). Salinity reduced the percentage of seed germination, seedling vigor index, α-amylase activity of canola seedling (Farhoudi, 2012). Another study indicated that salinity induced changes in α-amylase activity in three cotton cultivar during germination and early seedling growth. Thus, it was observed that the increase in NaCl concentration leads to decrease in α-amylase activity (Ashraf et al., 2002).

In our experiment, after treatment with NaCl the α-amylase activity at rapeseed seedling decrease comparatively with control at both intervals studied, more pronounced in 4-day-old seedling (Fig. 1). The same result was founded by Sangeetha, 2013, who studied the effect of salinity on α-amylase activity in corn germinating seeds.

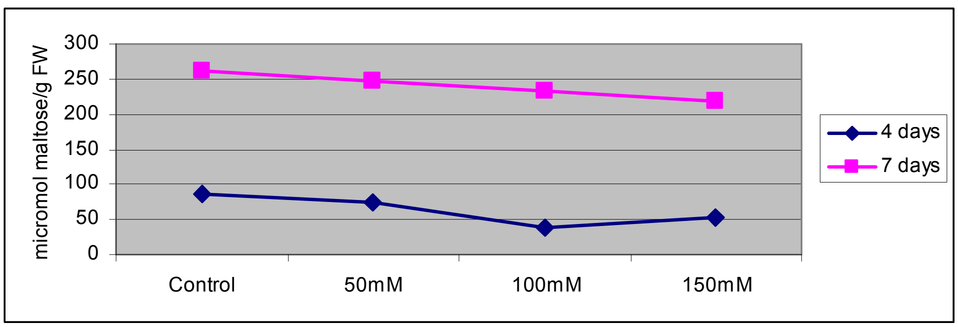

Fig. 1 - Variation of α–amylase activity at rapeseed seedling under NaCl stress

Activities of acid and alkaline phosphatases enzymes show significant alteration in plants exposed to abiotic stressful conditions such as soil salinity (Ehsanpour and Amini, 2003). Salt stress found to have profound effects on various hydrolases. Thus, Singh and Ramasare, (2009) reported a decreasing of several hydrolases activities, gradually with increasing salt stress in groundnut. Under salinity stress the decrease in acid phosphatase activity may imply fewer amounts of free PO_4^{-3} ions in cell wall. On the other hand, effect of salinity stress on canola caused an increase in both acid and alkaline phosphatase activities (Bybordi and Ebrahimian, 2011). Acid phosphatase hydrolyzes phosphatidyl groups into free fatty acids and soluble phosphorus in plant cells. In *B. napus* cv. Exgold seedling the early phase of saline stress (four days) the acid phosphatase activity decrease with the augmentation of NaCl stress (Fig. 2). The stimulant effect of salinity was in accordance with other results using *B. napus* cv. Exagone, where at seven days, the acid phosphatase activity increase with arise of NaCl stress (Oprica et al., 2011).

Plants exposed to various abiotic stresses, like salinity, initiate the cascade of changes starting with imbalanced water and nutrient uptake, stomatal closure, altered gaseous exchange, improper functioning of photosynthetic systems due to over-reduction of electron transport chains in chloroplast and mitocondria finishing with generation of ROS. The first line of cells defense who converting $O_2\bullet^-$ to H_2O_2 is superoxide dismutase. Therefore, it is important that H_2O_2 be scavenged rapidly by the antioxidative defence system to water and oxygen, this can be performed by catalase (CAT) and peroxidase (POD) enzymes (Guo et al., 2006). CAT is important in removal of H_2O_2 generated in peroxisomes by oxidases involved in β-oxidation of fatty acids, photorespiration and purine catabolism. CAT is known to have low affinity to H_2O_2 than POD, wich suggest that it is involved in mass scavenging H_2O_2, whereas POD is suggest to be involved in fine regulation of H_2O_2 (Sharma et al., 2012).

