Current Research Journal of Biological Sciences 4(1): 17-22, 2012
ISSN: 2041-0778

Submitted: September 12, 2011 Accepted: October 09, 2011 Published: January 20, 2012

The Effects of NaCl Stress on the Physiological and Oxidative Situation of Maize (*Zea mayz* L.) Plants in Hydroponic Culture

[1]Hamid Noorani Azad, [2]Mohammad Reza Hajibagheri, [1]Farshid Kafilzadeh and [2]Majid Shabani
[1]Department of Biology, Jahrom Branch, Islamic Azad University, Jahrom, Iran
[2]Department of Agriculture, Estahban Branch, Islamic Azad University, Estahban, Iran

Abstract: The effects of NaCl salinity on biomass, Malondialdehyde (MDA), peroxidase (POD), Catalase (CAT), Na^+, K^+, Ca^{2+} and proline in *Zea mays* L. seedlings were investigated under hydroponic condition. Seedlings were subjected to NaCl stress (0, 50, 100, 150 and 200 mM) for 14 days. A completely randomized design with four replicates for each treatment was used. Salinity stress affected on the growth and caused a reduction in root and shoot biomass. NaCl treatment caused a significant increase in root MDA content. NaCl at 100 mM and higher increased also the shoot MDA content significantly. Catalase activity of leaf was significantly increased at 100, 150 and 200 mM NaCl in comparison with the control. Peroxidase activity in leaf started to significant increase with the rise of NaCl content at 150 and 200 mM. The leaf Na^+ content, root and shoot proline concentrations increased with the increase in salinity stress. The leaf K^+ and Ca^{2+} amounts were significantly decreased with the rise of salinity stress in comparison with control.

Key words: Antioxidative enzymes, maize, MDA, proline, salt stress

INTRODUCTION

Salt stress in soil or water is one of the major abiotic stresses especially in arid and semi-arid regions and can severely limit plant growth and yield (Parvaiz and Satyawati, 2008). Damages caused by high salinity are often associated with three different mechanisms (Levinsh, 2006). First, ion toxicity is caused by excessive accumulation of Na^+ and Cl^- in the cytoplasm leading to an ionic imbalance. This can be counteracted by an increased transport intensity of the ions to the vacuole. Second, even if massive ion compartmentation occurred in the vacuole, the cytosol water potential must be lowered to balance a low-external water potential, thus allowing water intake in the plant cell and preventing macromolecule damages. Third, a high cellular NaCl concentration causes an increased formation of Reactive Oxygen Species (ROS) (Hernandez and Almansa, 2002). Salt stress can lead to stomatal closure, which reduces CO_2 availability in the leaves and inhibits carbon fixation, exposing chloroplasts to excessive excitation energy, which in turn could increase the generation of ROS and induce oxidative stress (Parvaiz and Satyawati, 2008). These species of oxygen are highly cytotoxic and can seriously react with vital biomolecules such as lipids, proteins, nucleic acid, etc, causing lipid peroxidation, protein denaturing and DNA mutation, respectively (Quiles and López, 2004). Plants have developed a series of enzymatic and non-enzymatic detoxification systems to counteract ROS, and protect cells from oxidative damage (Sairam and Tyagi, 2004). The Antioxidant enzymes such as CAT, and POD function in detoxification of super oxide and H_2O_2 (Mittler, 2002). Beside, Munns and James (2003) suggested that the assessment of cell membrane stability is an appropriate technique to screen plants under saline condition. Bandeoglu *et al.* (2004) found that salt stress increase MDA production and cell membrane damage in leaf of rice seedling. Reduction in cell membrane damage and lipid distribution (MDA production) improvement with antioxidant enzyme activity. Wahid and Close (2007) Reported that Proline accumulates in large quantities in response to environmental stresses acts as a compatible solute, and buffers cellular redox potential. ROS served as intermediate molecules in inducing salt resistance in the callus of *Populus euphratica*, by increasing the K^+/Na^+ ratio, which was dependent on the increased plasma membrane H^+-ATPase activity (Zhang *et al*., 2007). Salinity is known to induce K^+ deficiency in plants because of the competition of Na^+ and K^+ for binding sites in transport systems that mediate K^+ uptake (Shabala, 2003).

