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

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Abstract— This study was designed to examin 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 used to ameliorate the deleterious effect of salt stress and could be enhance the level of free amino acids, proline, glycine betaine, choline, glutathione, ascorbic acid, phenolic contents and total antioxidants 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 sever 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)**.

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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 a*l., 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 3/4way 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-Ciocalteau 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)*βcarotene* (5mM) and (d) *β-carotene*(10mM). Data are the mean of three replicates and error bars represent the standard errors of the means

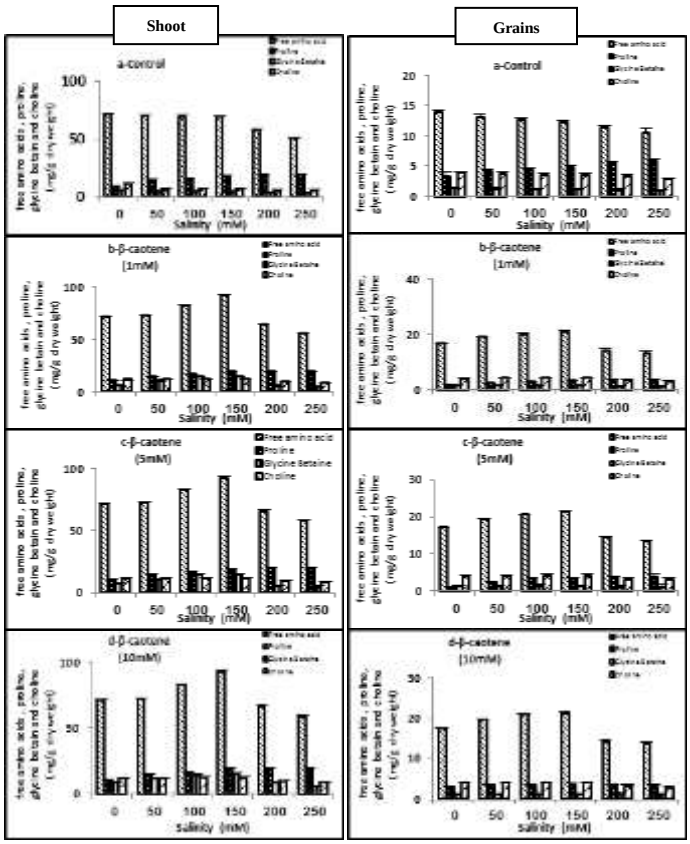
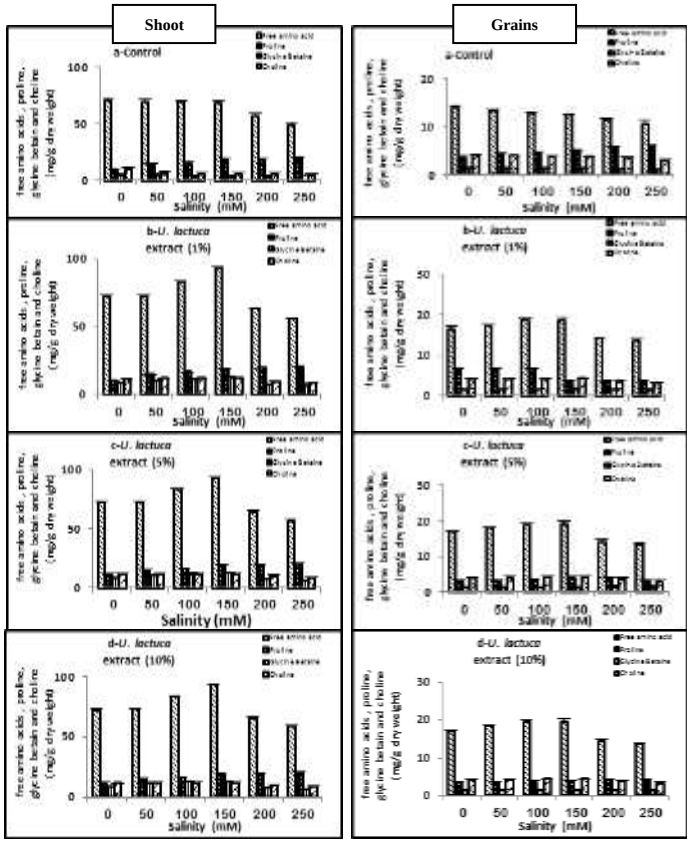


