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Effect of Salinity Stress on Activity of Enzymes Involved in Nitrogen and Phosphorous Metabolism Case Study: Canola (*Brassica napus* L.)

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ABSTRACT

Plant exposed to salinity stress exhibit changes in their physiology and metabolism. In general salinity reduces water availability and causes nutritional imbalance in plants. Since there is no enough information about effect of salinity stress on activity of enzymes involved in nitrogen and phosphorous metabolism we studied the effects of different levels of salinity (0, 50, 100, 150 and 200 mM NaCl) on nitrate reductase, acid phosphatase and alkaline phosphatase activity as well as nitrate and phosphate uptake and total nitrogen content in leaves of canola, so a hydroponically experiment was conducted. Canola seeds were sown in an experimental green house under non-saline conditions. After two month, healthy plants were selected for hydroponic culture in Hoagland's nutrient solution supplemented with NaCl. The plants were cultured in plastic pots filled up with perlite and then were put in green house. The plants were watered with fresh nutrient solution made every week. Leaf samples were clipped 0, 7, 14, 21, 30 and 45 days after exposure to salinity stress in order to nitrate reductase, acid phosphatase and alkaline phosphatase assay, measurement of nitrate uptake, phosphate uptake and total nitrogen content. The results showed that nitrate reductase activity was increased in treated plants with 100 mM NaCl, in contrast it was decreased gradually in plants that were exposure to 150 and 200 mM NaCl, in comparison with controls. Decrease of nitrate reductase activity due to salinity stress and nitrate uptake and follow them total nitrogen content were occurred at the same time. Reduction in nitrate reductase activity, nitrate content and total nitrogen content because of high salinity levels may be a physiological response in order to decrease in growth and extra biomass in canola. However, it was observed that salinity stress caused an increase in both acid phosphatase and alkaline phosphatase activity. We monitored phosphate levels in leaves and found that phosphate levels decreased significantly under salinity stress. These results suggest that the induction of acid phosphatase and alkaline phosphatase under salt stress may be due to a phosphorous deficiency.

Key words: Canola, acid phosphatase, nitrate reductase, salt stress, enzyme activity, nitrogen metabolism

INTRODUCTION

Salinity is one of the major abiotic stresses affecting plant productivity. Salinity decreases plant dry matter and leaf area (Amirjani, 2011) and finally decreases crop yield. Also it has reported that

salinity changes plant morphological traits (Zadeh and Naeini, 2007). Plants exposed to salt stress undergo changes in their metabolism with the changes taking place in their environment. In addition gene manipulation can improve salt stress tolerance by gene coding of related enzymes (Joseph and Jini, 2011). Nitrate reductase (NR, E.C.1.6.6.1), the first enzyme in the nitrate assimilation pathway, is a limiting factor of plant growth and development (Skriver and Mundy, 1990) also it is influenced by a variety of environmental factors (Solomonson and Barber, 1990) such as heavy metal like lead (Sengar *et al*., 2008). Many reports on the effects of salinity on nitrate reductase activity in different plants have frequently been contradictory. It has been reported that, NR activity increased with exogenous nitrate concentration (Jha *et al*., 2007). Bourgeais-Chaillou *et al*. (1992) reported that *in-vitro* conditions, NR activity in roots of soybean was higher than control plant as result of salt stress while leaf NR activity remained constant. Acid phosphatase (ACP, E.C 3.1.3.2) and alkaline phosphatase (ALP, E.C 3.1.3.1) are widely distributed in plant (Ourry *et al*., 1992). Alkaline phosphatase activity increases under conditions of salt, drought and osmotic stress. Hussain and Rai (1991) have been reported the depression of ALP activity under cadmium stress.

Athar *et al*. (2009) canola (*Brassica napus* L.) grown mainly for edible oil purpose is a moderately salt tolerant crop. Canola oil is the term given to the oil obtained from the seeds of several species of *Brassica* genus (Sakr and Arafa, 2009). In view of Bybordi *et al*. (2010a, b) increasing awareness of the health advantage of canola oil and existing salt tolerance potential, its demand has undoubtedly increased during the last two decades.

