WFL Publisher
Science and Technology

Meri-Rastilantie 3 B, FI-00980
Helsinki, Finland
e-mail: info@world-food.net

Journal of Food, Agriculture & Environment Vol.8 (2): 350-353. 2010

www.world-food.net

Effect of salinity and N sources on the activity of antioxidant enzymes in canola (*Brassica napus* L.)

Ahmad Bybordi

Faculty of Biology, Baku State University, 23 Z. Khalilov Street, 370148 Baku, Azerbaijan. e-mail: abaybordy@yahoo.com

Received 6 February 2010, accepted 12 April 2010.

Abstract

The effects of NaCl salinity (0 and 200 mM) and NH_4:NO_3 ratio (0:100, 25:75, 50:50 and 75:25) on the activities of antioxidant enzymes, viz. glutathione reductase (GR), superoxide dismutase (SOD), peroxidase (POD), catalase (CAT) and glutathione-S-transferase (GST), were investigated in leaves and roots of canola (*Brassica napus* L.). Salinity caused a reduction in plant biomass. The biomass production of plants at 75:25 NH_4:NO_3 was lower than that of plants at 0:100 NH_4:NO_3. The antioxidant enzymes exhibited higher activity in saline-treated plants. Changes in antioxidant enzyme activity caused by different NH_4:NO_3 ratios differed in leaves as well as in roots of canola. At 75:25 and 50:50 NH_4:NO_3 ratios plants showed higher CAT, GR and GST activity in leaves and roots, while POD and SOD activity was higher at 0:100 NH_4:NO_3.

Key words: Antioxidant enzymes, salt stress, NH_4: NO_3 ratio, canola.

Introduction

Salinity in the soil presents a stress condition for the growth of the plants. Under natural conditions of growth and development, plants are inevitably exposed to different types of stress, which may cause increased production of active oxygen species (AOS) [1]. These include superoxide radicals (O_2^{-o}), single oxygen (O_2), hydrogen peroxide (H_2O_2) and hydroxyl radical (OH^0), which cause tissue injury [2]. These compounds are strong oxidizing agents, and their possible accumulation in the cell is very dangerous since they damage the structure of membranes, proteins and DNA [3]. In a normally operating cell, there exists a dynamic equilibrium between the formation of ROS and their elimination. The capacity of the antioxidant defense system is often increased under stress condition [4]. In addition to ionic unbalance and hyperosmotic stress, high concentrations of NaCl induce oxidative stress, which is accompanied by the destruction of membranes and degradation of chlorophyll [5, 6]. The extent of the oxidative stress is usually assessed by the accumulation of malondialdehyde (MDA), a product of peroxide oxidation of membrane lipids [7-9, 11]. Catalase, which is located in peroxisomes, glyoxysomes and mitochondria, and is apparently absent in the chloroplasts, dismutates mostly photorespiratory/respiratory H_2O_2 into water and molecular O_2 [12, 13] whereas POD decomposes H_2O_2 by oxidation of co-substrates such as phenolic compounds and antioxidants. In biological systems, reduced glutathione (GSH) appears to be one of the most important antioxidants [3]. Salt stress can induce conditions of oxidative stress [14]. Changes in the activity of antioxidant enzymes in response to salinity [13, 15] are different in tolerant and sensitive cultivars [6, 10]. Recently, it has been suggested that the salt-tolerant cotton cultivar may exhibit better protection against AOS by increasing the activity of antioxidant enzymes under salt stress [6]. Many researchers have studied the combined effect of salinity and the nitrogen source added to the nutrient solution on productivity, photosynthesis and nitrogen metabolism [16, 17]. Recently, the effect of NO_3^- and NH_4^+ ions, and the combined effect of salinity with different N sources on certain biochemical adjustments to stress have been reported [18]. Results from these studies have suggested that plants grown with NH_4^+ as N source operate metabolic pathways, which generate more oxygen radicals [19, 20]. Recent studies have shown that ammonium and salinity increase the activity of the Mo-enzymes aldehyde oxidase and xanthine dehydrogenase, which take part in the early response of plant adaptation to stress [18]. Antioxidant enzymes play important roles in adaptation to stress condition. Consequently, the aim of this study was to examine whether these conditions affect the antioxidant defense system. In this investigation, the effect of salinity with a combination of different nitrogen (NO_3^- and NH_4^+ ratio) sources on the canola (*Brassica napus* L.) was examined. The developmental profiles of the antioxidative enzymes, superoxide dismutase (SOD), glutathione reductase (GR), catalase (CAT), peroxidase (POD) and glutathione-S-transferase (GST) were determined in all leaves and roots of canola.