Maize (*Zea mays* L.) is one of the most important crops in Iran, which plays a special role in people's nutrition. But unfortunately abiotic stresses, such as salinity, decrease maize growth and productivity by reducing water uptake and cause nutrient disorders and ion toxicity in this region. In the present study, the effects

Corresponding Author: Hamid Noorani Azad, Department of Biology, Jahrom Branch, Islamic Azad University, Jahrom, Iran

of increasing NaCl concentrations on biomass, MDA, POD, CAT, cation concentrations and proline content in *Zea mays* L. seedlings were investigated.

MATERIALS AND METHODS

Plant culture and treatments: The experiment was performed in a glasshouse of Islamic Azad University, Jahrom Branch, Iran, in 2010, under the following controlled conditions: photoperiod of 16/8 h (light/darkness), daily air temperature and relative humidity ranging 20-25ºC and 65-75%, respectively, and photosynthetically active radiations of 400 µmol/m^2.s. Mature seeds of maize (*Zea mays* L.) cultivars, single cross (No -704) obtained from vegetable research center, Fars, Iran. Seed were surface sterilized with a solution of mercuric chloride) 0.1% for 30 S (and were washed immediately with large volume of sterile distilled water, and germinated in plastic pots (12 cm diameter and 8 cm depth) filled with sterile sand and watered daily with distilled water until germination. Then, seedlings were irrigated for 2 weeks with Hoagland, s nutrient solution (1/2 strength sterile and pH = 7). Afterward, the seedlings were transferred to Hoagland solution containers and treated with adding different concentrations of NaCl: 0 (control), 50, 100, 150 and 200 mM for 14 d. A completely randomized design with four replicates for each treatment was used. After treatment for 14 days, the maize seedlings were sampled and transferred to liquid nitrogen and maintained at -70ºC until the measurement of variables under study.

Biomass assay: The dry weight (DW) of shoot and root were measured at each sampling. The DW is obtained after oven-drying the shoots and roots at 60ºC for 72 h.

Lipid peroxidation in leaf and root: Lipid peroxidation was determined by measuring the amount of malondialdehyde (MDA) formation, using the thiobarbituric acid method described by Valentovic *et al.* (2006). Briefly, 200 mg of fresh tissues leaf and root were homogenized in 2 mL of 0.1% (w/v) trichloroacetic acid (TCA) solution on ice. The homogenate was centrifuged at 14,000 rpm for 10 min, and 0.5 ml of the supernatant was added to 0.5 mL of 0.5% (w/v) TBA in 20% TCA. The mixture was incubated in boiling water for 20 min and the reaction was stopped by incubation in ice. Samples were then centrifuged for 5 min at 14,000 rpm, and the absorbance of the supernatant was measured at 532 nm, subtracting the value for nonspecific absorption at 600 nm (Valentovic *et al*., 2006).

Antioxidant enzymatic activities in leaf: Leaves (0.5 g) were homogenized in 2 mL of ice-cold potassium phosphate buffer (50 mM, pH = 7.0) containing 0.2 mM EDTA, 1.0% (w/v) polyvinylpyrrolidone and 0.1% triton. The homogenate was centrifuged at 10000g at 4 ºC for 20 min. Proteins were quantified using bovine serum albumin as standard (Bradford, 1976). Catalase (CAT) activity was determined by consumption of H_2O_2 in absorbance at 240 nm according to method of Aebi (1984). The measurements of peroxidase (POD) activity were done according to Fielding and Hall (1978). Enzyme extraction was added to reaction medium containing 5mM potassium phosphate buffer (pH = 7), 0.1 mM Na_2EDTA, 5 mM H_2O_2 and 30 mM gayacol. The increase in absorption was recorded at 470 nm.

Na^+, K^+ and Ca^{2+} contents in leaf: Cations were extracted from the desiccated leaf tissue with 0.5% (v/v) nitric acid. The concentration of $Na^+$and $K^+$was assayed by flame emission photometry. The concentrations of Ca^{2+} were measured by atomic absorption photometry (Hasna *et al.,* 2011).

Root and shoot proline contents: Extraction and determination of proline was performed according to the method of Bates *et al*. (1973). Root and shoot samples (0.5 g) were extracted with 3% sulfosalicylic acid. Extracts (2 mL) were held for 1 h in boiling water by adding 2 mL ninhydrin and 2 mL glacial acetic acid, after which cold toluene (4 mL) was added. Proline content was measured by a spectrophotometer (Shimadzu-UV-1601-Japan) at 520 nm and calculated as mmol/g FW against standard proline.

