Al-Azhar Bull. Sci. Vol. 24, No. 2 (Dec.): pp. 149-164, 2013.

EFFECT OF SALINITY ON DRY MATTER PRODUCTION, ION ACCUMULATION, SOME METABOLITES AND ANTIOXIDANT ENZYMES IN WHEAT AND BROAD BEAN

ABDEL KAREEM S.H. MOHAMED[1] and MOHAMED A.K. SHADDAD [2]

[1]*Botany and Microbiology Department, Faculty of Science, Al-Azhar University, Assiut 71524 Egypt.*
[2]*Botany Department, Faculty of Science, Assiut University, Assiut, 71516 Egypt.*

Abstract

Broad bean and wheat plants responded differently to salinity stress. The reduction in the dry weight of shoot of about 65% in wheat (cv. Giza 168) and about 35 % in broad bean (cv. Sakha 1) was associated with the more stability of photosynthetic pigment in broad bean than in wheat. At 150 mM of NaCl, carbohydrates and proteins accumulation in broad bean was evident than in wheat and this was accompanied by accumulation of sufficient amount of Ca^{2+} ions in broad been and Na^+ ions in the shoot and root of wheat. Consequently, lipid peroxides (MDA) content was higher in wheat compared to broad bean plants. The Na^+ uptake at the root surface was higher in broad bean than in wheat. However, broad bean showed lowest Na^+ translocation from root to shoot. The observable increase in the activity of catalase and proline content in broad bean more than in wheat could play the pivotal role in the detoxification of free radicals.

Keywords: Wheat- Broad bean - Salinity- MDA-Peroxidase- Catalase

Introduction

In Egypt, soil salinity is becoming an important constraint on crop production. High levels of salinity in the soil hinder the growth and development of crops and cause serious problems for world food production [1, 2]. Wheat, an important cereal crop in many parts of the world, is considered to be a moderately salt-resistant glycophyte species which reacts with the severe reduction of plant growth and yield under high salinity stress. Salinity lowers the water potential outside the roots, and causes reduction in growth rate [3-5]. Also, plants mainly suffer from salt stress due to continuous accumulation of toxic Na^+ levels inside plant tissues which leading to further growth reduction, [6].

Higher plants have evolved various genetically and physiologically complex strategies and mechanisms, affecting numerous plant processes at all levels of organization, to prevent the accumulation of Na^+ ions in leaf cells as well as to overcome the dominating and lasting osmotic stress [7]. Plant cell employs various mechanisms to maintain a high cytosolic K^+/Na^+ ratio and low cytosolic Na^+ concentrations under salinity stress [8, 9]. Whereas, salt-sensitive plant species mainly depend on Na^+ exclusion, salt-resistant plant species accumulate large amounts of Na^+ in the vacuole in order to avoid cytoplasm from toxic effects of the high Na^+ concentration [10]. Recently, [6] investigated that the high transcription level of tonoplast Na^+/H^+ antiporter of leaf maize hybrid plants was increased in the salt-resistant plants as compared to salt sensitive one, mediating lower

concentrations of Na^+ and higher K^+/Na^+ ratios in cytosol [11, 12]. Na^+ accumulation in the vacuole by Na^+/H^+ antiporters is supported by transmembrane proton gradients which are enhanced by increased activity of H^+ ATPases and V-PP_iases. Such Na^+ accumulation can decrease the cellular water potential [13, 9] and contributes to osmotic stress resistance [14].

Photosynthesis is considered the most important process that is been affected under saline conditions. There are strong evidences that salt affects photosynthetic pigments such as chl.a, chl.b and carotenoids [15-18]. However [19], observed the increase in the photosynthetic pigments with increase of salinity levels. Carotenoids act as antioxidant properties by protecting the photosystems where it's reacting with lipid proxidation and scavenging the reactive oxygen species (ROS) as reported by [20].

Accumulation of proline may play a role in combating salinity stress. Thus, in order to maintain osmotic homeostasis under salinity the accumulation of osmotically active substances such as proline, glycinebetaine [21-22] must be increased. These solutes are known to raise osmotic pressure in the cytoplasm, stabilize protein complexes and protect membranes during plant growth under stressful conditions, [23-24].

The present study investigates the response of the two plant species wheat and broad bean to high NaCl treatments (e.g. 150 mM) at early growth stages in terms of dry matter production, proline and lipid peroxides (MDA) content and antioxidant enzymes (catalase and peroxidase) caused by NaCl and the root and shoot concentrations of Na^+, K^+, and Ca^{2+}.

Materials and Methods:

Plant culture and treatments:

All the studies were carried out on more salt-sensitive cultivar (cv. Giza 168) wheat (*Triticum aesativum* L.) and less salt-sensitive cultivar (cv. Sakha 1) broad bean (*Vicia faba*). Plants were grown in normal clay soil in plastic pots (30 cm in diameter) in greenhouse under natural conditions. The two plant species were grown under control condition (1 mM NaCl) and different salinity levels, (50 mM and 150 mM NaCl). After Harvesting, shoots and roots were separated and freshly weight then oven-dried at 80°C for 24 hours for dry weight measurements.

Cation analysis:

For cation analysis about 200 mg of dried plant materials (shoots and roots) were weight and dry-ashed at 550°C over night in a forced-air oven. After cooling, plant materials were digested in 5 M HNO3 by heating prior to boiling. Filtrate was filled up to volume with double-distilled water and analyzed for Na^+, K^+, and Ca^{2+}concentrations by flame-photometer model (M7D).

Parameters of Na^+- exclusion:

Sodium uptake at the root surface was calculated as follows:

$$(1)\ Na^+ \text{ exclusion at the root surface} = \frac{\text{Total plant } Na^+ \text{ content}}{\text{Root dry weight}}$$

This parameter served to characterize Na^+ exclusion at the root surface. Sodium translocation from root to shoot was described by:

$$(2)\ Na^+ \text{ translocation from root to shoot} = \frac{\text{Shoot } Na^+ \text{ content}}{\text{Root } Na^+ \text{ content}}$$

This method was recommended by Sümer et al. [53].

Determination of photosynthetic pigments:

The fractions of pigments (chlorophyll a, chlorophyll b and carotenoids) were estimated using the spectrophotometric method recommended by Lichtenthaler [25]. Chlorophylls and carotenoids concentrations were calculated as mg/g FW at 663, 644 and 452 nm.