Materials and Methods

Pots of 50 cm in diameter were filled with the mix of perlite and vermiculite (1:1 v/v) and then the canola (*Brassica napus* L. cv. SLM_{046}) seeds were sown at a rate of 10 mg. For each salinized and non-salinized treatment, the experimental design was a completely randomized design with 4 replicates per treatment and 4 pots per unit of experiment. All pots received half strength of Hoagland's solution [21], according to field capacity moisture. The greenhouse was under natural sunlight during spring and summer and the temperature was set to 25±3°C and 18±3°C day and night,

respectively. The plants were subjected to two NaCl salinity treatments: non-salinized (0 mM) and salinized (200 mM). Both salinized and nonsalinized plants were fed with one of the following NO_3^- and NH_4^+ ratios: 0:100, 75:25, 50:50 and 25:75. The concentration of nutrients in the solutions was as follows (in mg l^{-1}): 330 K, 170 Ca, 50 Mg, 33 P, 1.5 B, 0.1 Cu, 2 Mn, 12 Fe (Fe-DTPA) and 0.1 Mo. Nitrogen at 200 mg l^{-1} was provided as NO_3^- and NH_4^+ forms to give NH_4^+:NO_3^- ratios of 0:100, 25:75, 50:50 and 75:25 (Table 1).

Table 1. The concentrations of salts (mM) used to prepare nutrient solutions at NH_4^+:NO_3^- ratio of 100:0, 75:25, 50:50 and 25:75.

Salt	NH_4^+:NO_3^- ratio in the solution			
	100 : 0	75: 25	50 : 50	25 : 75
KNO_3	5.7	5.4	0.0	0.0
$Ca(NO_3)_2$	4.3	2.7	3.6	1.8
$MgSO_4$	2.0	2.0	2.0	2.0
KH_2PO_4	1.0	0.0	0.0	0.0
NH_4HPO_4	0.0	1.0	1.0	1.0
NH_4Cl	0.0	2.6	6.2	9.8
KCl	1.0	2.3	7.7	7.7
$CaCl_2$	0.0	1.5	0.7	2.4

The electrical conductivity (EC) of nutrient solution was within the range of 2.7-2.8 dS m^{-1}. The balance of NH_4^+:NO_3^- ratio changes by varying the concentration of at least one more nutrient was inevitable due to charge balance constraint. However, difference in EC within these ranges is unlikely to influence significantly plant growth. The initial pH of the nutrient solutions containing NO_3^- and NH_4^+ was adjusted to 6.5-6.8 by adding H_2SO_4 or KH_2CO_3. The solutions were supplied once a day according to the nutrients and water taken up by the plant. The treatments were imposed when two true leaves of the canola plants were expanded. Plant growth (DW and FW) was evaluated in triplicate using twenty plants. At specified periods of growth, all tissue parts (leaf and root) were separated node-by-node, and fresh weights of these tissue parts were measured. For the determination of dry weight, these tissue parts were dried three days in an oven at 72°C and water content as a percentage of fresh weight (FW) was calculated using the formula: WC (%) = [(FW-DW)/FW]100.

Preparation of enzyme extracts: A crude enzyme extract was prepared by homogenizing 50 mg of tissue leaf and root in 0.1 M Tris HCl buffer, pH 7.5, 0.5 mM EDTA and 1% PVP (MW 360,000) at 4°C. The homogenates were centrifuged at 180,000 g for 30 min. The supernatant was used as the crude enzyme preparation.

