

Enhance salt stress tolerance in wheat (*Triticum aestivum* L.) plant using exogenous β -carotene or algal extract

Khaulood A. Hemida^{1*}, Refaat M. Ali¹ and Wael M. Ibrahim¹.

Abstract— This study was designed to examine the effect of exogenous application of β -carotene and algal extract to mitigate the deleterious effect of salt stress in wheat plants. Generally, salt stress induced changes in the contents of compatible osmolytes and some antioxidants compounds in the different plant parts of *T. aestivum*. The results indicated that applying of β -carotene or algal extract can be used to ameliorate the deleterious effect of salt stress and could be used to enhance the level of free amino acids, proline, glycine betaine, choline, glutathione, ascorbic acid, phenolic contents and total antioxidant activity in both shoot and grains.

Keywords— β -carotene, *Ulva lactuca*, wheat, osmolytes, antioxidants

I. Introduction

Plants are sessile organisms constantly challenged by a wide spectrum of biotic and abiotic stress (Zia *et al.*, 2006; Jamil *et al.*, 2010; Osakabe *et al.*, 2011).

Soil salinity is one among the major abiotic stresses that adversely affects plant productivity and metabolism (Parvaiz and Satyawati, 2008; Purty *et al.*, 2008, Ali *et al.*, 2008, 2009 and Hameed *et al.*, 2008).

Egypt is one of the countries that suffer from severe salinity problems.

One of the biochemical changes occurring when plants are subjected to environmental stresses is the production of reactive oxygen species such as superoxide, hydrogen peroxide and hydroxyl radicals (Poontariga *et al.*, 2003 and Rahnama and Ebrahimzadeh, 2006). These ROS have a high capacity for oxidation of lipids, nucleic acids and proteins, leading to extensive cell damage (Badawi *et al.*, 2004b; Mittova *et al.*, 2004 and Lu *et al.*, 2005).

¹-Khaulood A. Hemida
Botany Department, Faculty of Science, Fayoum University
Fayoum, Egypt.
Tel.: +201009690769-Fax.: +20846370025

²-Refaat M. Ali
Botany Department, Faculty of Science, Fayoum University
Fayoum, Egypt.
Tel.: +201285021116-Fax.: +20846370025

³- Wael M. Ibrahim
Botany Department, Faculty of Science, Fayoum University
Fayoum, Egypt.
Tel.: +0201098001146 -Fax.: +20846370025

Exogenous application of antioxidant has been shown to protect against various stress conditions such as drought and salinity (Zhang li xin *et al.*, 2011; Ejaz *et al.*, 2012 and Demiralay *et al.*, 2013). Among the different groups of naturally occurring antioxidant from plants, carotenoids (Han *et al.*, 2004; Tuna *et al.*, 2013).

Application of algae extracts could be provide protection against this oxidative stress by increase the antioxidant protective system, which involved as one of the factor responsible for salt tolerance of wheat plants (Abd El-Baky *et al.*, 2008).

A number of studies indicated that the degree of oxidative cellular damage in plants exposed to abiotic stress is controlled by the capacity of antioxidant systems (Omami *et al.*, 2006 and Gao *et al.*, 2008; Ali *et al.*, 2008, 2009). However, the response of foliar application to salinity on wheat plants was poorly investigated. Thus, the present work was conducted to study the response of β -carotene or algal extract foliar application to various levels of salinity. The interactive effect of salinity and foliar application of antioxidants (β -carotene and Algal extract) on chemical composition of the test plant *Triticum aestivum* L were considered in the current studies.

II. Materials and Methods

Grains of wheat (*Triticum aestivum* L.) which are used in this present study were obtained from Horticultural Research Institute, Agricultural Research Center, Ministry of Agriculture, Giza, Egypt.

Ulva lactuca were collected from Lake Qarun, (Fayoum government, Egypt) in summer season 2011. The collected algal species was identified according to Nasr (1940) and Jha *et al.* (2009). The alga was washed with tap water then distilled water several times to remove impurities. The washed algae were air dried for 10 days, then grind to very powder.

The powder was stored in airtight polyethylene bottles until; required.

β -carotene was obtained from Sigma Aldrich Company, Egypt.

A. Experimental Design:

A greenhouse pot experiment was conducted to study the antioxidant potential of chosen concentrations of β -Carotene or algal extract on some physiological changes associated with the growth of wheat plants irrigated by saline

solutions. The pot experiment was carried out under natural conditions, in the experimental greenhouse of Botany Department, Faculty of Science, Fayoum University, during winter season.

A group of 126 pots (25 cm diameter x 30 cm depth) were filled with 10 kg of a mixture of clay/sand (1:1, W/W) soil. The pots were divided into 3 sets, each set with 54 pots except control with 18 pots. The control set was left free from antioxidant treatment while the other two sets treated with β -Carotene or algal extract. Each set was subdivided into 6 groups, including the control and 5 saline treatments each with 3 replicates.

In order to regulate the distribution of the irrigation solution, a finely perforated plastic tube was inserted in each pot at 2 cm distance from the center of the pot and 3/4 way down in the soil. After a further 7 days, the Hoagland's nutrient solution (Hewitt, 1966) supplemented with different NaCl concentrations (0, 50, 100, 150, 200 and 250mM) were used for irrigation.

Antioxidant (β - Carotene) or algal extract were sprayed 3 times at 1, 5 and 10mM for β - Carotene and 1, 5 and 10% w/v for algal extract. Untreated plants were sprayed with distilled water. The first spray was made 30 days after planting and repeated at 60 and 90 days. The plants were sprayed with a manual pressure pump at an average 10 cc per plant. In order to prevent the accumulation of salts, the soil in each pot was leached every ten days with excessive amount of distilled water.