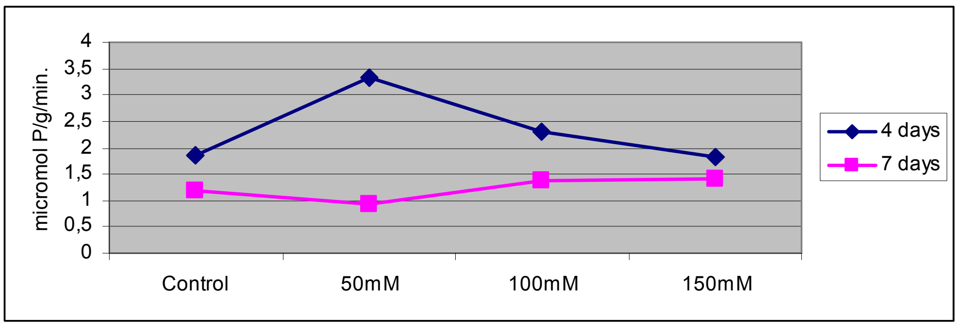

Fig. 2 - Acide phosphatase activity at rapeseed seedling under NaCl stress

The increase of catalase activity under abiotic stress has been observed in *Calendula officinalis* and *Lycopersicon esculentum* seedlings (Chaparzadeh et al., 2004) as soon as, *Cicer arietinum* leaves (Eyidogan and Oz, 2007) and roots of *C. arietinum* (Kukreja et al., 2005).

Our results indicated that in *Brassica napus* cv. Exgold seedling, catalase activity decreased in all NaCl concentrations at four days after saline treatment. At seven days old seedling, as salt stress was maintained, this enzyme activity increased and a significant increase was observed at 150 mM (Fig. 3). The decrease in catalase activity by salt stress is a phenomenon that occurs in many plant species, not only in the gramineous like rice and wheat (Erdal et al., 2011) but in pea plants (Sandalio et al., 2001) and in some rapeseeds seedling cultivar leaves (Zare and Pakniyat, 2012).

The effect of salinity on POD activity of *Brassica napus* cv. Exgold seedling was different after four and seven days exposure of salinity. The increased levels of POD and CAT activities in 7-days-old seedling were observed at 150 mM, which may result from the protective function of this enzyme (Fig. 4).

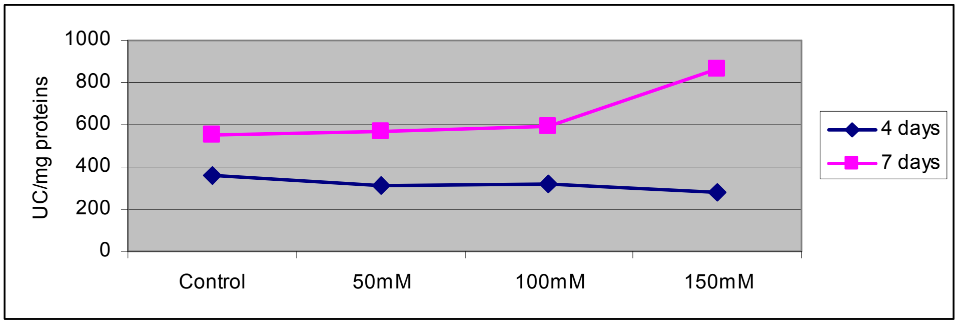

Fig. 3 - Catalase activity at rapeseed seedling under NaCl stress

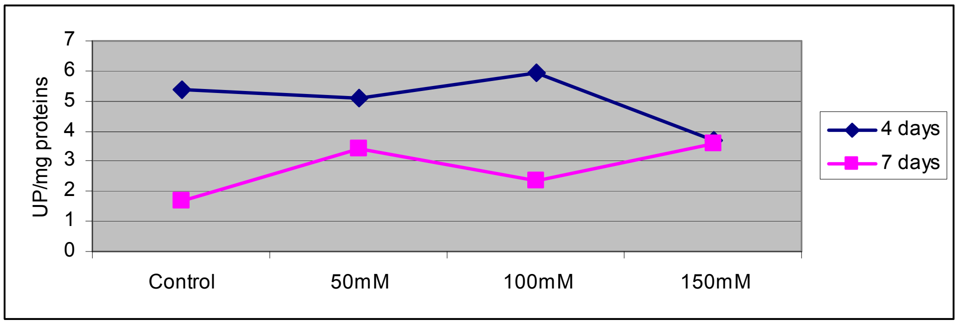

Fig. 4 - Peroxidase activity at rapeseed seedling under NaCl stress

CONCLUSIONS

1. The impact of salinity stress on physiological and biochemical indices of *Brassica napus* cv. Exgold seedling varied depending on the concentration of NaCl, the age of seedling and the treatment duration.

2. Salt stress was found to affect negatively the root length at both intervals studied. Hydrolytic enzymes have differently responded at saline stress at both ages of rapeseed seedling, being a reduced tendency of α-amylase activity but an increasing trend of acid phosphatase activity.

3. In four day after saline treatment the enzymes catalase and peroxidase activity, have registered a decline. As the abiotic factor was maintained the enzymes activities was intensified at seven day comparatively with the control.