Determination of enzyme activities: Glutathione reductase (EC 1.6.4.2) activity was assayed according to the method of Smith *et al.*[22] by following the increase in absorbance at 412 nm due to 5,5-dithiobis-2-nitro benzoic acid (DTNB) reduction by glutathione reduced form (GSH) generated from glutathione oxidized form (GSSG). The reaction mixture consisted of 0.2 M sodium phosphate buffer (pH 7.5), 1 mM EDTA, 0.75 mM DTNB in 0.01 M sodium phosphate buffer (pH 7.5), 0.1 mM NADPH and 1 mM GSSG. The reaction was started by the addition of enzyme extract. Glutathione-S-transferase (EC 1.8.1.7) activity was measured by the method of Mannervik and Gutenberg [23], following a decrease in absorbance at 340 nm due to glutathione (GSH) oxidation. The final assays volume of 1 ml contained 100 mM sodium phosphate buffer, pH 6.5, 1 mM GSH, 1 mM 1-chloro-2,4-dinitrobenzene (CDNB) in ethanol. Reaction was started by the addition of enzyme extract. Catalase (EC 1.11.1.6) activity was determined after the slight modification of Upadhyaya *et al.* [24]. The assay mixture contained 20 mM sodium phosphate buffer, pH 7.5, 0.025% H_2O_2 and enzyme extract. The decomposition of H_2O_2 was measured at 240 nm. Peroxidase (EC 1.11.1.17) activity was assayed in aliquots of crude enzyme preparation as described by Putter [25], with some modification. The assay mixture consisted of 25 mM guaiacol and 0.02% H_2O_2 in 0.1 M sodium phosphate buffer, pH 6.5 at 30°C. The product of the reaction was measured at 470 nm. Superoxide dismutase (EC 1.15.1.1) activity was measured by the photochemical method described by Giannopoliti and Ries [26], with slight modification. The reaction mixture consisted of 20 mM sodium phosphate buffer pH 7.5, 0.1 mM EDTA, 10 mM methionine, 0.1 mM p-nitro blue tetrazolium chloride (NBT) in ethanol, 0.005 mM riboflavin and enzyme extract. Blanks were kept in the dark and others were illuminated for 30 min. Total SOD activity was defined as the amount of enzyme required to cause 50% inhibition of the rate of NBT reduction at 500 nm. Protein was determined by the method of Lowry *et al*. [27], using crystalline bovine serum albumin as a reference.

Statistical methods: Each treatment was analyzed with at least three replicates, and a standard deviation (S.D.) was calculated. Statistical analysis was performed using the Student's t-test, and $P<0.05$ and $P<0.001$ were considered statistically significant and highly significant, respectively.

Results and Discussion

Canola (*Brassica napus* L. cv. SLM_{046}) plants accumulated less biomass over the same growth period in the presence of salinity (Table 2), and the water content of salinity-treated plants was lower compared to that of control plants. Salinity affected the growth of leaves more than that of roots. The fresh and dry weights of leaves and roots increased considerably in NO_3^--fed plants compared to the NH_4^+ fed plants. The plants grew much better in the presence of NO_3^- ($P<0.05$) than in the presence of NH_4^+. GR activity increased in roots as well as in leaves of salt-treated plants at 50:50 NH_4^+:NO_3^- ratio as compared to the other ratios of the non-saline controls (Table 3). GR activity was greater in all tissues at 75:25 NH_4^+:NO_3^- ratio. This increase was more pronounced in roots than in leaves. Plants fed by ammonium ions showed higher GST activity in leaves and roots (Table 3) in the absence as well as in the presence of 200 mM NaCl. CAT activity was highest at 75:25 NH_4^+:NO_3^- ratio in salt-treated plants (Table 3). CAT activity in all leaves and roots of ammonium-fed and salt-treated plants was higher than in nitrate-fed plants (Table 3). CAT activity in roots was significantly ($P<0.001$) higher than in leaves. The activity of SOD was higher in leaves and roots under saline conditions (Table 3). Significantly ($P<0.001$) higher POD activity was observed in roots than in leaves of canola (Table 3).