B. Determination of glutathione

Glutathione was extracted by grinding 0.5g of plant tissues in 1% picric acid (w/v) under cold condition. After centrifugation at 10,000g for 10 min, the supernatant was collected immediately for assay. Glutathione was estimated according to Anderson (1985).

C. Estimation of vitamin C (Ascorbate)

The total ascorbic acid content was estimated using Folin phenol reagent according to Jagota and Dani (1982).

3.2.2.5 Estimation of free amino acids:

The tissue extracted and FAA was determined using spectrophotometer at 580nm according to (Muting & Kaiser, 1963).

D. Determination of proline:

Extraction and determination of proline was made according to the method described by Bates et al. (1973).

(QACS) in plants (Glycine – Betaine and Choline) was determined according to (Arakawa et al., 1990).

E. Determination of phenolic constituents:

Phenolic compounds were extracted from dried tissues according to method adopted by Sauvesty et al. (1992).

The Folin-Ciocalteu phenol method (Lowe, 1993) was used for phenolics determination.

F. Determination of antioxidant activity:

Determination of antioxidant activity using DPPH radical scavenging method For the 1, 1-diphenyl-2-picrylhydrazyl (DPPH) is according to modified Abe et al. (1998) assay.

G. Statistical analysis

The experimental design was a random complete block, with three replications. The data were analyzed by the STATGRAPHICS (Statistical Graphics Corporation, Princeton USA) statistical package by the t-test and ANOVA functions to assess significant differences among means.

III. Results

A. Compatible solutes:

The content of total free amino acid, glycine betaine and choline in shoot and grains of wheat decreasing significantly with the rise of salinization level. However, the content of proline in shoots and grains significantly increased with increasing salinity level Figure (1-2)

Foliar application with β -carotene or algal extract significantly increased proline, glycine betaine and choline while the free amino acid increased when treated with β -carotene but decreased when treated with algal extract in shoot. In grains when treated with β -carotene, free amino acid and choline increased but proline and glycine betaine decreased. On the other hand, in grains when treated with algal extract significantly increased free amino acid and proline only at concentration of 1% but glycine betaine and choline increased with increasing algal extract concentration.

Figure (1): Effect of NaCl on free amino acids, proline, glycine betaine and choline content (mg/g dry weight) of *Triticum aestivum* shoot and grains treated with different concentrations of β -carotene (a) control, (b) β -carotene(1mM), (c) β carotene (5mM) and (d) β -carotene(10mM). Data are the mean of three replicates and error bars represent the standard errors of the means

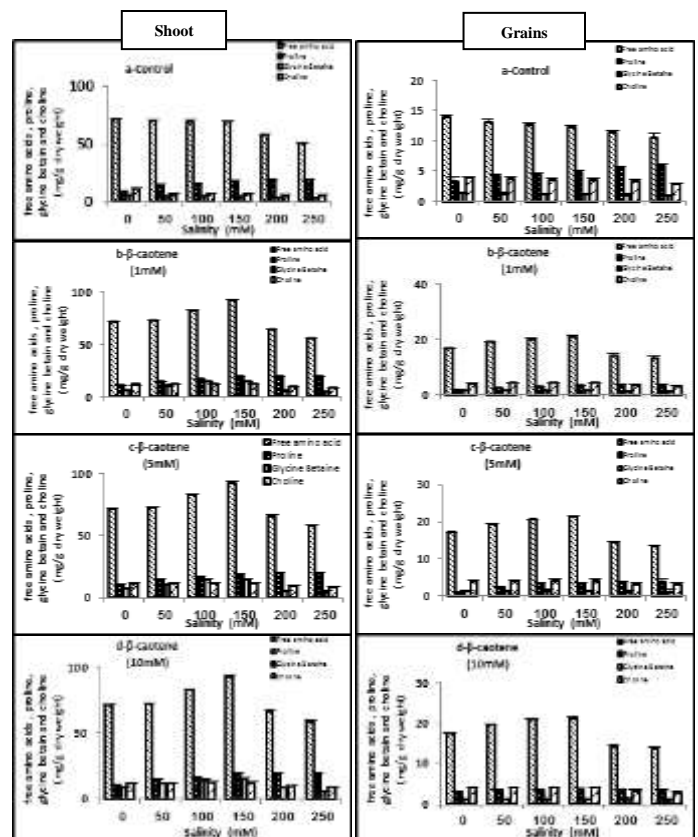
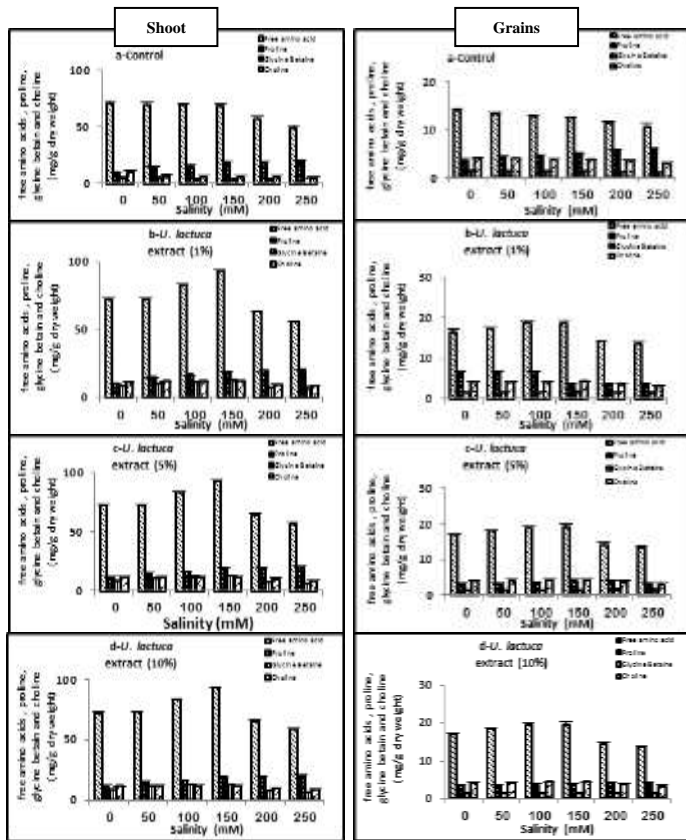