The statistics method: Statistical analyses were carried out by analysis of variance (ANOVA) using SAS 9 software. Differences between treatments were analyzed by the Duncan's multiple range test.

RESULTS

Effects of salt stress on biomass: The biomass of shoots and roots, estimated on the basis of their dry weight, decreased significantly (**$p<0.01$) in salt-treated plants, when compared with control plants (Fig. 1a, b). Therefore, salinity in maize seedlings caused inhibition of shoots and roots growth and reduction in the biomass of organs.

Effect on the MDA contents: A raise in MDA, an indicator of membrane damage was observed under NaCl salinity. Salt stress for 14 d increased MDA contents in root and leaf of *Zea mays* L. seedlings. However, the difference between control and 50 mM NaCl was not significant in leaf. Treatments with NaCl salinity induced significant (**$p<0.01$) increases in root MDA content (Fig. 1c, d). The increase of MDA level in root was greater than in leaf.

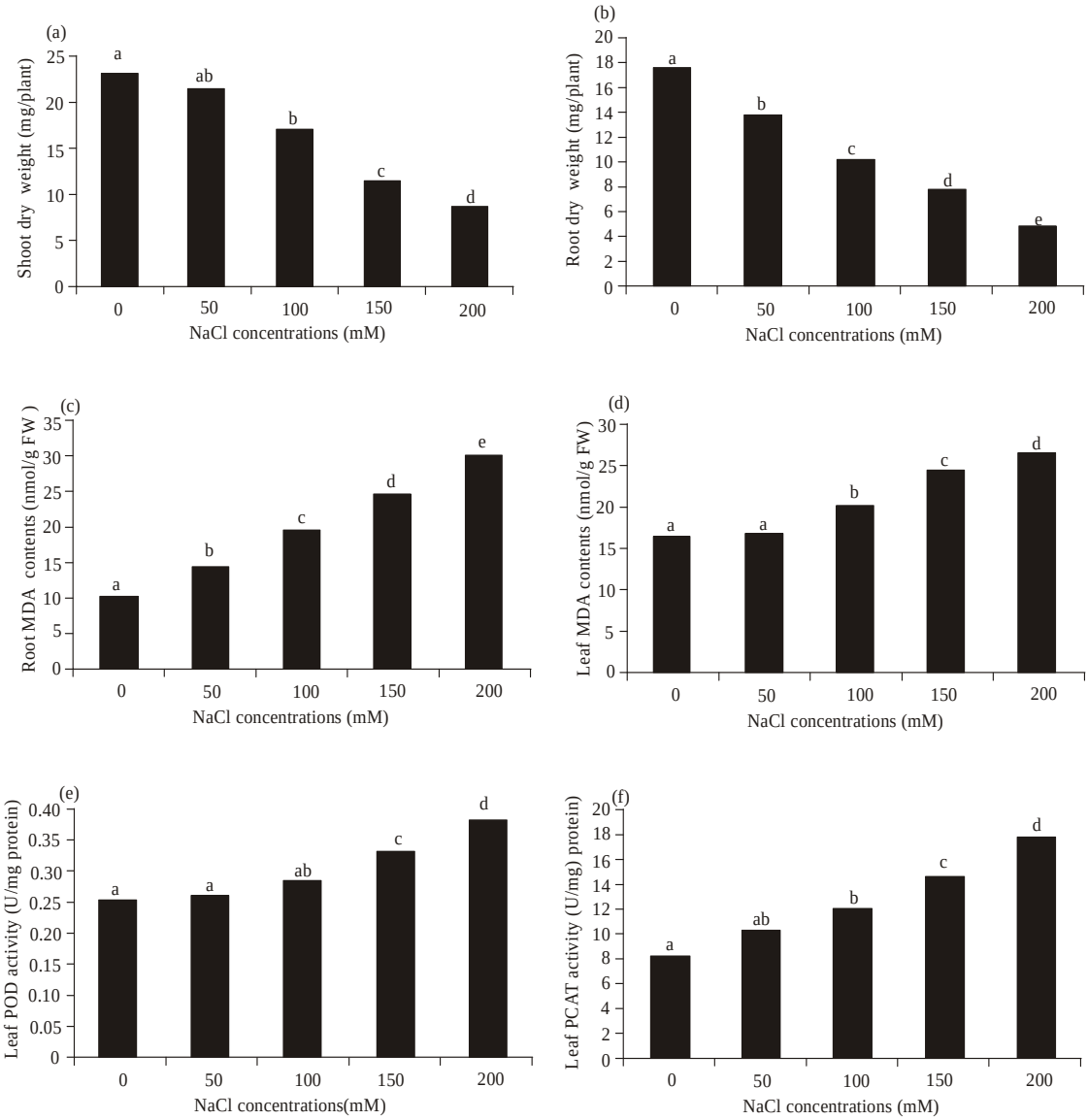

Fig. 1: Effects of NaCl on shoot dry weight, (a) root ry weight, (b) root MDA contents, (c) leaf MDA contents, (d) leaf POD activity, (e) leaf CAT activity, (f) in maize