The effect of nitrogen ratio on roots was similar with leaves, leading to higher ($P<0.001$) enzyme activity at 0:100 NH_4^+:NO_3^- ratio, salinity and 75:25 NH_4^+:NO_3^- ratio both reduced the growth

Table 2. Effect of salinity and NH_4: NO_3 ratio on fresh weight, dry weight and water content in canola.

Salinity	NH_4: NO_3 ratio	Leaf			Root		
		FW (g)	DW(g)	WC(%)	FW(g)	DW(g)	WC(%)
Non- saline (0 mM NaCl)	0:100	150 ± 6.5	75 ± 3.2	50 ± 2.2	80 ± 2.5	41 ± 1.89	48 ± 2.1
	25:75	140 ± 6.2	72 ± 3.1	48 ± 2.1	70 ± 2.2	40 ± 1.85	42 ± 2.2
	50:50	136 ± 6.1	73 ± 3.3	46 ± 2.0	74 ± 2.3	39 ± 1.84	47 ± 2.1
	75:25	130 ± 6.3	74 ± 3.4	43 ± 2.2	70 ± 2.1	38 ± 1.83	45 ± 2.3
Saline condition (200 mM NaCl)	0:100	89 ± 5.5	46 ± 2.1	48 ± 1.96	40 ± 2.1	28 ± 1.76	30 ± 1.96
	25:75	80 ± 5.6	44 ± 2.2	45 ± 1.98	36 ± 2.3	28 ± 1.75	20 ± 1.98
	50:50	78 ± 5.4	43 ± 2.1	44 ± 1.97	36 ± 2.1	24 ± 1.72	33 ± 1.96
	75:25	70 ± 5.2	43 ± 2.0	38 ± 1.92	35 ± 2.1	23 ± 1.71	34 ± 1.96

Each value represents mean±S.D.of three replicates Data are statistically significant at $P<0.05$.

Table 3. Activity of antioxidant enzymes in leaves and roots during plant growth, as affected by salinity and NH_4:NO_3 ratio.

Salinity		NH_4: NO_3 ratio	POD activity [µmol guaiacol (protein)min^{-1}]	CAT activity [µmol H_2O_2 mg^{-1} (protein)min^{-1}]	GR activity [µmol NADPH mg^{-1} (protein)min^{-1}]	SOD [mg^{-1} (protein) min^{-1}]	GST [mg^{-1} (protein) min^{-1}]
Non- saline (0 mM NaCl)	Leaf	0:100	5.50 ± 0.32	10.50 ± 1.66	0.11 ± 0.02	20.1 ± 2.18	0.15 ± 0.02
		25:75	5.20 ± 0.31	12.20 ± 1.62	0.13 ± 0.03	20.0 ± 2.19	0.10 ± 0.04
		50:50	5.00 ± 0.30	12.50 ± 1.61	0.15 ± 0.05	19.5 ± 2.20	0.18 ± 0.05
		75:25	4.50 ± 0.30	12.80 ± 1.60	0.17 ± 0.04	18.4 ± 2.21	0.20 ± 0.07
	Root	0:100	12.50 ± 1.86	20.20 ± 2.22	0.35 ± 0.04	65.2 ± 4.86	0.45 ± 0.05
		25:75	12.20 ± 1.89	20.00 ± 2.21	0.37 ± 0.06	60.2 ± 4.96	0.46 ± 0.06
		50:50	12.10 ± 1.87	19.80 ± 2.20	0.42 ± 0.07	51.6 ± 4.22	0.48 ± 0.08
		75:25	12.00 ± 1.85	19.90 ± 2.19	0.45 ± 0.08	45.3 ± 4.95	0.49 ± 0.08
Saline condition (200 mM NaCl)	Leaf	0:100	12.20 ± 1.76	12.20 ± 1.72	0.18 ± 0.01	55.2 ± 3.99	0.18 ± 0.03
		25:75	11.20 ± 1.75	13.80 ± 1.73	0.19 ± 0.02	50.1 ± 3.98	0.20 ± 0.05
		50:50	10.50 ± 1.74	14.50 ± 1.75	0.20 ± 0.04	45.3 ± 4.02	0.35 ± 0.06
		75:25	10.00 ± 1.71	16.20 ± 1.76	0.22 ± 0.06	40.1 ± 3.97	0.42 ± 0.08
	Root	0:100	35.50 ± 2.26	23.20 ± 2.25	0.39 ± 0.03	99.8 ± 6.71	0.65 ± 0.08
		25:75	30.20 ± 2.22	24.10 ± 2.27	0.40 ± 0.05	90.4 ± 6.41	0.72 ± 0.09
		50:50	15.20 ± 2.25	25.50 ± 2.28	0.58 ± 0.06	62.6 ± 6.31	0.82 ± 0.10
		75:25	14.00 ± 2.28	29.80 ± 2.26	0.75 ± 0.05	51.3 ± 6.41	0.96 ± 0.12