Determination of lipid peroxidation:

Lipid peroxidation was determined by measuring malondialdehyde (MDA) formation using the thiobarbituric acid reaction as described by Madhava Rao and Sresty [26]. The concentration of MDA was calculated by using an extinction coefficient (1 55 mM^{-1} cm^{-1}) and the results expressed as SM MDA/g FW.

Determination of free proline content:

Free proline content was determined according to Bates et al. [27]. Proline concentration was determined using calibration curve of proline and expressed as mg/g DW.

Determination of total carbohydrates:

The anthrone-sulphuric acid method [28, 29] was used for the determination of carbohydrates. A calibration curve using pure glucose was made and the data expressed as mg glucose/g DW.

Determination of total proteins:

Total proteins were determined using alkaline reagent solution according to the method of Lowery et. al. [30]. A calibration curve was constructed using egg albumin and the data were expressed as mg protein/ g DW.

Catalase activity (EC 1.11.1.6):

Catalase activity was determined Spectrophotometrically by measuring the rate of H_2O_2 conversion to O_2. The Bioassay medium contained 2.9 ml of bioassay media (K-phosphate buffer at pH 7.5) containing 3 mM H_2O_2, 0.1ml of sample tissue extract. The decrease in absorbance at 240 nm was monitored and the resulted of catatase activity as (Δ abs240 mg^{-1} dry weight).

Peroxidase activity (EC 1.11.1.7):

Peroxidase activity was determined spectrophotometrically according to Adam et al. [31] with some modifications. The assay medium contained 2.5 ml of 100 mM K-phosphate buffer at (pH 5.5), 100 µl of 1 mM guiaicol and 0.1ml of enzyme

extract. The reaction was started by addition of 300 ml of 1.3 mM H_2O_2. The increase in absorbance at 470 nm was recorded. The resulted of peroxidase activity as (Δ abs470 mg^{-1} dry weight).

Results and discussion:

Shoots and roots dry matter production:

The dry matter production of both wheat and broad bean shoots and roots was highly decreased by salt stress. While, this reduction in wheat dry matter was much higher in shoots (about 50%) than in roots (about 35%) at the level of 150 mM NaCl (Fig. 1A). In broad bean plants, the magnitude of this reduction at the same NaCl concentration treatment was in shoots of about 67% and in roots of about 35% (Fig. 1B) in comparing with the control plants. These differences in salt tolerance among wheat and bean reveal the contrasting situation between both shoots and roots reduction in dry matter in the two plant species.

Salinity had adverse effects not only on the dry matter production of both shoot and root, but also on other morphological parameters such as plant fresh weight and plant height (data not show). Accordingly, the root /shoot ratio was much higher in wheat than in broad bean which reflecting that broad bean plants was less sensitive than wheat. Some authors reported that salinity reduces shoot and root weights [32, 33]. Accordingly, while wheat shoot, considered more sensitive than root, the opposite is true in broad bean whereas roots was more sensitive than shoots. This difference in the salt tolerance among species and genotype plants was also recorded [34, and 6]. Other plant growth criteria such as yield, leaf damage and plant height have been used for identifying salinity tolerance [35].

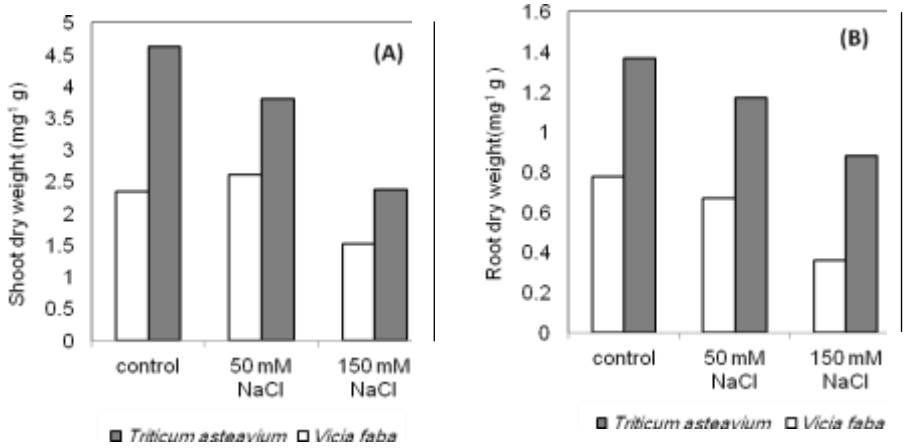

Figure (1): Absolute shoot dry matter production (A) and root dry matter production (B) of two plant species wheat and bean grown under different salinity levels (50 mM and 150 mM NaCl) and without NaCl treatment. Data represent means of 3 independent replications.

Cation analysis:

(A). Shoot and root Na^+ concentration:

Salinity tolerance was greatly conformed by the difference in the accumulation of Na^+, K^+ and Ca^{+2} ions in the plant organs under salt stress conditions. In our results, Na^+ concentration was increased in both wheat and broad bean as was as the plant organs. There are great differences in the accumulation and the compartmentalization of Na^+ among the different organs. In wheat, the absolute amount of Na^+ in shoot and root was 23.70 mg g^{-1} dry weight and 45.66 mg g^{-1} dry weight, respectively (Fig. 2A) while in bean, the absolute amount of Na^+ in shoot and root was 15.57 and 26.11 mg g^{-1} dry weight, respectively (Fig. 2B).The variations in Na^+ concentration accumulation means that broad bean plants can avoid Na^+ partially rather than wheat. The absorption and accumulation of Na^+ ion in wheat increased by more than 50 % than broad bean in the two plant organs (shoot and root). Such avoidance mechanism is an important strategy for increasing the salt tolerance in glycophtic plants, thus, it could reduce the toxicity of Na^+ ions of shoots in broad bean, which in turn may be responsible for stability of Chl.a and Chl.b under salt stress condition.