Figure (2): Effect of NaCl on free amino acids, proline, glycine betaine and choline content (mg/g dry weight) of *Triticum aestivum* shoot and grains treated with different concentrations of *Ulva lactuca* (a) control, (b) algal extract (1%), (c) algal extract (5%) and (d) algal extract (10%).
 Data are the mean of three replicates and error bars represent the standard errors of the means.



Application of β -carotene and treatment with NaCl increased free amino acid, glycine betaine and choline in shoot while increased free amino acids and choline in grains when compared with corresponding level of NaCl. Furthermore the proline content increased in shoots but in grains proline and glycine betaine decreased comparing to resembling levels of NaCl. On the other hand proline content increased in shoot but decreased in grains when compared with corresponding treatments with NaCl.

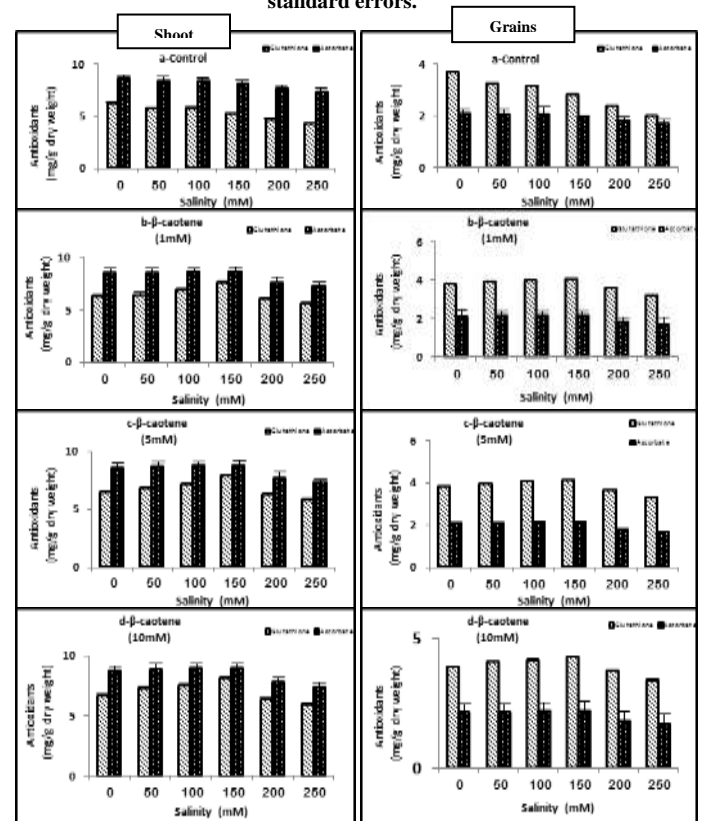
B. Antioxidant metabolites:

The contents of glutathione and ascorbate in shoot and grains of wheat plants decreased significantly with increasing NaCl level Figure (3-4).

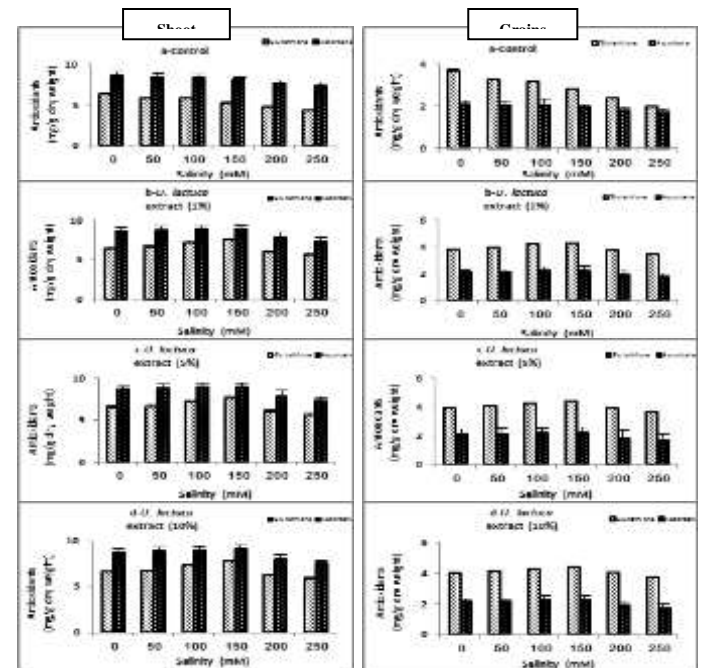
Foliar application with different concentrations of β -carotene or algal extract increased the glutathione and ascorbate in shoots and grains.

Application of β -carotene or algal extract in combination with NaCl treatments increased significantly the glutathione and ascorbate contents in shoot and grains when compared with corresponding levels of NaCl.

Figure (3): The effect of treatment with different concentrations of NaCl and β -carotene on glutathione and ascorbic acid (mg/g dry weight) of *Triticum aestivum* seedlings (a) control, (b) β -carotene(1mM), (c) β -carotene (5mM) and (d) β -carotene(10mM)..
 Data are the mean of three replicates and error bars represent the standard errors.