Each value represents mean ± S.D.

rate of canola plants (Table 3). Misra *et al.* [28], however, did not find any NH_4^+ effect on the plant growth. Canola plants grow well in presence of NO_3^-. Depletion in plant growth (FW and DW) under saline condition (200 mM NaCl) is attributed to decreased water uptake followed by limited hydrolysis of food reserves from storage tissue, as well as due to impaired translocation of food reserves from storage tissue to the developing embryo axis [15, 29]. Under optimal conditions, many metabolic reactions produce active oxygen species (AOS). However, plants possess an efficient system for scavenging AOS, which protects them from destructive oxidative reaction [2, 30]. As part of this system, antioxidant enzymes play important roles in the defense mechanism. Many changes have been found in the activities of antioxidant enzymes in plants under salinity. It has been reported that salt-tolerant plants are able to regulate the ion and water movement for effective removal of AOS [31]. The activity of antioxidant enzymes has been reported to increase under salt stress in cucumber [32] and wheat in shoot [33] but decreased in wheat roots [13]. The increased production of AOS in chloroplasts of plants under salt stress has been previously reported [14]. Changes in SOD, POD, GR, GST and CAT enzyme activity in 1-month-old salt-stressed plants suggest that oxidative stress may be an influential component of possible environmental stresses on canola. Higher enzyme activity was observed in all plant tissue parts under saline conditions, and most markedly in the roots, in contrast to the opposite trend previously observed in wheat [15]. The mechanisms of antioxidative enzymes under salinity stress are not yet clear. The GR, GST and CAT activity in all leaves and roots was higher at 75:25 NH_4^+:NO_3^- ratio in comparison to 0:100 NH_4^+:NO_3^- ratio. This may suggest a higher rate of production of active oxygen species in the presence of ammonium ions. Recently, it has been reported that ammonium or one of its assimilation products may serve as a stress signal and ammonium-fed plants operate metabolic pathways that generate more oxygen radicals [34]. POD activity did not show a significant increase at 75:25 NH_4^+:NO_3^- ratio. However, POD activity was higher in all leaves and roots of 0:100 NH_4^+:NO_3^- ratio plants. SOD activity followed a similar pattern, caused by their connection in the substrate and product. The higher POD activity indicated that

it had a higher capacity for the decomposition of H_2O_2 generated by SOD. POD and SOD do not seem to play a key role in the antioxidative process in NH_4^+-fed plants. Many researchers have reported that the form of nitrogen supplied to plant roots has influence on their growth only under non-saline conditions, but there is also evidence to indicate that the nitrogen form affects salinity tolerance under other conditions [33]. Results confirming this were found in the case of GR, GST and CAT, which showed higher enzyme activities under salinity at 75:25 and 50:50 NH_4^+:NO_3^- ratio than at 0:100 NH_4^+:NO_3^-. Similar results have been reported for Mo-enzymes in maize [34] and ryegrass [18]. The NH_4^+ ion may represent a stress signal that triggers the activation of enzymes responsible for some adaptation mechanism to stress [35].