Changes in antioxidant enzymes activities: In the leaf of *Zea mays* L. seedlings antioxidant enzyme activities increased at the salt stress. Peroxidase activity in leaf started to significant increase with the rise of NaCl content at 150 and 200 mM in comparison with the control (**p<0.01) (Fig. 1e). Catalase activity was significantly (**p<0.01) increased in leaf at 100, 150 and 200 mM NaCl in comparison with the control (Fig. 1f).

Effects of salt stress on cation contents: Figures. 2a, b and C illustrate Na^+, K^+ and Ca^{2+}changes in leaves of maize seedlings by increase in NaCl salinity. The concentrations of Na^+ increased in salt-treated plants of *Zea mays* L. when compared with control plants (**p<0.01). K^+ and Ca^{2+} contents in leaf were significantly decreased with the rise of salinity stress in comparison with control conditions (**p<0.01). Decrease in K+ concentration was significant in all treatments except 50 mM NaCl.

Root and shoot proline contents: As can be seen in Fig. 2d, e, Proline accumulation in root and shoot increased in NaCl treatments. Howevere, In case of root it was significant for all treatments (**p<0.01), except 50 mM NaCl, but in shoot there was a significant increase only in 150 and 200 mM treatments.

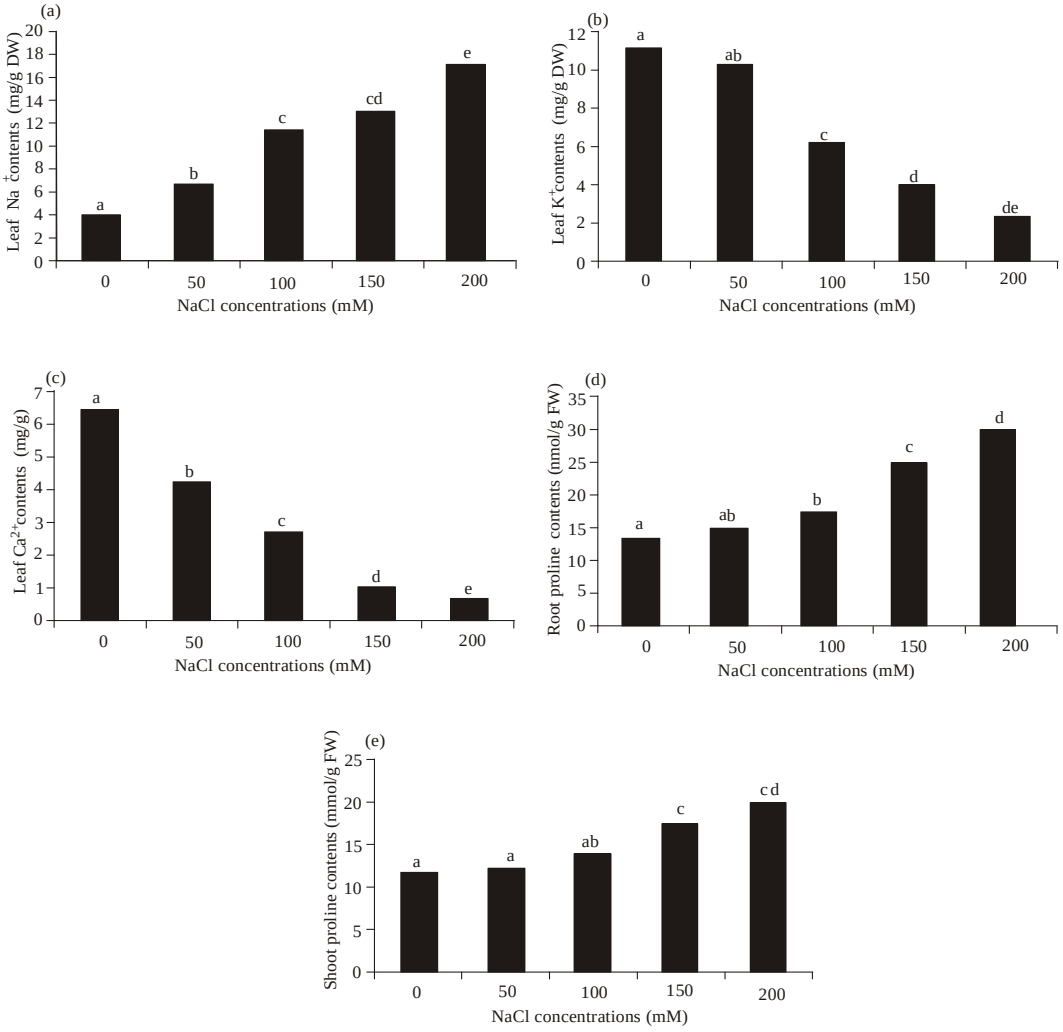

Fig. 2: Effects of NaCl on leaf Na^+ contents, (a), leaf K^+ contents, (b), leaf Ca^{2+} contents, (c), root proline contents, (d) shoot proline contents, (e) in maize

DISCUSSION

Salinity stress causes ion toxicity and osmotic imbalances, leading to oxidative stress in plants (Farouk, 2011). Soil salinity is a current abiotic stress for plants. Growth inhibition is a common response to salinity and plant growth is one of the most important agricultural indices of salt stress tolerance as indicated by different studies (Parida and Das, 2005). Our results showed that the growth of *Zea mays* L. seedlings started to decrease significantly in 14 d salinity treatment. Reduction in shoot and root growth rates, as well as biomass observed in salt stressed seedlings are in conformity with proven effects of salinity stress. Salinity affects development processes such as seed germination, seedling growth and vigor, vegetative growth, flowering and fruit set (Sairam and Tyagi, 2004).

Hasna *et al.* (2011) reported that reduction in biomass and growth can be due to higher Na^+ concentration in the shoots of *Arabidopsis thaliana* and ionic toxicity and decreases osmotic potential.

It hase been shown salinity increased cellular accumulation of ROS (Hernandez and Almansa, 2002). Unless controlled, ROS may oxidize and eventually cause damage to proteins, lipids and nucleic acids. However, some of them can also serve as signaling molecules, if