Figure(4): The effect of treatment with different concentrations of NaCl and algal extract on glutathione and ascorbic acid (mg/g dry weight) of *Triticum aestivum* seedlings (a) control, (b) algal extract (1%), (c) algal extract (5%) and (d) algal extract (10%).
 Data are the mean of three replicates and error bars represent the standard errors of the means.



C. Phenolic Content and total antioxidants:

The changes in the phenolic constituents of wheat plants after growth period as of treatment with different concentration of NaCl apart or in combination with different concentrations of either antioxidant or algal extract were represented in Figures (5-6).

Generally, the phenolic aglycone, total phenolic and total antioxidants in shoot and grains significantly increased with increasing NaCl levels. While, phenolic glycone decreased significantly.

Foliar application of either β -carotene or algal extract significantly increased the phenolic aglycone and total antioxidants content in both shoot and grains. However, when treated with β -carotene the phenolic glycone and total phenolic increased significantly in both shoot and grains with increasing antioxidants levels.

On the other, when treated with algal extract phenolic glycone in shoot and grains significantly decreased while total phenolic in shoot and grains significantly increased with increasing algal extract concentrations.

Figure (5): Effect of NaCl on phenolic and total antioxidants content (mg/g dry weight) of *Triticum aestivum* shoot and grains treated with different concentrations of β -carotene (a) control, (b) β -carotene(1mM), (c) β carotene (5mM) and (d) β -carotene(10mM).

Data are the mean of three replicates and error bars represent the standard errors of the means

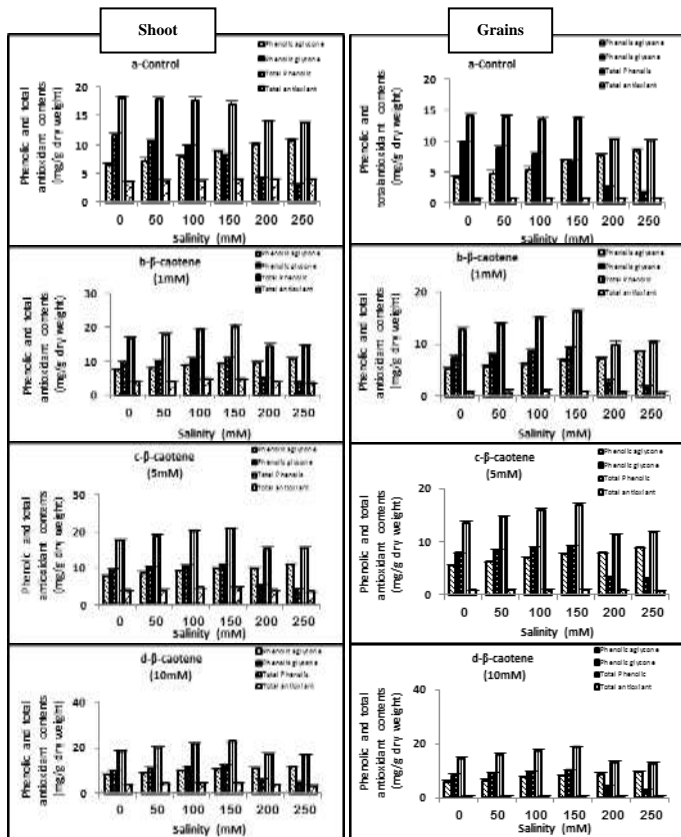
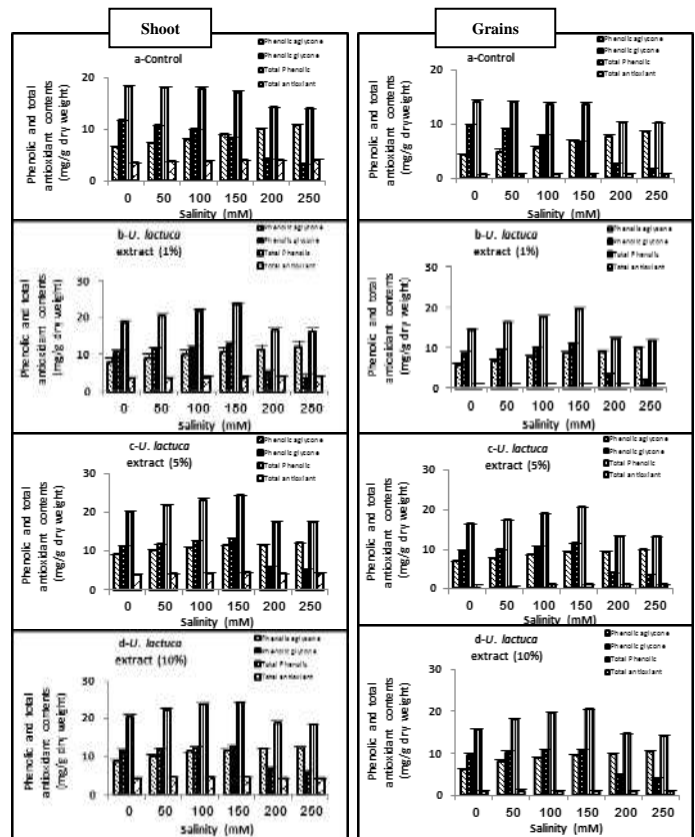


Figure (6): Effect of NaCl on phenolic and total antioxidants content (mg/g dry weight) of *Triticum aestivum* shoot and grains treated with different concentrations of algal extract (a) control, (b) algal extract (1%), (c) algal extract (5%) and (d) algal extract (10%).