This has resulted into increased cultivation of canola on soils where salinity problems already exist. Thus, there is a need for further improvement in the salt tolerance of canola. Bybordi *et al*. (2010c) during salt stress some plants may have a better protection against reactive oxygen species, at least in part, by increasing the activity of antioxidant enzymes under salt stress.

In this study, we made an attempt to investigate the effect of NaCl stress on nitrate reductase activity and some physiological and other related enzymes in nitrogen and phosphorous metabolism in canola plants.

MATERIALS AND METHODS

In order to study the effects of salt stress on nitrogen and phosphorous metabolism in canola (*Brassica napus* L. CV SLM_{046}) an experiment was conducted in Baku State University, Faculty of Biology in 2009. Seedlings were raised in an experimental green house under non-saline conditions. After two month, healthy and uniform plants were selected for hydroponic culture in Hoagland's nutrient solution supplemented with NaCl (0, 50, 100, 150 and 200 mM). The plants were cultured in plastic pots filled up with perlite and then were put in green house. The greenhouse was under natural sunlight and temperature was set up to 25°C 3 and 75% relative humidity. The plants were watered with fresh nutrient solution made every week. The pair of leaves from the apex of the growing shoots was harvested 0, 7, 14, 21, 30 and 45 days after exposure to salinity stress in order to nitrate reductase, acid phosphatase and alkaline phosphatase assay, measurement of nitrate uptake, phosphate uptake and total nitrogen content. During each sampling, a new set of plants was used. Nitrate reductase was assayed based on the methods of Sagi *et al*. (1997). Protein concentration in the enzyme extract was determined following the procedure of Bradford (1976) using Bovine Serum Albumin (BSA) as a standard. Total nitrogen

contents in leaves of control and stressed plants were determined following the colorimetric procedure of Baethgen and Alley (1989).

Assay of acid phosphatase: Activity of ACP (EC, 3.1.3.2) was determined as described by Joyce and Grisolia (1960).

Assay of alkaline phosphatase: The activity of ALP (E.C.3.1.3.1) was determined following the procedure of Ihlenfeldt and Gibson (1975).

One unit of ACP or ALP is the number of enzyme required to liberate 1 μ Mole of P-nitro phenol per min. Activity staining of an acid phosphatase native polyaeryl amide gel electrophoresis was performed following the method of Laemmli (1970).

Measurement of phosphate level: Phosphate uptake in control and salinity stressed leaves was measured as method of Ames method (Ames, 1966).

Statistical analysis: Statistical analysis of the results was carried out according to Duncan's Multiple Range Tests. Data were subjected to a two-way ANOVA following the method of Sokal and Rohlf (1995).

RESULTS AND DISCUSSION

Nitrate reductase activity was increased significantly in those plants were subjected to 100 mM NaCl, while it was decreased gradually under 150 and 200 mM treatments compared to control treatment (Table 1). After 45 days of exposure, NR activity decreased in 150 and 200 mM NaCl treated plants, respectively, as compared to control treatment. Decreasing of NR activity due to salinity stress was also accompanied with significant decrease in nitrate uptake and total nitrogen content. According to Table 1, both nitrate and total nitrogen content as well as NR activity were increased because of 100 mM salinity stress in treated plants and decreased in 150 and 200 mM treatments in comparison with control treatment, moreover, total nitrogen content dramatically increased in 100 mM treatment and decreased in 150 and 200 mM, respectively, in comparison with control during the 45 days period of experiment (Table 1). Similarly, in comparison with control treatment, nitrate content increased in treated plants with 50 mM and decreased in treated plants with 150 and 200 mM NaCl, respectively (Table 1). Effects of NaCl on ACP and ALP activity and phosphate uptake in canola leaves through the duration of treatment are shown in Table 2. Forty five days after salinity induction, NaCl caused a significant increase in ACP activity in treated plants with 150 and 200 mM NaCl in comparison with control treatment. It's worth mentioning that, there were no significant changes in ACP activity in treated plants with 50 and 100 mM NaCl. In contrast, ALP activity was increased in treated plant with 50, 100, 150 and 200 mM NaCl in comparison with control (Table 2).