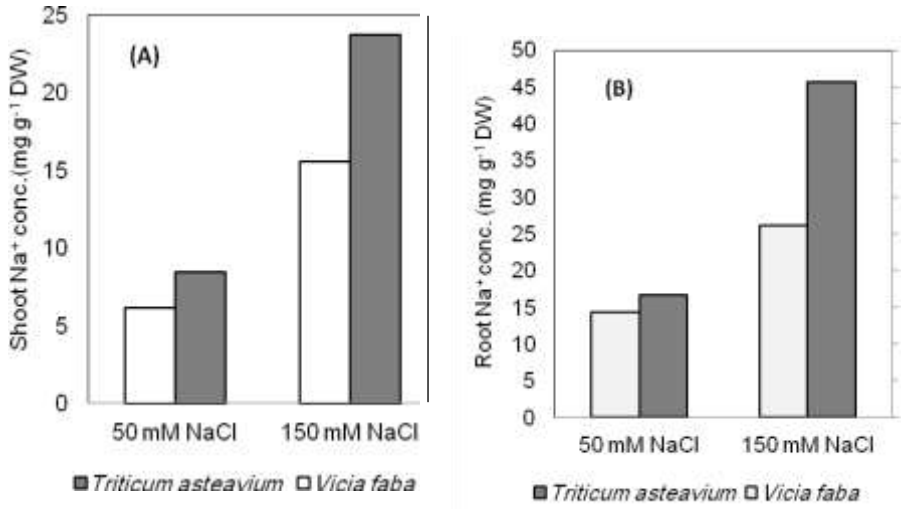

Figure (2): Sodium ion concentration in shoot and root dry weights of two plant species wheat and bean grown under different salinity treatment (50 and 150 mM NaCl). Data are means of three replicates ± SE.

(B). Na^+ uptake and translocation:

Na^+ uptake by the roots and translocation of Na^+ from root to shoot was studied under low and high salt treatment (50 mM and 150 mM NaCl) (Fig. 3A and B). In contrast to low salinity, a high salinity treatment led to significantly increased Na^+ uptake and Na^+ translocation in two plant species. At 50 mM NaCl, Na^+ uptake and translocation showed unchanged in the two tested plant species. On the other hand, under high salinity (150 mM NaCl) the wheat plant showed the higher Na^+ uptake

by the roots (92.28 mg g-1 DW) and also the highest Na^+ translocation to the shoot (2.53) (Fig. 3B), as compared to the broad bean plant which showed lowest Na^+ translocation from root to shoot (1.41) but at the same time showed highest Na^+ uptake by the roots (109.81 mg g-1 DW) (Fig. 3A). These results are in agreement with results of Munns [3], Saneoka et al. [21] and Hasegawa et al. [13], they observed that, Na^+ accumulation causes osmotic stress by decreasing root water potential and in order to maintain osmotic homeostasis in the cytosol of plant cell under salinity the accumulation of osmotic active substances must be increased. Therefore, Mimura et al.; Pitann et al. and Belkheiria and Mulas [49, 6, 50], suggested that a typical strategy of glycophytes is the inclusion of Na^+ into vacuoles mediated by tonoplast Na^+/H^+ antiporters and which its use as osmotic regulation. Such strategy may contribute to avoid excess Na^+ uptake at root level in broad bean. The Na^+ accumulation in root was higher than in shoot in both plant species, suggesting a possible role of Na^+ inclusion into root vacuoles for limiting Na^+ transport to the root xylem then into the shoot of both plant species (Fig.3).

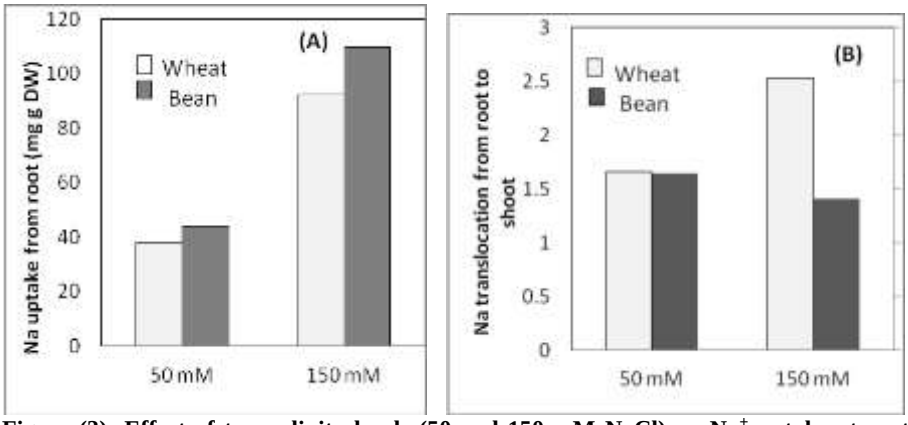

Figure (3): Effect of two salinity levels (50 and 150 mM NaCl) on Na^+ uptake at root surface (A) and Na^+ translocation to the shoot (B) of the two plant species wheat and broad bean. The results represent means ± SE of the three replicates.

(C). Shoot and root K^+ concentration:

The concentration of K^+ highly decreased significantly and suddenly by salt stress in wheat and bean plants (Fig. 4A and B). Unlike Na^+, excessive reduction in K^+ content was much higher in roots than shoots in both plant species. Interestingly, the absolute amount of K^+ concentration was much higher in wheat than in bean as was the case of Na^+ accumulation; a similar result were reported by [51], where they found that the salt sensitive wheat genotype showed higher K^+ concentration than the salt tolerance one.

On the other hand, the trend of the accumulation and compartmentalization of K^+ ions seem to be the same in the two plant species. Similarly, Pittan et al., [6]

found no significant differences in the accumulation of K^+ ion in the plant species maize hybrids that were grown under high salinity level (200 mM NaCl). According to our results, the high accumulation of K^+ in wheat more than in broad bean did not beneficial in this plant species, thus confirming the problematic role of K^+ under stress conditions reported by Maathuis and Amtmann, [52] who suggested that due to physicochemical similarities between Na^+ and K^+, excess Na^+ antagonistically competes with K^+ uptake leading to Na^+ will passively influxes into the cytosol of plant cell.

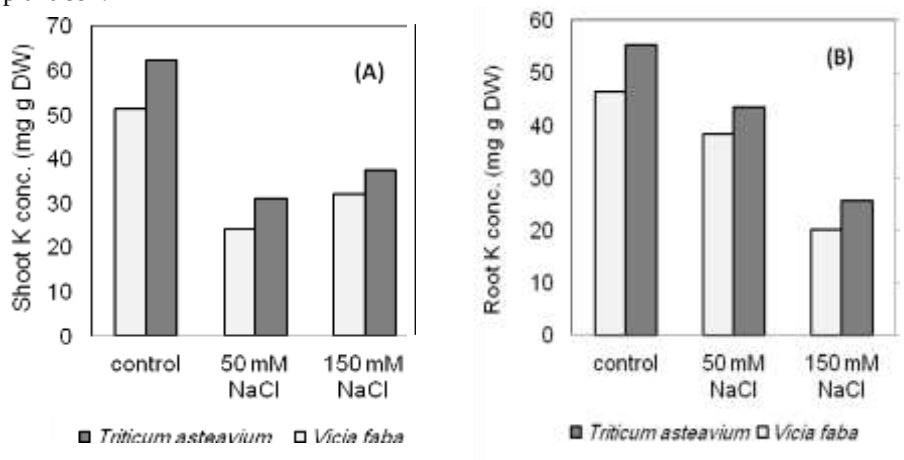

Figure (4): Potassium concentration in the shoot (A) and root dry weights (B), of two plant species wheat and broad bean grown under control (1 mM NaCl) and different salinity treatment (50 and 150 mM NaCl). Data are means of three replicates ± SE.