Data are the mean of three replicates and error bars represent the standard errors of the means.



Foliar application with β -carotene and treatment with various concentrations of NaCl significantly increased phenolic aglycone up to 250mM. While, total phenolic and total antioxidant in shoot and grains increased significantly up to 150mM of NaCl and above that decreased but, the phenolic glycone in shoot and grains significantly decreased with increasing NaCl concentrations.

Application of algal extract and treatment with various concentrations of NaCl significantly increased phenolic aglycone and total antioxidants up to 250mM while, phenolic glycone and total phenolic significantly increased with increasing salinity up to 150mM both in shoot and grains and then decreased.

iv. Discussion

Antioxidants are involved in the regulation of growth and stress responses (Shao *et al.*, 2008 and Wu *et al.*, 2010). Also, several authors reported that exogenous application of antioxidants (Verma and Mishra, 2005 and Yaşar Akça and Esra Samsunlu, 2012) or algal extract (Hassan and Ghareib, 2009 ; Abd El Baky *et al.*, 2012 and Abd El Baky and El-Baroty, 2013a) can overcome harmful effects of NaCl stress.

Algae are considered as a rich source of several fine chemicals of economic value such as vitamins, carotenoids, phycobiliprotein, polysaccharides, fatty acids which possess varied biological properties and showed antibacterial, antifungal, antioxidant, anticancer and immune modulator agents (**Abd El Baky et al., 2012 and Abd El Baky and El-Baroty, 2013a**) However, many studies indicated that some natural compounds might play an important role in enhancing plant tolerance to some abiotic stresses such as salt, drought and extreme temperatures (**Ashraf, 2010 and Abd El Baky et al., 2010**).

Considerable changes in the accumulation of proline, other amino acids, glycine betaine, choline, glutathione, ascorbate, phenolic compounds in the different organs of the tested plant were induced by various levels of salinity.

The content of total free amino acid, glycine betaine and choline in shoot and grains of wheat decreasing significantly with the rise of salinization level this was in accordance to the result obtained by (**Debouba et al., 2006; Dluzniewska et al., 2007 and Surabhi et al., 2008**). However, the content of proline in shoots and grains significantly increased with increasing salinity level is the same as that obtained by (**Taji et al., 2004; Kant et al., 2006 and Pang et al., 2010**).

A Considerable changes in the accumulation of total free amino acid, glycine betaine and choline in shoot and grains of wheat shoot and grains as a result of application of either β -carotene or algal extract were noticed and this in accordance to the result obtained by (**Verma and Mishra, 2005 and Pise and Sabale, 2010**).

Plants synthesize proline under arid and salinity stress conditions in order to protect themselves and to regulate their physiological status (**Chen and Dickman, 2005**). Hence, it can be stated that plants and their cultivars which synthesize large amounts of proline are more tolerant to stress conditions (**De Lacerda, et al., 2005; Demiral & Türkan, 2005 and Mehdi et al., 2010**).

Treatment application with different concentrations of either β -carotene or algal extract and irrigation with different concentrations of NaCl more or less showed significant increase in total free amino acid, proline, glycine betaine and choline in shoot while these contents may decreased in grains those all when compared with corresponding treatments with NaCl (**Verma and Mishra, 2005 and Abd El-baky, et al., 2008**).

The non-enzymatic anti-oxidative mechanisms like ASA and GSH responded differently to NaCl treatments. The contents of glutathione and ascorbate in shoot and grains of wheat plants decreased significantly with increasing NaCl level (**Lu et al., 2006; Anjum et al., 2008a and Arshi et al., 2010**).

The ascorbate and glutathione contents in shoot and grains of wheat plants exhibited a significant increase with the rising of either β -carotene or algal extract concentrations A response of significance in connection with the role of foliar application of β -carotene or algal extract in modifying the salt stress induced changes was also revealed in the present

investigation with respect to the biosynthesis of ascorbate and glutathione. These results are in accordance with results obtained by (**Nagesh and Devaraj, 2008 and Chen et al., 2010b**).

There is some evidence of the induction of phenolic metabolism in plants as a response to multiple stresses (including salt stress) (**Michalak, 2006**). Phenolics including various flavonoids play pivotal roles in absorbing free radicals, quenching singlet oxygen, and decomposing peroxides.

Generally, the phenolic aglycone, total phenolic and total antioxidants in shoot and grains significantly increased with increasing NaCl levels. While, phenolic glycone decreased significantly.

Processes leading to an increase in phenolic compounds have been reported by (**Ayaz et al., 2000 and Posmyk et al., 2009**). In addition, the ability to respond to salt stress by the synthesis of phenolic compounds has been observed in the tolerant and sensitive strawberry genotypes **Keutgen and Pawelzik (2009)**.

DPPH free radical scavenging, TBA and chemiluminescence assays were often used to evaluate the radical scavenging activity (antioxidant capacity) of various compounds in medicinal plants (**Sariahmetoglu et al., 2003, Güvenc et al., 2005 and Chen et al., 2006**). The antioxidant properties on DPPH radical scavenging was thought to be due to their hydrogen-donating ability.

The antioxidant capacity estimated by DPPH assay could be thus contributed by phenolic compounds. So, to counteract NaCl-induced oxidative stress, DPPH radical scavenging property is involved in salt tolerance (**Xie et al., 2008**). The close correlation between antioxidant activity and phenolic content has been demonstrated by the other workers (**Liu et al., 2007, Verzellon et al., 2007 and Naciye et al., 2008**).