they are produced transiently but do not accumulate (Foyer and Noctor, 2005). Their function as signaling molecules or the degree of damage caused by ROS ultimately depends on the balance between their formation and removal by the antioxidative scavenging systems (Hernandez and Almansa, 2002). In this study, we present the evidence that salt stress is able to excessive

generation of MDA in the root and leaf of *Zea mays* L. seedlings. Increase in the level of MDA, produced during peroxidation of membrane lipids, is often used as an indicator of oxidative damage. Free radical-induced peroxidation of lipid membranes is a reflection of stress-induced damage at the cellular level (Nagesh and Devaraj, 2008). As a sequel, lipid peroxidation products such as MDA, will accumulate and severe membrane damage will inevitably occur. Similar results were observed in salt-sensitive plants such as cotton (Gossett *et al*., 1994) and maize (Arora *et al*., 2008) under salinity. MDA concentration and cell membrane damage increased under salt stress condition because of elevating of ROS production. Enzymatic antioxidant defense system can protect plant cells from oxidative injury. POD and CAT are the most important protective enzymes to remove reactive oxygen species. Our results showed that in the leaves of maize seedlings antioxidant enzyme (POD and CAT) activities increased at the salt stress. The highest antioxidant activity help plants under environmental stress like salt stress (Munns and James, 2003). A correlation between the antioxidant enzyme activities and salinity tolerance were reported in many plants (Ashraf and Ali, 2008). Bandeoglu *et al.* (2004) reported that antioxidant enzyme activities like CAT and POD helps plant to decrease MDA concentration and cell damage under salinity stress. In our study, the increased activities of these enzymes were suggested to be insufficient to overcome lipid peroxidation and thus salt-induced oxidative stress in *Zea mays* L. seedlings.Yamazaki *et al.* (2003) showed that High activity of CAT and POD decrease H_2O_2 level in cell and increase the stability of membranes and CO_2 fixation because several enzymes of the Calvin cycle within chloroplasts are extremely sensitive to H_2O_2. A high level of H_2O_2 directly inhibits CO_2 fixation. In this study reduction of biomass and growth in maize seedlings could be attributed to oxidative stressess and inhibition in photosynthetic CO_2 fixation. Moreover, salt stress induced a greater increase of MDA in roots than in leaves of *Zea mays* L., suggesting some degree of oxidative stress, probably due to the high NaCl concentration and higher lipid peroxidation in roots. Therefore, in *Zea mays* L. salt toxicity was mainly concentrated in roots, a strong decrease of plant growth was observed, associated with oxidative stress in the roots at the highest NaCl supply. Also, higher biomass reduction in roots could be related to the severe increase of the oxidative stress in roots in response to NaCl.