In conclusion, this study showed that differences in GR, GST, CAT, SOD and POD activities in all leaves and roots of canola (*Brassica napus* L.) plants could be attributed to differences in mechanisms underlying oxidation stress injury. Subsequently, with the supplementation of significantly higher antioxidant enzyme activity more tolerance to salinity was observed in roots than in leaves.

References

[1]Smirnoff, N.1993. The role of active oxygen in the response of plants to water deficity and desiccation. New Phytol. **125**:27-58.

[2]Foyer, C. H., Descourvieres, P. and Kunert, K. J. 1994. Protection against oxygen radicals: An important defense mechanism study in transgenic plants. Plant Cell Environ. **17**:507-523.

[3]Noctor, G. and Foyer, C. H. 1998. Ascorbate and glutathione: Keeping active oxygen under control. Annu. Rev. Plant Physiol. Plant Mol. Biol. **49**:249-279.

[4]Gressel, J. and Galun, E. 1994. Genetic controls of photo oxidant tolerance. In Foyer, C.H. and Mullineaux, P.M. (eds). Causes of Photo Oxidative Stress and Amelioration of Defense Systems in Plant. CRC Press, Boca Raton, pp. 237-274.

[5]Gossett, D. R., Millhollon, E. P. and Lucas, M. C. 1994. Antioxidant response to NaCl in salt-tolerant and salt-sensitive cultivars of cotton. Crop Sci. **34**:706-714.

[6]Meloni, D. A., Oliva, M. A., Martinez, C. A. and Cambraia, J. 2003. Photosynthesis and activity of superoxide dismutase, peroxidase and glutathione reductase in cotton under salt stress. Environ. Exp. Bot. **49**:69-76.

[7]Mitteler, R. 2002. Oxidative stress, antioxidants and stress tolerance. Trends Plant Sci. **7**:405-414.

[8]Prasad, K. V. S. K., Saradhi, P. P. and Sharmila, P. 1999.Concerated action of antioxidant enzymes and curtailed growth under zinc toxicity in *Brassica juncea*. Environ. Exp. Bot. **42**:1-10.

[9]Sairam, R. K., Srivastava, G. C., Agarwal, S. and Meena, R.C. 2005. Differences in antioxidant activity in response to salinity stress in tolerant and susceptible wheat genotype. Biol. Planta. **49**(1):85-91.

[10]Sairam, R. K., Veerabhadra Rao, K. and Srivastava, G. C. 2002. Differential response of wheat genotype to long term salinity stress in relation to oxidative stress, antioxidant activity and osmolyte concentration. Plant Sci. **163**:1037-1046.

[11]Sudhakar, C., Lakshmi, A. and Giridarakumar, S. 2002. Changes in the antioxidant enzyme efficacy in two high yielding genotypes a mulberry (*Morus alba* L.) under NaCl salinity. Plant Sci. **161**:613-619.

[12]Asada, K. 1992. Ascorbate peroxidase - A hydrogen peroxide scavenging enzyme in plants. Physiol. Plant. **85**:235-241.

[13]Wiilekens, H., Chamnongpol, S., Schraudner, M., Langebartels, C., Van Montagu, M., Inze, D. and Van Camp, W. 1997. Catalase is a sink for H_2O_2 and is indispensable for stress defense in C_3 plants. EMBO J. **16**:4806-4816.

[14]Zhu, J. K. 2000. Genetic analysis of plant salt tolerance using Arabidopsis. Plant Physiol. **124**:941-948.

[15]Meneguzzo, S., Navari-Izzo, F. and Izzo, R.1999. Antioxidative responses of shoots and roots of wheat to increasing NaCl concentrations. J. Plant Physiol. **155**:274-280.

[16]Hawkins, H. J. and Lewis, O. A. M. 1993. Effect of NaCl salinity, nitrogen form, calcium and potassium concentration on nitrogen uptake and kinetics in *Triticum aestivum* L. cv Gamtoos. New Phytol. **124**:171-177.