Application of algal extracts increased the levels of phenolic compounds, ascorbic acid and α -tocopherol, in wheat plants irrigated sea water to protect the membrane by preventing or reducing oxidative damage by ROS (**Abd El Baky and El-Baroty, 2008**). However, it is hypothesized a cycle where H₂O₂ scavenged by phenolic compounds to produce phenoxyl radicals, this radical reduces the ascorbic acid into mono (OH)-dehydroascorbate (**Abd El Baky and El-Baroty, 2008 and Abd El Baky et al., 2014**).

References

- [1] **Abd El Baky H.H. and El-Baroty G.S. (2013a)**: Healthy benefit of microalgal bioactive substances. *J.Aquatic Sci.*, 1: 11-23.
- [2] **Abd El Baky H.H., El-Baroty G.S., Bouaid A., Martinez M. and Aracil J. (2012)**: Enhancement of lipid accumulation in *Scenedesmus obliquus* by Optimizing CO₂ and Fe³⁺ levels for biodiesel production. *Bioresource Technol.*, 119: 429-432.
- [3] **Abd El Baky H.H., El-Baz F.K. and El-Baroty G.S. (2010)**: Enhancing antioxidant availability in wheat grains from plants grown under seawater stress in response to microalgae extract treatments. *J. Sci.Food Agric.*, 90: 299-303.