(D). Shoot and root Ca^{+2} concentrations:

Even Ca^{+2} contents remained almost unchanged at 50 mM NaCl in shoots and roots of both plants, great reductions were calculated at 150 mM NaCl. Interestingly, the reduction of Ca^{+2} contents in shoot and root of wheat plants was ca. 60% and ca. 56%, respectively, It was only about ca. 37% in shoot and root of bean (Fig. 5A and B). While Ca^{+2} is reported [53] to play a major role in salt tolerance, most workers, [53-55] observed a depressive effect of Na^+ and Ca^{+2} uptake which they may compete much more for the common uptake sites, this may explain the great reduction in Ca^+ with the increase in NaCl concentration.

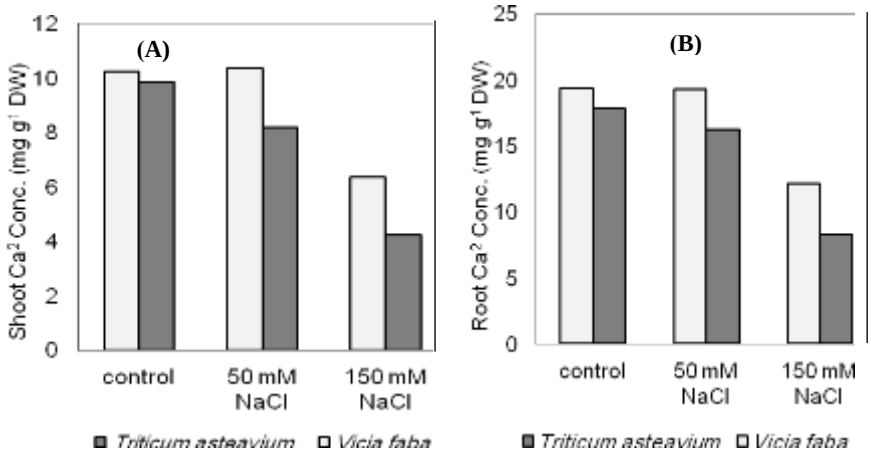

Figure (5): Calcium concentration in the shoot and root dry weights of two plant species wheat and broad bean under control (1 mM NaCl) and different salinity treatment (50 and 150 mM NaCl). Data are means of three replicates ± SE.

Photosynthetic pigments:

The data of photosynthetic pigments in bean plant revealed that while Chl.a and Chl.b almost unchanged, carotenes were reduced gradually by increasing salinity stress comparing to control plants (Fig. 5A). The total photosynthetic pigments reduced slightly, the Chl.a/Chl.b ratio did not affected and it remained about the control values. In wheat plants, the data reveals that the concentration of all photosynthetic pigments did not affected by 50 mM NaCl but they were markedly reduced at at 150 mM NaCl (Fig. 5A). Both Chl.b and carotnoids were much reduced than Chl.a and the Chl.a/Chl.b ratio was remarkably elevated at that concentration, the pervious results indicated that, the stability of Chl.a is more than Chl.b under severs salinity stress.

Similar data were obtained by Shaddad et al. and Ahmed et al. [36, 2], they observed that, chlorophyll content in plants was decreased after exposure to high salinity stress. Therefore, the difference in Chl.a and Chl.b among the two salinizied plant species could be used as suitable selection criterion for the differences in the salt tolerance among broad bean and wheat plants. In this respect, the reduction in photosynthetic pigments has been suggested to be related to the activation of chlorophyllase enzyme which catalyses the catabolism of chlorophyll as reported by [37]. This is in harmony with the difference in the accumulation of carbohydrates and proteins among the two tested plant species which has been observed later in this study.

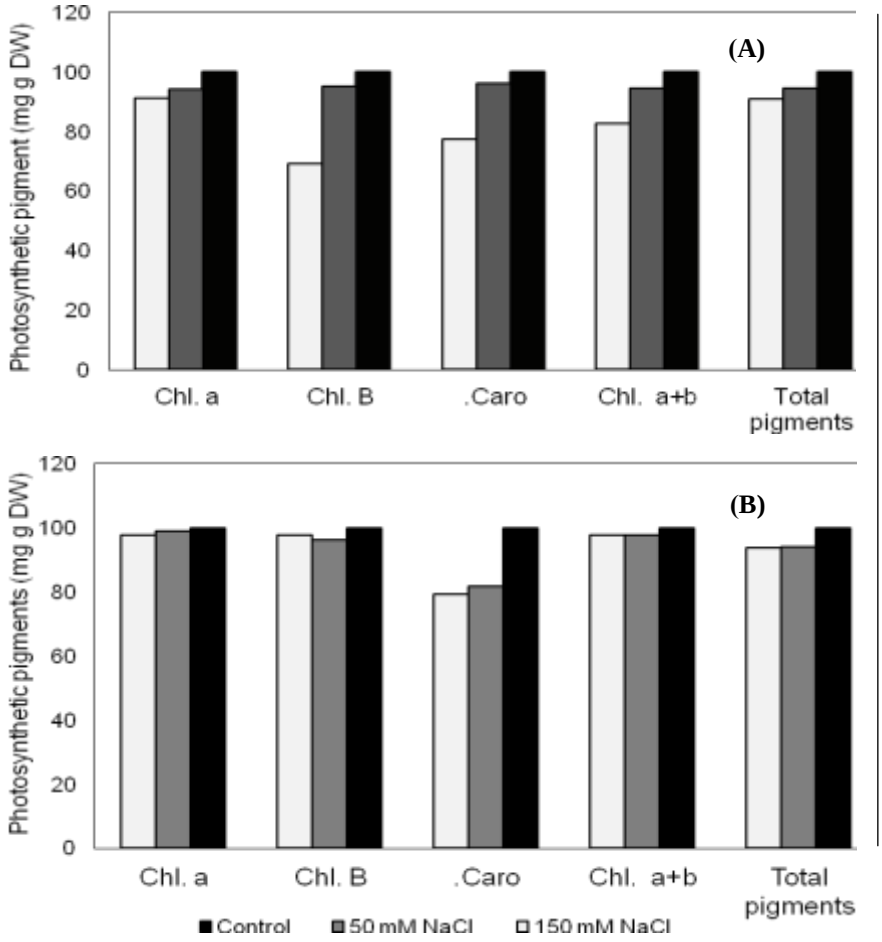

Figure (6): Effects of salinity stress on photosynthetic pigments of (cv. Giza 168) wheat (*Triticum aestivium* L.), (A) and (cv. Sakha 1) broad bean (*Vicia faba*), (B) grown under control (1 mM NaCl) and different salinity treatment (50 and 150 mM NaCl). Data are means of three replicates ± SE.