It this experiment, Na^+concentration increased, while K^+ and Ca^{2+} contents in leaf were significantly decreased with the rise of salinity stress. Salt stress is known to induce K^+ deficiency in plants because of the competition of Na^+ and K^+ for binding sites in transport systems that mediate K^+ uptake (Shabala, 2003). Maathius and Amtmann (1999) reported that for most other species, Na^+ is not necessary for growth. The availability of some Na^+ is beneficial to the plant, but too much will case damage. Volkmar *et al*. (1998) showed that Na+ competing for K^+ binding sites in the cytoplasm inhibit metabolic processes that depend on $K^{+.}$

It is usually assumed that Na^+ can negatively affect plant Ca^{2+} relations. Na^+ can replace electrostatically bound Ca^{2+}in cell walls and cell membranes. In addition, Ca^{2+} nutrition can be deregulated because the osmotic effects of salinity stress lead to a reduction in transpiration, particularly affecting relatively immobile ions such as Ca^{2+} in their root-shoot translocation (Maathuis, 2006).

We show in our study that salinity stress increased proline accumulation in root and shoot of *Zea mays* L. seedlings. Proline accumulation occurs normally in cytosol in response to drought and salinity stress. Apart from its role as osmolyte, proline contributes to stabilization of sub-cellular structures, scavenging ROS, and buffering cellular redox potential under stress (Ashraf and Foolad, 2006). Recent studies suggest that proline may play as an enzyme stabilizing role in canola (Bhattacharjee and Mukherjee, 2002) and reduce lipid peroxidation in rice cultivars (Farhoudi *et al*., 2007) under salt stress. Therefore, the increase in the proline concentration of *Zea mays* L. seedlings especially in roots, can maintain osmoregulation and prevent enzyme destruction under salt stress.

CONCLUSION

In conclusion, this study showed that salt stress decrease biomass and growth in maize plants due to ionic toxicity and decrease osmotic potential and oxidative stress. Increase in the level of MDA, produced during peroxidation of membrane lipids, is as an indicator of oxidative damage. Enzymatic antioxidant defense system can protect plant cells from oxidative injury. Salt stress is induced K^+ deficiency in maize plants because of the competition of Na^+ and K^+ for binding sites in transport systems that mediate K^+ uptake. The increase in the proline concentration of. seedlings especially in roots, can maintain osmoregulation and prevent enzyme destruction under salt stress.

REFERENCES

Aebi, H., 1984. Catalase in vitro. Method Enzymol., 105: 121-126.

Arora, N., R. Bhardwaj, P. Sharma and H.K. Arora, 2008. 28-Homobrassinolide alleviates oxidative stress in salttreated maize (*Zea mays* L.) plants. Braz. J. Plant Physiol., 20(2): 153-157.

Ashraf, M. and M.R. Foolad, 2006. Roles of glycine betaine and proline in improving plant abiotic stress resistance. Environ. Exptl. Bot., 59: 206-216.

Ashraf, M. and Q. Ali, 2008. Relative membrane permeability and activities of some antioxidant enzymes as the key determinants of salt tolerance in canola (*Brassica napus* L.)Environ. Exp. Bot., 63: 266-273.

Bandeoglu, E., F. Eyidogan, M. Yucel and H.A. Oktem, 2004. Antioxidant response of shoots and roots of lentil to NACL Salinity stress. Plant Grth. Regu., 42: 69-77.

Bates, L.S., S.P. Waldren and I.D. Teare, 1973. Rapid determination of free proline for water-stress studies. Plant Soil, 39: 205-208.

Bhattacharjee, S. and A.K. Mukherjee, 2002. Salt stress induced cytosolute accumulation, antioxidant response and membrane deterioration in three rice cultivars during early germination. Seed Sci. Tech., 30: 279-287.