- [4] **Abd El-Baky H.H., El Baz F.K. and El-Baroty G.S. (2008).** Evaluation of marine alga *Ulva lactuca* L. as a source of natural preservative ingredient. *EJEAFChE*, 7:3353–3367.
- [5] **Abe N., Murata T. and Hirota A. (1998):** Novel DPPH radical scavengers, bisorbicillinol and demethyltrichodimerol, from a Fungus. *Biotechnol. Biochem.* 62: 661-666.
- [6] **Ali Q., Ashraf M. and Anwar F. (2009):** Physico-chemical attributes of seed oil from drought stressed sunflower (*Helianthus annuus* L.) plants. *Grasas y Aceites*, 60:475-481.
- [7] **Ali Q., Ashraf M., Shahbaz M. and Humera H. (2008):** Ameliorating effect of foliar applied proline on nutrient uptake in water stressed maize (*Zea mays* L.) plants. *Pak. J. Bot.*, 40: 211-219.
- [8] **Anderson M.E. (1985):** Determination of glutathione and glutathione disulfide in biological samples. *Methods Enzymol* 113:548–555.
- [9] **Anjum N.A., Umar S., Ahmad A. and Iqbal M. (2008a):** Responses of components of antioxidant system in mungbean [*Vigna radiata* (L.) Wilczek] genotypes to cadmium stress. *Communications in Soil Science and Plant Analysis Journal* 39:2469-2483.
- [10] **Arakawa, K., Katayama, M. and Takabe, T. (1990):** Levels of betaine and betaine aldehyde dehydrogenase activity in the green leaves, and etiolated leaves and roots of barley. *Plant Cell Physiol.* 31: 797–803.
- [11] **Arshi A., Ahmed A., Aref M., Iqbal M. (2010):** Effect of calcium against salinity-induced inhibition in growth, ion accumulation and proline contents in *Cichorium intybus* L *Journal Environ. Biol.*, 31 (6) : 939–944.
- [12] **Ashraf M. (2010):** Biotechnological approach of improving plant salt tolerance using antioxidants as markers. *Biotechnol. Adv.*, 27: 84-93.
- [13] **Ayaz F.A., Kucukislamoglu M., and Reunanen M. (2000):** Sugar, Non-volatile and Phenolic Acids Composition of Strawberry Tree (*Arbutus unedo* L. var. *ellipsoidea*) *Fruits. J. Food Compos. Anal.* 13 (2): 171-177.
- [14] **Badawi G.H., Yamauchi Y., Shimada E., Sasaki R., Kawano N., Tanaka K. and Tanaka K. (2004b):** Enhanced tolerance to salt stress and water deficit by overexpressing superoxide Dismutase in tobacco (*Nicotiana tabacum*) chloroplasts. *Plant Science* 166: 919-928.
- [15] **Bates L.S., Waldern R.P. and Tear I.D. (1973):** Rapid determination of free proline for water-stress studies. *Plant and Soil.* 39: 205-207.
- [16] **Chen C. and Dickman M. B. (2005):** Proline suppresses apoptosis in the fungal pathogen *Colletotrichum trifolii*, *PNAS* 102: 3459-3464.
- [17] **Chen J. H., Jiang H. W., Hsieh E. J., Chen H. Y., Chien C. T., Hsieh H. L. and et al. (2012b).** Drought and salt stress tolerance of an Arabidopsis glutathione S-transferase U17 knockout mutant are attributed to the combined effect of glutathione and abscisic acid. *Plant Physiol.* 158: 340–351.
- [18] **Chen, P.W.; Chiang, C.M.; Tseng, T.H. and Yu, S.M. (2006):** Interaction between rice MYBGA and the gibberellin response element controls tissue-specific sugar sensitivity of α -amylase genes. *The Plant Cell* 18:2326-2340.
- [19] **De Lacerda C.F., Cambraia J.M., Oliva A., and Ruiz H.A. (2005):** Changes in growth and in solute concentrations in sorghum leaves and roots during salt stress recovery. *Environ. Exp. Bot.*, 54:69-76.
- [20] **Debouba M., Gouia H., Suzuki A. and Ghorbel M.H. (2006):** NaCl stress effects on enzymes involved in nitrogen assimilation pathway in tomato "*Lycopersicon esculentum*" seedlings. *J. Plant Physiol.*, 163: 1247–1258 .
- [21] **Demiral T. and Türkan İ. (2005):** Comparative lipid peroxidation, antioxidant defense systems and proline content in roots of two rice cultivars differing in salt tolerance. *Environ. Exp. Bot.*, 53:247-257.
- [22] **Demiralay M., Sağlam A. and Kadioğlu A. (2013):** Salicylic acid delays leaf rolling by inducing antioxidant enzymes and modulating osmoprotectant content in *Ctenanthe setosa* under osmotic stress. *Turk J Biol* 37: 49–59.
- [23] **Dluzniewska P., Gessler A., Dietrich H., Schnitzler J.P., Teuber M., and Rennenberg H., (2007):** Nitrogen uptake and metabolism in *Populus canescens* as affected by salinity. *New Phytol.* 173: 279–293.
- [24] **Ejaz B., Sajid Z.A. and Aftab F. (2012):** Effect of exogenous application of ascorbic acid on antioxidant enzyme activities, proline contents, and growth parameters of *Saccharum* spp. hybrid cv. HSF-240 under salt stress. *Turkish Journal of Biology* 36:630-640.
- [25] **Gao W.R., Wang X.S., Liu Q.Y., Peng H., Chen C., Li J.G., Zhang J.S., Hu S.N. and Ma H. (2008):** Comparative analysis of ESTs in response to drought stress in chickpea (*C. arietinum* L.). *Biochem Biophys Res Commun* 376, 578-583.
- [26] **Güvenc A., Houghton P.J., Duman H., Coskun M., Sahin P. (2005):** Antioxidant activity studies on selected *Sideritis* species native to Turkey, *Pharm Biol* 43(2), 173-177.
- [27] **Hameed M., Naz N., Ahmad M.S.A., Islam-ud-Din. and Riaz A. (2008):** Morphological adaptations of some grasses from the salt range, Pakistan. *Pak. J. Bot.*, 40: 1571-1578.
- [28] **Han B., Yoon S., Su J.L., Han H.R., Wang M., Qu W.J., and Zhong D.B. (2004):** Effects of selenium, copper and magnesium on antioxidant enzymes and lipid peroxidation in bovine fluorosis. *Asian-Aust. J. Anim. Sci.* 17:1695-1699.
- [29] **Hassan S.M. and Ghareib H.R. (2009):** Bioactivity of *Ulva lactuca* acetone extracts on germination and growth of lettuce and tomato plants. *African Journal of Biotechnology* 8: 3832-3838.
- [30] **Hewitt E.J. (1966):** Sand and Water Culture Methods Used in the Study of Plant Nutrition. Technical Communication No. 22. Commonwealth Bureau, London
- [31] **Jagota S. K. and Dani H. M. (1982):** A new calorimetric technique for the estimation of vitamin C using Folin phenol reagent. *Analytical Biochemistry* 127:178-182.
- [32] **Jamil M., Iqbal W., Bangash A., Rehman S., Imran Q.M., and Rha E.S. (2010):** Constitutive expression of OSC3H33, OSC3H50 and OSC3H37 genes in rice under salt stress. *Pak.J. Bot.*, 42: 4003-4009.
- [33] **Jha B., Reddy C.R.K., Thakur M.C. and Rao M.U. (2009):** Seaweeds of India: The diversity and distribution of seaweeds of the Gujarat coast. Springer, Dordrecht Heidelberg London New York. 216.
- [34] **Kant S., Kant P., Raveh E. and Barak S. (2006):** Evidence that differential gene expression between the halophyte, *Thellungiella halophila*, and *Arabidopsis thaliana* is responsible for higher levels of the compatible osmolyte proline and tight control of Na⁺ uptake in *T. halophila*. *Plant Cell Environ.* 29:1220-1234.
- [35] **Keutgen A.J. and Pawelzik E. (2009):** Impacts of NaCl stress on plant growth and mineral nutrient assimilation in two cultivars of strawberry. *Environ. Exp. Bot.* 65:170-176.
- [36] **Liu X., Dong M., Chen X., Jiang M., Lv X., and Yan G. (2007):** Antioxidant activity and phenolic of an endophytic *Xylaria* sp. From *Ginkgo biloba*. *Food chemistry.* 105:548-554.
- [37] **Lowe L.E. (1993):** In: Lewis Publishers (Eds.), Boca Raton, FL, USA, 409-412.
- [38] **Lu C., Bravo R., Caltabiano L.M., Irish R.M., Weerasekera G. and Barr D.B. (2005):** The presence of dialkylphosphates in fresh fruit juices: implication for organophosphorus pesticide exposure and risk assessments. *J Toxicol Environ Health* 68:209–227.
- [39] **Lu Y., et al. (2006):** Identifying cycling genes by combining sequence homology and expression data. *Bioinformatics.* 22: 314-322.
- [40] **Mehdi H., Chaabouni A.C., Boujnah D. and Boukhris M. (2010):** The response of young pistachio trees grown under saline conditions depends on the rootstock, A no. XX, 2010—XIV GREMPA, Meeting on pistachios and almonds.
- [41] **Michalak A. (2006):** Phenolic compounds and their antioxidant activity in plants growing under heavy metal stress. *Pol. J. Environ. Stud.* 15:523-530.
- [42] **Mittova V., Guy M., Tal M., and Volokita M. (2004):** Salinity upregulates the antioxidative system in root mitochondria and peroxisomes of the wild salt-tolerant tomato species *Lycopersicon pennellii*. *J. Exp. Bot.* 55: 1105-1113.
- [43] **Muting D. and Kaiser E. (1963):** Spectrophotometric method of determining of alfa-amino-N in biological material by means of the ninhydrin reaction. *Hoppe-Seyler's Zschr Physiol Chem.* 332: 276-289
- [44] **Nagesh Babu R. and Devaraj V.R. (2008):** High temperature and salt stress response in French bean (*Phaseolus vulgaris*). *Aust J Crop Sci* 2(2):40–48.