Carbohydrate and protein contents:

The results reported in this paper showed that, the carbohydrate contents were increased progressively in both plant species (wheat and broad bean). The absolute amount of carbohydrates in wheat was much higher than in broad bean even under the normal conditions. The data of protein content indicated that in wheat protein content increased marginally up to 50 mM NaCl then sharp increased was recorded. Protein content was increased by about 60% over the control at 150 mM NaCl. In broad bean protein content increased slightly up to 50 mM NaCl and more markedly at 150 mM NaCl. Also, as in the case of carbohydrates, protein content was much higher in wheat than in bean. Such increased level of carbohydrates and proteins, in

the two plant species under salt stress condition was accompanied with observed reduction in plant growth. This means that the two plants have ability to transport carbohydrates and proteins from state of growth to state of survival (osmo-regulation).

Also, the production of carbohydrates and proteins in broad bean was much higher than in wheat. Thus, the up-regulation of photosynthetic pigments in broad bean could increase the efficiently of photosynthetic apparatus in broad bean which leads to increasing the food manufacturing (carbohydrates and proteins) in broad bean than in wheat. Other investigators, [38] reported that, the increased carbohydrate contents under salt stress could be good parameter to identify salt-tolerant wheat plants. This increasing in the total carbohydrate contents act as osmoregulation mechanism [39, 40] and also it used to protect the plants from oxidative stress and maintain the protein and membrane structure [41].

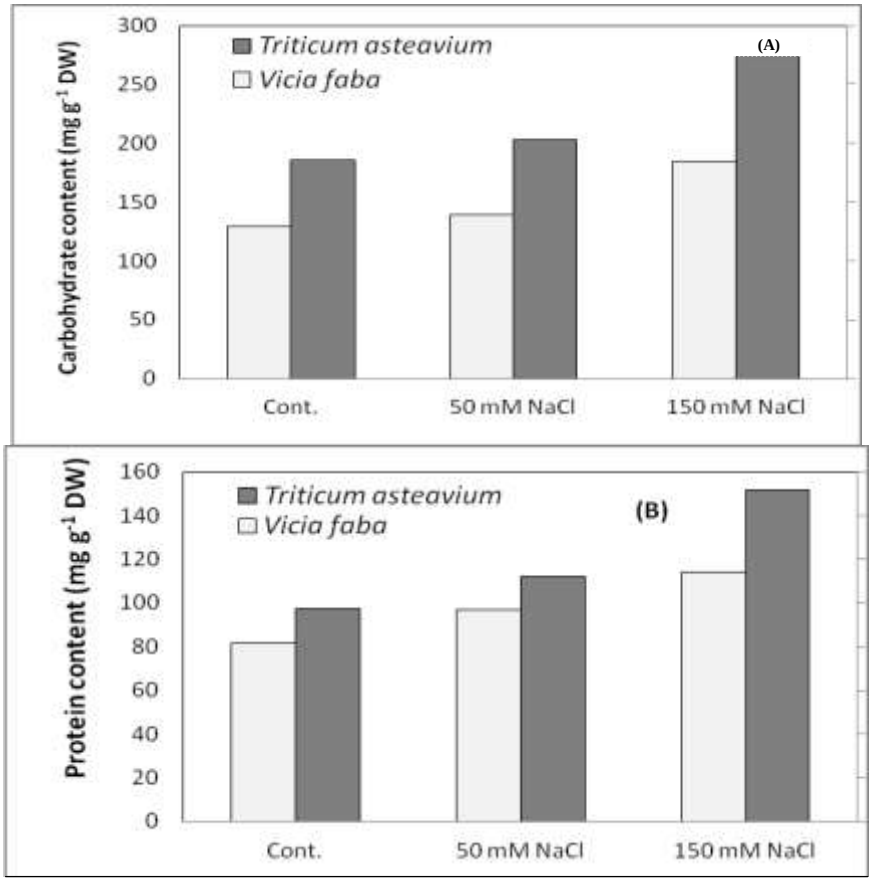

Figure (7): Effects of salinity stress on the total carbohydrate content (A) and protein (B) in the two plant species wheat and broad bean grown under (1 mM NaCl) and different salinity treatment (50 and 150 mM NaCl).

Proline and Lipid peroxides (MDA):

There is markedly and progressively increasing trend in proline content as the salinity stress increased in the shoot of both plant species. However, the accumulation percentage of proline was much higher in broad bean than in wheat, regardless of the plant organ had been tested and the salinity level had been used (Fig. 8A). At the level of 150 mN NaCl, proline percentages increased and they were 153% and 256% in both wheat and bean, respectively.

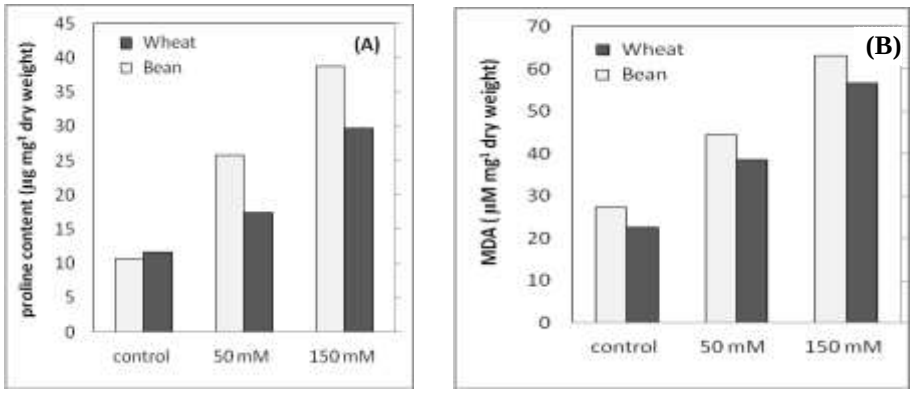

Figure 8: Proline content (A) and MDA content (B) of wheat and broad bean plants grown under control and different salinity levels (50 mM and 150 mM NaCl). Data are means of three replicates ± SE.