[17]Dat, J., Vandenabeele, S., Vranjva, E., Van Montagu, M., Inze, D. and Van Breusegem, F. 2000. Dual action of the active oxygen species during plant stress responses. Cell Mol. Life Sci. **57**:779-795.

[18]Sagi, M., Omarov, R. T. and Herman, S. H. 1998. The Mo-hydroxylases xanthine dehydrogenase and alhehyde oxidase in ryegrass as affected by nitrogen and salinity. Plant Sci. **135**:125-135.

[19]Rios-Gonzalez, K., Erdei, L. and Lips, S. H. 2002. The activity of antioxidant enzymes in maize and sunflower seedlings as affected by salinity and different nitrogen sources. Plant Sci. **162**:923-930.

[20]Bybordi, A., Tabatabaei, S. J. and Ahmedov, A. 2010. The influence of salinity stress on antioxidant activity in canola cultivars (*Brassica napus* L.). J. Food, Agri & Environ. **8**(1):122-129.

[21]Hoagland, D. R. and Arnon, D. S. 1950. The water culture method for growing plants without soil. Calif. Agric. Exp. Stat. Circ. **374**:1-32.

[22]Smith, I. K., Vierheller, T. L. and Thorne, C. A. 1988. Assay of glutathione reductase in crude tissue homogenates using 5,5-dithiobis-(2-nitrobenzoic acid). Anal. Biochem. **175**:408-413.

[23]Mannervik, B. and Gutenberg, C.1987. Glutathion transferase. Methods Enzymol. **77**:231-235.

[24]Upadhyaya, A., Sankhla, D., Davis, T. D., Sankhla, N. and Smith, B. N. 1985. Effect of paclobutrazol on the activited oxygen metabolism and lipid peroxidation in senescing soybean leaves. J. Plant Physiol. **121**:543-461.

[25]Putter, J. 1974. Methods of Enzymatic Analysis. Vol.2. Verlag Chemie, Academic Press, Weinheim, New York, 685 p.

[26]Giannopoliti, C. N. and Ries, S. K. 1977. Superoxide dismutases. Plant Physiol. **59**:309-314.

[27]Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. 1951. Protein measurement with the folin phenol reagent. J. Biol. Chem. **193**:265-272.

[28]Misra, N., Varsha Dubey, V. S., Srivastava, A. K., Luthra, R. and Kumar, S. 2002. Modulation of growth, nitrate reductase activity and indole alkaloid content in leaves by nitrate supplementation. J. Aromatic Med. Plants **24**:383-388.

[29]Olmes, E., Hernandez, J. A., Sevilla, F. and Hellin, E.1994. Indication of several antioxidant enzymes in the selection of a salt tolerant cell line of *Pisum sativum*. J. Plant Physiol. **144**:594-598.

[30]Sharata, A. and Tal, M. 1998. The effect of salt stress on lipid peroxidation and antioxidants in the leaf of the cultivated tomato and its wild salt-tolerant relation *Lycopersicon penelli*. Physiol. Plant. **104**:169-174.

[31]Rout, N. P. and Shaw, B. P. 2001. Salt tolerance in aquatic macrophytes: Possible involvement of the antioxidative enzymes. Plant Sci. **160**:415-423.

[32]Lechno, S., Zamski, E. and Tel-or, E. 1997. Salt stress induced responses in cucumber plants. J. Plant Physiol. **150**: 206-211.

[33]Lewis, O. A. M., Leidi, E. O. and Lips, S. H. 1980. Effect of nitrogen source on growth response to salinity stress in maize and wheat. New Phytol. **111**:155-160.

[34]Barabas, N. K., Omarov, R. T., Erdei, L. and Lips, S. H. 2000. Distribution of the Mo-enzymes aldehyde oxidase, xanthine dehydrogenase and nitrate reductase in maize (*Zea mays* L.) nodal roots as affected by nitrogen and salinity. Plant Sci.**155**:49-58.

[35]Polesskaya, O. G., Kashirina, E. I. and Alekhina, N. D. 2006. Effect of salt stress on antioxidant system of plants as related to nitrogen nutrition. Russ. J. Plant Physiol. **53**(2):186-192.