- [45] **Nasr A.H. (1940):** A report on some marine algae collected from the vicinity of Alexandria. Fouad I Institute of Hydrobiology and Fisheries, Government Press, Bulaq, Cairo.1 (36): 1-33.
- [46] **Omami E.N., Hammes P.S. and Robbertse P.J. (2006):** Differences in salinity tolerance for growth and water-use efficiency in some amaranth (*Amaranthus* spp.) genotypes. *New Zealand J. Crop Hortic. Sci.* 34: 11–22.
- [47] **Osakabe Y., Kajita S. and Osakabe K. (2011):** Genetic engineering of woody plants: current and future targets in a stressful environment. *Physiologia Plantarum* 142: 105–117.
- [48] **Pang Q., Chen S., Dai S., et al. (2010):** Comparative proteomics of salt tolerance in *Arabidopsis thaliana* and *Thellungiella halophila*. *J Proteome Res* 9(5):2584-2599.
- [49] **Parvaiz A. and Satyawati S. (2008):** Salt stress and phyto-biochemical responses of plants-a review. *Plant Soil Environ* 54:89-99.
- [50] **Pise N.M. and Sabale A.B. (2010):** Effect of seaweed concentrates on the growth and biochemical constituents of *Trigonella foenum-graecum* L. *Journal of Phytology* 2: 50-56.
- [51] **Poontariga H., Darinee P., Kannarat, R. and Rangsi, C. (2003):** "Science Asia", 29:109-113.
- [52] **Posmyk, M.M., R. Kontek and K.M. Janas. (2009):** Antioxidant enzymes activity and phenolic compounds content in red cabbage seedlings exposed to copper stress. *Ecotoxicol. Env. Safety*, 72: 596-602.
- [53] **Purty R.S., Kumar G., Singla-Pareek S.L. and Pareek A. (2008):** Towards salinity tolerance in Brassica: an overview. *Physiology and Molecular Biology of Plants*. 14:39–49.
- [54] **Rahnema H. and Ebrahimzadeh H. (2006):** Antioxidant Isozymes Activities in Potato Plants (*Solanum tuberosum*) Under Salt Stress. *Journal of Science*, 17: 225-230.
- [55] **Sariahmetoglu M., Wheatley R.A., Cakici I., Kanzik I. and Townshend A. (2003):** Flow injection analysis for monitoring antioxidant effects on luminol chemiluminescence of reactive oxygen species, *Anal Lett* 36:749-765.
- [56] **Sauvesty, A., Page, F. and Huot, J. (1992):** A simple method for extracting plant phenolic compounds. *Canadian Journal of Forest Research* 22: 654–659.
- [57] **Shao H.B., Chu L.Y., Lu Z.H. and Kang C.M. (2008):** Primary antioxidant free radical scavenging and redox signaling pathways in higher plant cells. *Int. J. Biol. Sci.* 4: 8-14.
- [58] **Surabhi G.K., Reddy A.M., Kumasi G.J. and Sudhakar C. (2008):** Modulations in key enzymes of nitrogen metabolism in two high yielding genotypes of mulberry (*Morus alba* L.) with differential sensitivity to salt stress. *Environ. Exp. Bot.* 64: 171-179.
- [59] **Taji T., Seki M., Satou M., Sakurai T., Kobayashi M., Ishiyama K. and et al. (2004):** Comparative genomics in salt tolerance between *Arabidopsis* and *Arabidopsis*-related halophyte salt cress using *Arabidopsis* microarray. *Plant Physiol.* 135: 1–13.
- [60] **Tuna A.L., Kaya C., Altunlu H. and Ashraf M. (2013):** Mitigation effects of non-enzymatic antioxidants in maize (*Zea mays* L.) plants under salinity stress. *Australian Journal of Crop Science* 7(8)1181-1188.
- [61] **Verma S., and Mishra S.N. (2005):** Putrescine alleviation of growth in salt stressed *Brassica juncea* by inducing antioxidative defence system. *J. Plant Physiol.*, 162: 669-677.
- [62] **Verzellan E., Tagliazucchi D., and Conte A. (2007):** Relationship between the antioxidant properties and the phenolic and flavonoid content in traditional balsam vinegar. *Food. chem.*, 105: 564-571.
- [63] **Wu X., Zhu W., Zhang H., Ding H. and Zhang H.J. (2010):** Exogenous nitric oxide protects against salt-induced oxidative stress in the leaves from two genotypes of tomato (*Lycopersicon esculentum* Mill.). *Acta Physiol Plant* 10: 1007–1173
- [64] **Xie Z., Huang J., Xu X., and Jin Z. (2008):** Antioxidant activity of peptides isolated from alfalfa leaf protein hydrolysate. *Food Chem.*, 111: 370-376.
- [65] **Yaşar Akça. and Esra Samsunlu U. (2012):** The effect of salt stress on growth, chlorophyll content, proline and nutrient accumulation, and K/Na ratio in walnut. *Pak. J. Bot.*, 44(5): 1513-1520
- [66] **Zhang L.X., Gao M., Li S.Q., Li S.X. and Liang Z.S. (2011):** Modulation of plant growth, water status and antioxidative system of two maize (*Zea mays* L.) cultivars induced by exogenous glycinebetaine under long term mild drought stress. *Pak. J. Bot.*, 43(3): 1587-1594.
- [67] **Zia M.J., Ahmad R., Khaliq I., Ahmad A. and Irshad M. (2006):** Micronutrient status and management in orchard soil: applied aspects. *Soil. Environ.* 25(1): 6-16J.