The accumulation of both proline content and lipid peroxides (MDA) showed higher values in broad bean than in wheat under all NaCl concentrations (Fig. 8B). Such results suggested that salinity increased markedly the proline accumulation in different salt-treated plant species which is supposed to positive correlate with the adaptation to salinity as reported by [42, 43]. Also, [44] found the increased levels of proline in the two wheat species under sever salt stress. Other investigators have reported that proline accumulation may be suitable parameter to selected salt tolerance plants from salt sensitive ones, [45, 46, 2]. It was reported that the higher concentration of proline under salt stress increases the stress tolerance of the plants through such functions as the protection of enzymes against denaturation, the stabilization of protein synthesis and osmoregulation and increase membrane stability under various conditions [47]. It may also help in non-enzymatic free radical detoxifications [48].

Catalase and peroxidase activity

Data of catalase activity of two plant species wheat and broad bean showed no difference between the two plant species grown under control and 50 mM NaCl. At 150 mM NaCl catalase activity increased in broad bean but not in wheat (Fig. 9A). While catalase activity was increased when plant grown under 150 mM NaCl, preoxidase activity was increased slightly at 150 mM NaCl, (Fig.9 B). However, there is no difference between the two plants for increasing the activity of catalase and peroxidase enzymes especially at 50 mM NaCl, (Fig. 9A and B).

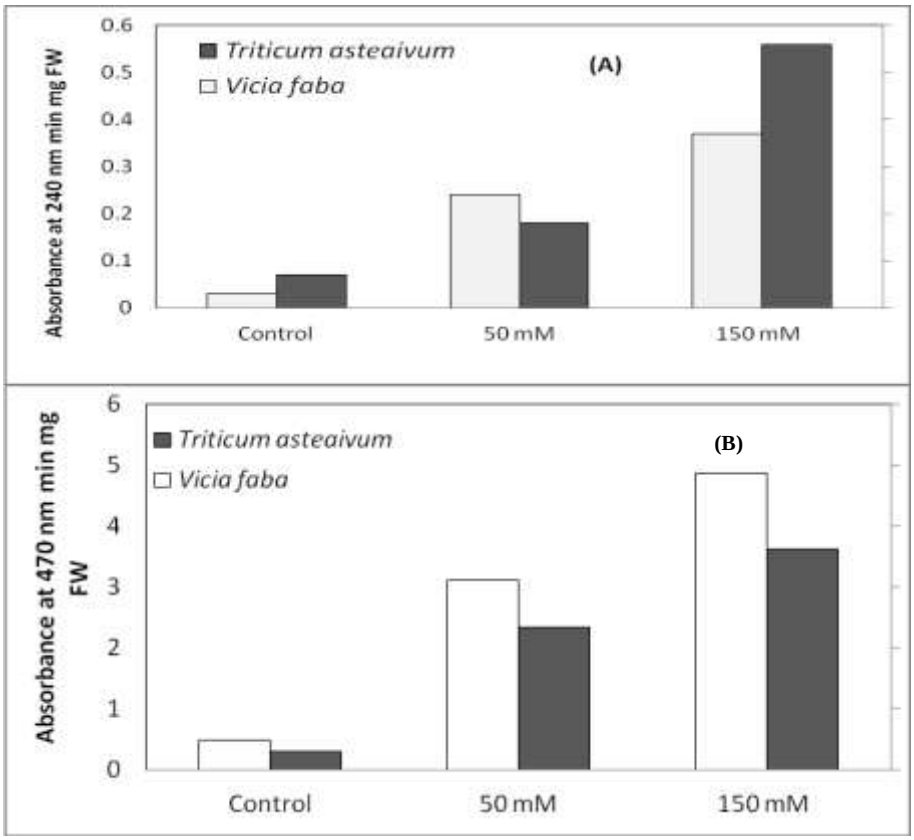

Figure (9): Catalase activity (A) and Peroxidase activity (B) of two plant species wheat (*Triticum asteavium*) and broad bean (*Vicia faba* cv. Sakha 1) grown at control (1 mM NaCl) and different salinity levels (50 mM and 150 mM NaCl.

The activity of catalase and peroxidase varied among the two plant species as well as the salinity level used. It's worth mention that, Catalase enzyme was higher in broad bean than in wheat. Accompanied with higher accumulation of proline in broad bean interestingly leads to the reduction in MDA content in comparison to wheat. This means that both catalase enzyme and proline play pivotal role in scavenging the free radicals which decrease the membrane stability and cause partial up-regulation of cell membrane. Interestingly, the activity of peroxidase enzyme was much higher in wheat than in broad bean. Therefore, peroxidase enzyme is a sign for sensitive rather than tolerance. Accordingly, proline as non enzymatic antioxidant component and catalase as an antioxidant enzyme could be used as a suitable selection parameter for the difference between salt tolerances among the two plant species wheat and broad bean.

Conclusion

The most striking feature in this work is that not only the two plant species responded differentially to the salt stress but also their plant organs. The reduction of the dry matter in both shoots and roots, fresh weight, plant height and root/shoot ratio, yield, leaf damage and plant height have been used for identifying salinity tolerance. In this study, we adapted other salinity tolerance criteria such photosynthetic pigments data and a/b ratio which had been suggested to be related to the activation of chlorophyllase enzyme which catalyses the catabolism of chlorophyll. This in a line with the difference in the accumulation of carbohydrates and proteins among the two tested plant species, Thus, the up-regulation of photosynthetic pigments in broad bean could increase the efficiently of photosynthetic apparatus in broad bean which leads to increasing the food manufacturing (carbohydrates and proteins) in broad bean than in wheat. The increased level of carbohydrates and proteins, in the two plant species under salt stress condition was accompanied with observed reduction in plant growth. This means that the two plants have ability to transporter carbohydrates and proteins from state of growth to state of survival (osmo-regulation). This was also confirmed by the accumulation of proline especially in broad bean which is supposed to positively correlate with the adaptation to salinity.