REFERENCES

1. **Artenie V., Ungureanu E., Negura Anca Mihaela, 2008 -** *Metode de investigare a metabolismului glucidic si lipidic*, Ed. Pim.
2. **Asada K., 1999 -** *The water–water cycle in chloroplasts: scavenging of active oxygens and dissipation of excess photons*, Annu. Rev. Plant Physiol. Plant Mol. Biol. 50, 601–639.

3. Ashraf M.Y., Sarwar G., Ashraf M., Afaf R., Sattar A., 2002 - *Salinity induced changes in α-amylase activity during germination and early cotton seedling growth*, Biologia Plantarum 45 (4):589-591.
4. Bradford M, 1976 - *A rapid and sensitive method for microgram quantities of protein utilizing the principle of protein-dye binding*. Anal Biochem. 7(72):248–254.
5. Bybordi A., Elnaz E., 2011 - *Effect of Salinity Stress on Activity of Enzymes Involved in Nitrogen and Phosphorous Metabolism Case Study: Canola (Brassica napus L.),* Asian Journal of Agricultural Research, 5(3), 208-214.
6. Chaparzadeh, N., Amico, R.A., Khavari, Najad, R., Navarizzo, F., 2004 **-** *Antioxidative responses of Calendula officinalis under salinity conditions,* Plant Physiol. Biochem., 42, 695-701.
7. Ehsanpour AA, Amini F., 2003 - *Effect of salt and drought stress on acid phosphatase activities in alfalfa (Medicago sativa L.) explants under in vitro culture*. Afr. J. Biotechnol. 2 (5), 133-135.
8. El-Beltagi H.S., Mohamed A.A., 2010 - *Variations in fatty acid composition, glucosinolate profile and some phytochemical contents in selected oil seed rapeseed (Brassica napus L.) cultivars*. Fats Oil, 61(2): 143-150.
9. Erdal S., Aydın M., Genisel M., Taspınar MS., Dumlupinar R. Kaya O., Gorcek Z., 2011 - *Effects of salicylic acid on wheat salt sensitivity,* African Journal of Biotechnology 10 (30), 5713-5718.
10. Eyidogan F., Oz M.T., 2007 - *Effect of salinity on antioxidant responses of chickpea seedlings,* Acta Physiol. Plant. 29, 485-493.
11. ***Farhoudi R., 2012*** **-** *Effect of salinity stress on α-amylase activity, cell membrane leakage and seedling growth of canola cultivars*, J. of Plant Process and Function, 1(1),13-24.
12. Guo Z., Ou W., Lu S., Zhong Q., 2006 - *Differential responses of antioxidative system to chilling and drought in four rice cultivars differing in sensitivity.* Plant Physiology and Biochemistry, 44: 828–836.
13. Kukreja S., A.S. Nandval, N. Kumar, S.K. Sharma, S.K. Sharma, V. Unvi, P.K. Sharma, 2005 - *Plant water status, H_2O_2 scavenging enzymes, ethylene evolution and membrane integrity of Cicer arietinum roots as affected by salinity,* Biol. Plant. 49, 305-308.
14. Munns, R., 2002 **-** *Comparative physiology of salt and water stress*. Plant Cell Environ. 25:239–250.
15. Oprică Lăcrămioara, Zenovia Olteanu, Elena Truță, Gabriela Vochița, 2011 - *Early biochemical responses of Brasica napus cv. Exagone seed germination at salt treatment*, An. Şt. Univ. Al. I. Cuza, Secț. Genetică si Biologie Moleculară, XII (4), 95-103.
16. Saeidnia S., Gohari A. R., 2012 - *Importance of Brassica napus as a medicinal food plant,* Journal of Medicinal Plants Research, 6(14), pp. 2700-2703.
17. Sairam R.K., Tyagi A., 2004 - *Physiology and molecular biology of salinity stress tolerance in plants.* Curr. Sci., 86: 407–421.
18. Sandalio L.M., Dalurzo H.C., Gomez M., Romero-Puetras M.C., del Rio L.A., 2001 - *Cadmium-induced changes in the growth and oxidative metabolism of pea plants.* Experiment. Bot., 364: 2115-2126.
19. Sangeetha. R, 2013 - *Effect of salinity induced stress and its alleviation on the activity of amylase in the germinating seeds of Zea mays*, International Journal of Basic and Life Science, 1 (1), 1-9.
20. Sharma P., Ambuj Bhushan Jha, Rama Shanker Dubey, Pessarakli M., 2012 - *Reactive oxygen species, oxidative damage, and antioxidative defense mechanism in plants under stressful conditions*, Journal of Botany, 1-26.
21. Singh A., Ramasare Prased, 2009 - *Salt stress effects growth and cell wall bound enzymes in Arachis hypogaea L. seedlings*, International Journal of Integrative Biology, 7 (2), 117-123.