The results in Table 1 show a concentration-dependant decrease in NR activity, nitrate and nitrogen content. Lacuesta *et al.* (1990) discussed that as NR is a substrate inducible enzyme, it's decreased by some researchers to decreased nitrate uptake by plant under salt stress. The decrease of nitrate uptake is accompanied with a high Cl^- uptake and translocation to the shoot (Flores *et al.*, 2000). Since presence of Cl- into the soil inhibits uptake, NR activity would be affected by flux into the metabolic pools (Abd-El-Baki *et al.*, 2000). We observed that there was no

Table 1: Effects of different levels of salinity stress on NR activity, nitrate uptake and total nitrogen levels in leaves of canola as a function of days of NaCl treatment

Duration of NaCl (day)	Salinity levels (NaCl mM)	NR, Umg/Protein	Nitrate M mol/g fwt	Total nitrogen mg g^{-1} fwt
0	0	0.30^{ab}	2.80^{ab}	2.50^{ab}
	50	0.28^{b}	2.70^{ab}	2.40^{ab}
	100	0.26^{b}	2.60^{ab}	2.45^{ab}
	150	0.29^{b}	2.59^{ab}	2.44^{ab}
	200	0.30^{ab}	2.56^{b}	2.43^{b}
7	0	0.29^{bc}	2.75^{ab}	2.48^{ab}
	50	0.33^{b}	2.96^{a}	2.66^{ab}
	100	0.38^{a}	2.90^{a}	2.67^{a}
	150	0.25^{c}	2.66^{ab}	2.36^{b}
	200	0.24^{c}	2.52^{b}	2.33^{b}
14	0	0.30^{b}	2.77^{ab}	2.49^{ab}
	50	0.36^{ab}	2.98^{a}	2.77^{a}
	100	0.38^{a}	2.97^{a}	2.76^{a}
	150	0.27^{c}	2.60^{ab}	2.30^{b}
	200	0.25^{c}	2.56^{b}	2.26^{b}
21	0	0.29^{b}	2.75^{ab}	2.48^{ab}
	50	0.32^{ab}	2.99^{a}	2.79^{a}
	100	0.38^{a}	2.97^{a}	2.78^{a}
	150	0.21^{c}	2.46^{b}	2.26^{b}
	200	0.20^{c}	2.39^{b}	2.22^{b}
30	0	0.28^{b}	2.77^{ab}	2.46^{ab}
	50	0.37^{ab}	2.98^{a}	2.82^{a}
	100	0.41^{a}	2.94^{a}	2.80^{a}
	150	0.20^{d}	2.32^{b}	2.25^{b}
	200	0.18^{d}	2.22^{b}	2.21^{b}
45	0	0.28^{b}	2.76^{ab}	2.45^{ab}
	50	0.39^{ab}	2.99^{a}	2.86^{a}
	100	0.42^{a}	2.98^{a}	2.85^{a}
	150	0.18^{c}	2.30^{b}	1.90^{c}
	200	0.15^{c}	2.12^{c}	1.20^{d}

Values in columns with the same letter are no significantly different $p<0.05$

significant differences between the two cases on NR activity; these observations suggest that decreasing of NR activity is not due to direct inhibitory of salinity stress and it may be due to a reduction in nitrate uptake in these plants.

Flores *et al.* (2000) reported that salinity inhibits nitrate uptake and NR activity in leaves of Zea mays. Higher levels of total nitrogen in those plants which were treated with 50 mM may be a natural response for favorable growth of the plants at this concentration, similar these results have been reported earlier. The results appeared in Table 2 show an increase in both ACP and ALP activity due to increase of salinity level also a noticeable change in phosphorus level was observed in stressed plants. Abd-El-Baki *et al.* (2000) has been reported that activity of ACP and ALP increased in plants were grown under conditions of phosphorus deficiency.