This was greatly marauded by great difference in the accumulation of Na^+, K^+ and Ca^{+2} ions in the plant organs under salt stress conditions. Avoidance mechanism of broad bean plants could reduce the toxicity of Na^+ ions of shoots and also could be responsible for stability of Chl.a and Chl.b under salt stress condition, which consequently increased production of organic matter in broad bean than in wheat. Also, Bean plants absorb and accumulate sufficient amount of Ca^{+2} more than wheat plants and the trend of accumulation of K^+ ions seem to be the same in both plant species. On the other hand, the activity of catalase and peroxidase varied among the two plant species and this was accompanied with higher accumulation of proline in broad bean which interestingly leads to the reduction in MDA content in comparison to wheat. Based on our results, we recommended that the cultivation of broad bean is the moderate saline soil in Egypt.

Reference:

1. Wang H., Wu Z., Zhou Y., Han J. and Shi D. (2012): Effects of salt stress on ion balance and nitrogen metabolism in rice. Plant Soil Environ. 58 (2):62-67.
2. Ahmed I.M., Dai H., Zheng W., Cao F., Zhang W., Sui and Wu F. (2013): Genotypic difference in physiological characteristic in the tolerance to drought and salinity combined stress between Tibetan wild and cultivar barley. Plant Physiol. Biochem., 63: 49-60.
3. Munns R. (2002): Comparative physiology of salt and water stress. Plant, Cell &Environ. 25(2): 239-250.
4. Hajlaoui H., El Ayeb N., Garrec J.P. and Denden M. (2010): Differential effects of salt stress on osmotic adjustment and solutes allocation on the basis of root and leaf tissue senescence of two silage maize (Zea mays L.) varieties. Indus.Crops. Prod., 31: 122-130.

5. Hasanuzzaman M., Nahar K., and Fujita M. (2013): Plant Response to Salt Stress and Role of Exogenous Protectants to Mitigate Salt-Induced Damages. P. Ahmed et al.(eds.), ecophysiology and response of plant under salt stress, 1: 133-147.

6. Pitann B., Mohamed A., Neubert A. and Schubert S. (2013): Tonoplast Na^+/H^+ antiporters of newly developed maize (Zea mays) hybrids contribute to salt resistance during the second phase of salt stress. J. Plant Nutr. Soil Sci., 176 (2): 148-156.

7. Schubert S., Neubert A., Schierholt A., Sűmer A. and Zörb C. (2009): Development of salt-resistant maize hybrids: The combination of physiological strategies using conventional breeding methods. Plant Sci., 17:196–202

8. Tester M. and Davenport R. (2003): Na^+ tolerance and Na^+ transport in higher plants, Ann. Bot. 91: 503–527.

9. Blumwald E. (2000): Sodium transport in plant cells. Biochim. Biophys. Acta 1465, 140-151.

10. Blumwald E., Aharon G.S. and Apse M.P. (2000): Na^+ transport in plant cells. Biochim. Biophys. Acta 1465, 140–151.

11. He T. and Cramer G.R. (1993): Salt tolerance of rapid cycling Brassica species, in relation to potassium sodium ratio and selectivity at the whole plant and callus level. J. Plant Nutr. 16: 1263-1277.

12. Yamaguchi T., Aharon G.S., Sottosanto J.B. and Blumwald E. (2005): Vacuolar Na^+/H^+ antiporter cation selectivity is regulated by calmodulin from within the vacuole in a Ca^{2+}-and pH-dependent manner. PNAS 102, 16107-16112.

13. Hasegawa P.M., Bressan R.A., Zhu J-K. and Bohnert H.J. (2000): Plant cellular and molecular responses to high salinity. Annu. Rev. Plant Physiol. Plant Mol. Biol. 51, 463-499.

14. Brini F., Hanin M., Mezghani I., Berkowitz G.A. and Masmoudi K. (2007): Overexpression of wheat Na^+/H^+ antiporter TNHX1 and H^+-pyrophosphatase TVP1 improve salt- and drought-stress tolerance in Arabidopsis thaliana plants. J. Exp. Bot. 58, 301-308.

15. Stepien P. and Klobus. G. (2006): Water relations and photosynthesis in Cucumis sativus L. leaves under salt stress. Biol. Plant., 50: 610-616.

16. Shaddad M.A.K., Abd El-Samad H.M. and Ragaei M.M. (2008): Drought Tolerance of Wheat Genotypes at the Early Growth Stage. Assiut Univ. J. Bot. 37(2):93-103.

17. Ahuja I., Vos C.H., Bones M. and Robert D. H. (2010): Plant molecular stress responses face climate change. Trands plant Sci., 15:664-674.

18. Mane A.V., Karadge B.A., and Samant J.S. (2010): Salinity induced change in photosynthetic pigments and polyphenols of Cymbopogon Nardus (L.) Rendle. J. Chem. And Pharm. Resea., 2 (3): 338-352.

19. Romero Aranda R., Soria T. and Cuartero J. (2001): Tomato plant water uptake and plant water relationships undergrowth condition. Plant Sci., 160: 265-272.

20. De Pascale S., Maggio A., Fogliano V., Ambrosino P. and Ritieni A. (2001): Irrigation with saline water improves carotenoids content and antioxidant activity of tomato. J. Hort. Sci. Biotec. 76:447-453.

21. Saneoka H., Nagasaka C., Hahn D.T., Yang W.J., Premachandra G.S., Joly R.J. and Rhodes D. (1995): Salt resistance of glycinebetaine-deficient and -containing maize lines. Plant Physiol. 107, 631-638.

22. Wang H., Miyazaki S., Kawai K., Deyholos M., Galbraith D.W. and Bohnert H.J. (2003): Temporal progression of gene expression responses to salt shock in maize roots. Plant Mol. Biol. 52, 873-891.

23. Yancey P.H. (1994): Compatible and counteracting solutes. In: K Strange (ed.) Cellular and Molecular Physiology of Cell Volume Regulation. CRC Press, Boca Raton, FL, 81-109.

24. Sakamoto A. and Murata N. (2000): Genetic engineering of glycinebetaine synthesis in plants: current status and implications for enhancement of stress tolerance. J. Exp. Bot. 51, 81-88.

25. Lichtenthaler H.K. (1987): Chlorophylls and caroteniods pigments of photosynthetic biomembranes in Methods in Enzymology vol. 148, 183-350. Academic Press Orlando F.I., USA.

26. Madhava Rao K.V., and Sresty T.V.S. (1989): Antioxidative parameters in the seedlings of pigeonpea in response to Zn and Ni stresses. Plant Sci., 2000, 157: 113-128.