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

Farhoudi, R., F. Sharifzadeh, K. Poustini, M.T. Makkizadeh and M. Kochakpor, 2007. The effects of NACL priming on salt tolerance in canola (*Brassica napus*) seedlings grown under saline conditions. Seed Sci. Tech., 35: 754-759.

Farouk, S., 2011. Osmotic adjustment in wheat flag leaf in relation to flag leaf area and grain yield per plant. J. Plant Physiol. Biochem., 7(2): 117-138.

Fielding, J.L. and J. Hall, 1978. A biochemical and cytochemical study of peroxidase activity in roots of *Pisum sativum*. J. Exp. Bot., 29: 981-989.

Foyer, C.H. and G. Noctor, 2005. Oxidant and antioxidant signal ling in plants: A re-evaluation of the concept of oxidative stress in a physiological context. Plant Cell Environ., 28: 1056-1071.

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

Hasna, E., B.H. Karim, C. Jana, M.B. Sergi and A. Chedly, 2011. Early effects of salt stress on the physiological and oxidative status of *Cakile maritima* (halophyte) and *Arabidopsis thaliana* (glycophyte). Physiol. Plantarum., 142: 128-143.

Hernandez, J.A. and M.S. Almansa, 2002. Short-term effects of salt stress on antioxidant systems and leaf water relations of pea leaves. Physiol. Plant, 115: 251-257.

Levinsh, G., 2006. Biological basis of biological diversity: Physiological adaptations of plants to heterogeneous habitats along a sea coast. Act. Univ. Lat., 710: 53-79.

Maathius, F.J.M. and A. Amtmann, 1999. K^+ nutrition and Na^+ toxicity: The basis of cellular K^+/Na^+ ratios. Ann. Bot., 84: 123-133.

Maathuis, F.J., 2006. The role of monovalant cation transporters in plant response to salinity. J. Exp. Bot., 57: 1137-1147.

Mittler, R., 2002. Oxidative stress; antioxidants and stress tolerance. Trend. Plant Sci., 7: 405-410.

Munns, R. and R.A. James, 2003. Screening methods for salinity tolerance: Acase study with tetraploid wheat. Plant Soil, 253: 201-218.

Nagesh, B.R. and V.R. Devaraj, 2008. High temperature and salt stress response in French bean (*Phaseolus vulgaris*). Aust. J. Crop Sci., 2(2): 40-48.

Parida, A.K. and A.B. Das, 2005. Salt tolerance and salinity effect on plants: A review. Ecotoxicol. Environ. Saf., 60: 324-349.

Parvaiz, A. and S. Satyawati, 2008. Salt stress and phytobiochemical responses of plants: A review. Plant Soil Environ., 54: 89-99.

Quiles, M.J. and N.I. López, 2004. Photoinhibition of photosystems I and II induced by exposure to high light intensity during oat plant grown effects on the chloroplastic NADH dehydrogenase complex. Plant Sci., 166: 815-823.

Sairam R.K. and A. Tyagi, 2004. Physiological and molecular biology of salinity stress tolerance in plants. Curr. Sci., 86: 407-420.

Shabala, S., 2003. Regulation of potassium transport in leaves: From molecular to tissue level. Ann. Bot., 92: 627-634.

Valentovic, P., M. Luxova, L. Kolarovi and O. Gasparikora, 2006. Effect of osmotic stress on compatible solutes content, memberane stability and water relation in two maize. Plant Soil Environ., 52(4): 186-191.

Volkmar, K.M., Y. Hu and H. Steppuhn, 1998. Physiological responses of plants to salinity: A review. Can. J. Plant Sci., 78: 19-27.

Wahid, A. and T.J. Close, 2007. Expression of dehydrins under heat stress and their relationship with water relations of sugarcane leaves. Biol. Plant., 51: 104-109.

Yamazaki, J., A. Ohashi, Y. Hashimoto, E. Negishi, S. Kumagai, T. Kubo, T. Oikawa, E. Maruta and Y. Kamimura, 2003. Effects of high light and low temperature during harsh winter on needle photodamage of *Abies mariesii* growing at the forest limit on Mt. Norikura in Central Japan. Plant Sci., 165: 257-264.

Zhang, F., Y. Wang, Y. Yang, H. Wu, D. Wang and J. Liu, 2007. Involvement of hydrogen peroxide and nitric oxide in salt resistance in the calluses from *Populus euphratica*. Plant Cell Environ., 30: 775-785.