Table 2: The Effects of different salinity levels on activity of ACP, ALP, and phosphate levels in leaves of canola measured as a function of days of NaCl treatment

Duration of NaCl (day)	Salinity levels (NaCl mM)	ACP, Umg/Protein	ALP, Umg/Protein	Phosphate M mol/g fw
0	0	1.81^{c}	0.80^{b}	235^{a}
	50	1.82^{c}	0.81^{b}	236^{a}
	100	1.83^{c}	0.82^{b}	237^{a}
	150	1.82^{c}	0.83^{b}	238^{a}
	200	1.79^{c}	0.83^{b}	239^{a}
7	0	1.82^{c}	0.82^{b}	230^{ab}
	50	1.83^{c}	0.83^{b}	240^{a}
	100	1.85^{c}	0.84^{b}	228^{ab}
	150	2.01^{bc}	0.95^{ab}	220^{ab}
	200	2.05^{bc}	0.99^{ab}	218^{ab}
14	0	1.85^{c}	0.85^{b}	233^{ab}
	50	1.86^{c}	0.86^{b}	230^{ab}
	100	1.87^{c}	0.87^{b}	228^{ab}
	150	2.22^{bc}	1.10^{ab}	210^{b}
	200	2.53^{b}	1.12^{ab}	200^{b}
21	0	1.84^{c}	0.86^{b}	230^{ab}
	50	1.85^{c}	0.87^{b}	228^{ab}
	100	1.86^{c}	0.88^{b}	226^{ab}
	150	2.18^{bc}	1.15^{ab}	200^{b}
	200	2.21^{bc}	1.18^{ab}	196^{bc}
30	0	1.83^{c}	0.86^{b}	228^{ab}
	50	1.85^{c}	0.87^{b}	226^{ab}
	100	1.86^{c}	0.88^{b}	225^{ab}
	150	3.33^{b}	1.88^{ab}	196^{bc}
	200	3.54^{a}	1.20^{a}	190^{c}
45	0	1.84^{c}	0.87^{b}	227^{ab}
	50	1.85^{c}	0.88^{b}	226^{ab}
	100	1.86^{c}	0.89^{b}	210^{b}
	150	3.35^{ab}	1.22^{a}	195^{bc}
	200	4.68^{a}	1.44^{a}	190^{c}

Values in columns with the same letter are no significantly different $p<0.05$

CONCLUSION

In conclusion, present results suggest that high level of salinity causes a depression in NR activity, nitrate uptake and total nitrogen content in canola leaves. In addition, salinity leads to significant decrease in phosphate level, thus, salinity induces increase in activity of both ACP and ALP, and it is might be due to inhibition of phosphate uptake. In addition we conclude that uptake is inhibited by high salinity level in canola and also at low availability of nitrate, activity of NR will decrease. The low level of nitrogen in this plant under conditions of high level of salinity may affect on protect synthesis mechanism and then on growth and development (Cramer and Lips, 1995; Cram, 1973; Lefebvre *et al.*, 1990).

REFERENCES

Abd-El-Baki, G.K., F. Siefritz, H.M. Man, H. Weiner, R. Kaldenhoff and W.M. Kaiser, 2000. Nitrate reductase in *Zea mays* L. under salinity. Plant. Cell. Environ., 23: 515-521.

Ames, B.N., 1966. Assay of inorganic phosphate, total phosphate and phosphatases. Methods Enzymol., 8: 115-118.

Amirjani, M.R., 2011. Effect of salinity stress on growth, sugar content, pigments and enzyme activity of rice. Int. J. Bot., 7: 73-81.

Athar, H.U.R., M. Ashraf, A. Wahid and A. Jamil, 2009. Inducing salt tolerance in canola (*Brassica napus* L.) by exogenous application of glycinebetaine and proline. Pak. J. Bot., 41: 1311-1319.

Baethgen, W.E. and M.M. Alley, 1989. A manual colorimetric method of measuring ammonium N in soil and plant keelhaul digest. Commun. Soil. Sci., 20: 991-999.

Bourgeais-Chaillou, P., F. Perez-Alfocea and G. Guerrier, 1992. Comparative effect on N-sources on growth and physiological responses of soybean exposed to NaCl-stress. J. Exp. Bot., 254: 1225-1233.

Bradford, M.M., 1976. A rapid and sensitive method for the quantification of microgram quantities of proteins utilizing the principle of protein-dye binding. Anal. Biochem., 72: 248-254.

Bybordi, A., S.J. Tabatabaei and A. Ahmadev, 2010a. Effect of salinity on the growth and peroxidase and IAA oxidase activities in canola. J. Food Agric. Environ., 8: 109-112.

Bybordi, A., S.J. Tabatabaei and A. Ahmadev, 2010b. Effects of salinity on fatty acid composition of canola (*Brassica napus* L.). J. Food Agric. Environ., 8: 113-115.