27. Bates L.S., Waldren, R.P. and Teare I.D. (1973): Rapid determination of free proline for water stress studies. Plant Soil 39: 205-207.

28. Fales F.W. (1951): The assimilation and degradation of carbohydrates by yeast cells. J. Biol chem., 193: 113-118.

29. Schlegel H.G. (1956): Verwertung organischeer sauren durch chlorella in lichta Planta. 47: 510- 526.

30 Lowery O.H., Rosebough N.J., Farr A.L., Randall R.J. (1951): Protein measurement with foline phenol reagent. J. Biol.Chem., 1951, 193: 265-275.

31. Adam A.L., Bestwick C.L., Barna B. and Mansfield J.W. (1995): Enzymes regulating the accumulation of active oxygen species during the hypersensitive reaction of bean to Pseudomonas syringae pv. Phaseolicola. Planta. 197: 240-249.

32. Tavakkoli E., Fatehi F., Conentry S., Rengasamy P. and McDonald G.K. (2011): Additive effects of Na and Cl ions on barley growth under salinity stress. J. Exp. Bot., 62: 2189-2203.

33. Orsini F., Alnayef M., Bona A., Maggio, A. and Gianquinto, G. (2012): low stomatal density and reduced transpiration facilitate strawberry adaptation to salinity. Environ. And Experim. Bot., 81: 1-10.

34. Schubert S. and Zörb C. (2005): The physiological basis for improving salt resistance in maize. In: Plant Nutrition for Food Security, Human Health and Environmental Protection (CJ Li et al., eds.) Tsinghua University Press, Beijing, China, 540-541.

35. Eker S., Gönül C., Ömer K., , Ahmet Ü., Öztürk C. and Çakmak L.Ý. (2006): Effect of salinity stress on dry matter production and ion accumulation in hybrid maize varieties.Turkish J. Agric. and Forestry, 30 (5): 365-373.

36. Shaddad M.A.K, Abd El-Samad M.H. and Mohammed H.T. (2011): Interactive Effects of Drought Stress and Phytohormones or Polyamines on Growth and Yield of Two M (Zea maize L.) Genotypes. Am. J. Plant Sci. Am. J. Plant Sci. 2:790-807.

37. Majumdar S., Ghosh S., Glick B.R. and Dumbroff E.B. (1991): Activities of chlorophyllase, phosphoenolpyruvate carboxylase and ribulose-1,5-bisphosphate carboxylase in the primary leaves of soybean during senescence and drought. 81 (4): 473-480.

38. Naureen G., and Naqvi F. N. (2010): Salt tolerance classification in wheat genotypes using reducing sugar accumulation and growth characteristics. Emir. J. Food and Agri., 22(4): 308-317.

39. Shaddad M. A., Radi A. F., Abdel-Rahman A. M. and Azooz M. M. (1990): Response of seeds of Lupinus termis and Vicia faba to the interactive effect of salinity and ascorbic acid or pyridoxine. Plant and Soil, 122(2):177-183.

40. Siringam K., Niran J., Suriyan C., Thanapol B. and Copyright K. C. (2012): Salt tolerance enhancement in indica rice (Oryza sativa L. spp. indica) seedlings using exogenous sucrose supplementation. Plant Omics J., 5(1): 52-59.

41. Hajihashem S., Kiarostami K., Enteshari S. and Saboora A. (2006): The Effects of Salt Stress and Paclobutrazol on Some Physiological Parameters of Two Salt-tolerant and Salt-sensitive Cultivars of Wheat. Pakist. J. Bio. Sci. 9: 1370-1374.

42. Asharf M. and Harris, P.J.C. (2004): Potential biochemical indicators of salinity tolerance in plants. Plant Sci., 166: 3-16.

43. Mansour M.M., Salama K.H., Ali F.Z. and Abou Hadid A.F. (2005): Cell and plant response to NaCl in Zea Mays L. cultivars differeing in salt tolerance. Gen. Appl. Plant Physiol. 31: 29-41.

44. Khatkar G. and Kuhad M.S. (2000): Short-trem salinity induced in two wheat cultivars at different growth stages. Bio. Plantu. 43 (4): 629-632.

45. Azooz M. M., Youssef A.M. and Ahmed P. (2011): Evaluation of salicylic acid (SA) application on growth, osmotic solutes and antioxidant enzyme activities on broad bean seedling grown under diluted seawater. Int. J. Plant Physiol. Biochem., 3 (4): 253-264.

46. Hussein O.S., Ahmed A.H.H., Ghalab A.R. and El-Hefny A.M. (2012): Some active ingredients, total protein and amino acids in plants produced from irradiated Ambrosia maritima seeds growing under different soil salinity levels. Amer. J. of Plant Physiol., 7 (2): 70-83.

47. Desingh, R. and Kanagaraj G. (2007): Influence of salinity stress on photosynthesis and ant oxidative systems in two cotton varieties. Gen Appl. Plant Phyiol., 33 (3-4): 221-234.

48. Khan M.A., Shirazi M.U., Khan M.A., Mujtaba S. M., Islam E., Mumtaz S., Shereen A., Ansari R.U. and Yasin M. (2009): Role of proline, K Na ratio and chlorophyll content in salt tolerance of wheat (Triticum aestivum L.). Pak. J. Bot., 41 (2): 633-638.

49. Mimura T. Kura-Hotta M., Tsujimura T., Ohnishi M., Miura M., Okazaki Y., Mimura M., Maeshima M., and Washitani-Nemoto S. (2003): Rapid increase of vascular volume in response to salt stress. Planta, 216: 397- 402.

50. Belkheiria O., and Mulas M. (2013): The effects of salt stress on growth, water relations and ion accumulation in two halophyte Atriplex species. Environ. Experi. Bot., 17–28.

51. Cuin T.A., Betts S.A., Chalmandrier R. and Shabala S. (2008): A root's ability to retain K^+ correlates with salt tolerance in wheat. J. Exper. Bot. 59 (10): 2697-2706.

52. Maathuis J.M. and Amtmann N.A. (1999): K^+ Nutrition and Na^+ Toxicity: The Basis of Cellular K^+/Na^+ Ratios. Ann. Bot. 84: 123-133.

53. Sümer A., Zörb C. and Schubert S. (2004): Evidence of Na^+ toxicity for the vegetative growth of maize (Zea mays L.) during the first phase of salt stress. J. Appl. Bot. 78: 135-139.