Bybordi, A., S.J. Tabatabaei and A. Ahmadev, 2010c. The influence of salinity stress on antioxidant activity in canola cultivars(*Brassica napus* L.). J. Food Agric. Environ., 8: 122-127.

Cram, W.J., 1973. Internal factors regulating nitrate and chloride influx in plant cells. J. Exp. Bot., 75: 328-341.

Cramer, M.D. and S.H. Lips, 1995. Enriched rhizosphere concentration canamelio rate the influence of salinity on hydroponically grown tomato plants. Physiol. Plant., 94: 425-433.

Flores, P., M.A. Botella, V. Martinez and A. Verda, 2000. Ionic and osmotic effects on nitrate reductase activity in tomato seedlings. J. Plant Physiol., 156: 552-557.

Hussain, Y. and L.C. Rai, 1991. Studies on nitrogen and phosphorous metabolism and the photosynthetic electron transport system of Nostoc linckia u nder cadmium stress. J. Plant Physiol., 138: 429-435.

Ihlenfeldt, M.J.A. and J. Gibson, 1975. Phosphate utilization and alkaline phosphatase activity in anacystis nidulans (*Synechococcus*). Arch Microbiol., 102: 23-28.

Jha, P., A. Ali and N. Raghuram, 2007. Nitrate-induction of nitrate reductase and its inhibition by nitrite and ammonium ions in *Spirulina platensis*. Physiol. Mol. Biol. Plants, 13: 163-167.

Joseph, B. and D. Jini, 2011. Development of salt stress-tolerant plants by gene manipulation of antioxidant enzymes. Asian J. Agric. Res., 5: 17-27.

Joyce, B.K. and S. Grisolia, 1960. Purification and properties of a nonspecific acid phosphatase from wheat germ. J. Biol. Chem., 235: 2278-2281.

Lacuesta, M., B Gonzalez-Moro, C. Gonzale-Murua and A. Munoz-Rueda, 1990. Temporal study of the effect of phosphinothricin on the activity of glutamine synthetase glutamate dehydrogenase and nitrate reductase in *Medicago sativa* L. J. Plant. Physiol., 136: 410-414.

Laemmli, U.K., 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T_4. Nature, 227: 680-685.

Lefebvre, D.D., S.M.G. Duff, C. Fife, O.C. Julien-Inalsingh and W.C. Plaxton, 1990. Response to phosphate deprivation in *Brassica nigra* suspension cell, enhancement of intracellular cell surface and secreted in Pi-absorption rate. Plant Physiol., 93: 504-511.

Ourry, A., S. Mesle and J. Boucaud, 1992. Effects of osmotic stress on nitrate uptake, translocation, storage a reduction in ryegrass (*Lolium perenne* L.). New Phytol., 120: 275-280.

Sagi, M., N.A. Savidov, N.P. lvov and S.H. Lips, 1997. Nitrate reductase and molybdenum cofactor as affected by salinity and nitrogen source. Physiol. Plant., 99: 546-553.

Sakr, M.T. and A.A. Arafa, 2009. Effect of some antioxidants on canola plants grown under soil salt stress condition. Pak. J. Biol. Sci., 12: 582-588.

Sengar, R.S., M. Gautam, S.K. Garg, R. Chaudhary and K. Sengar, 2008. Effect of lead on seed germination, seedling growth, chlorophyll content and nitrate reductase activity in mung bean (*Vigna radiata*). Res. J. Phytochem., 2: 61-68.

Skriver, K. and J. Mundy, 1990. Gene expression in response to abscisic acid and osmotic stress. Plant Cell, 2: 503-512.

Sokal, R.R. and F.J. Rohlf, 1995. Biometry: The Principles and Practice of Statistics in Biological Research. W.H. Freeman and Company, New York, USA., pp: 271-356.

Solomonson, L.P. and M.J. Barber, 1990. Assimilatory nitrate reductase: Functional properties and regulation. Annu. Rev. Plant Physiol., 41: 225-253.

Zadeh, H.M. and M.B. Naeini, 2007. Effects of salinity stress on the morphology and yield of two cultivars of canola (*Brassica napus* L.). J. Agron., 6: 409-414.