Figure (2): Effect of NaCl on free amino acids, proline, glycine betaine and choline content (mg/g dry weight) of *Triticum aestivum* shoot and grains treated with different concentrations of *Ulva lactuca* (a) control, (b) algal extract (1%), (c) algal extract (5%) and (d) algal extract (10%). Data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

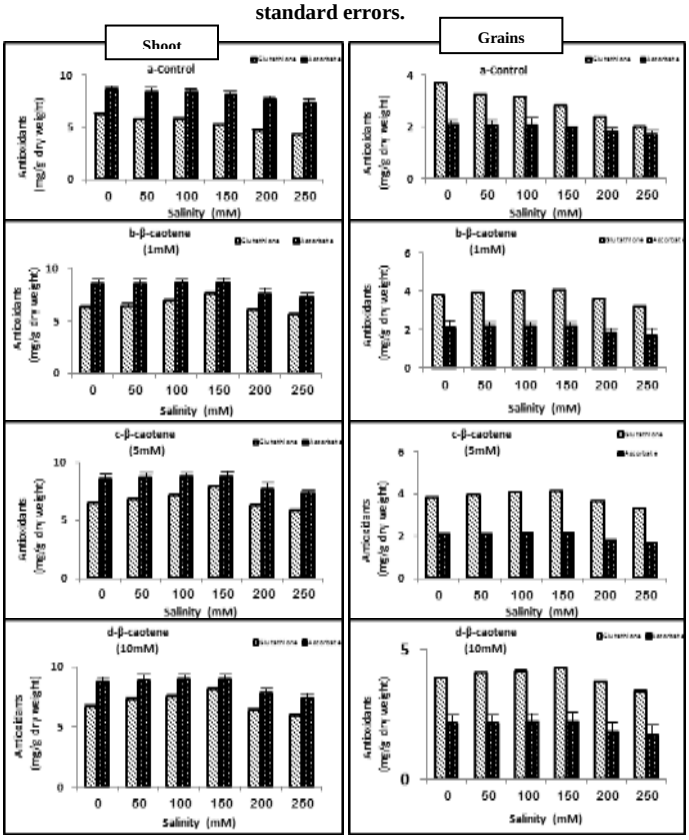
B. Antioxidant metabolites:

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

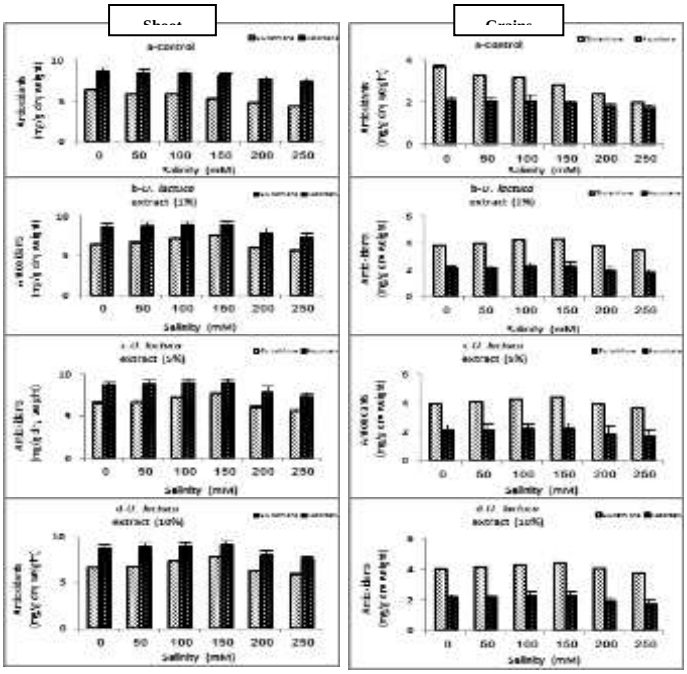
Foliar application with different concentrations of β-carotene or algal extract increased the glutathione and ascorbate in shoots and grains.

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)*βcarotene* (5mM) and (d) *β-carotene*(10mM). Data are the mean of three replicates and error bars represent the standard errors of the means

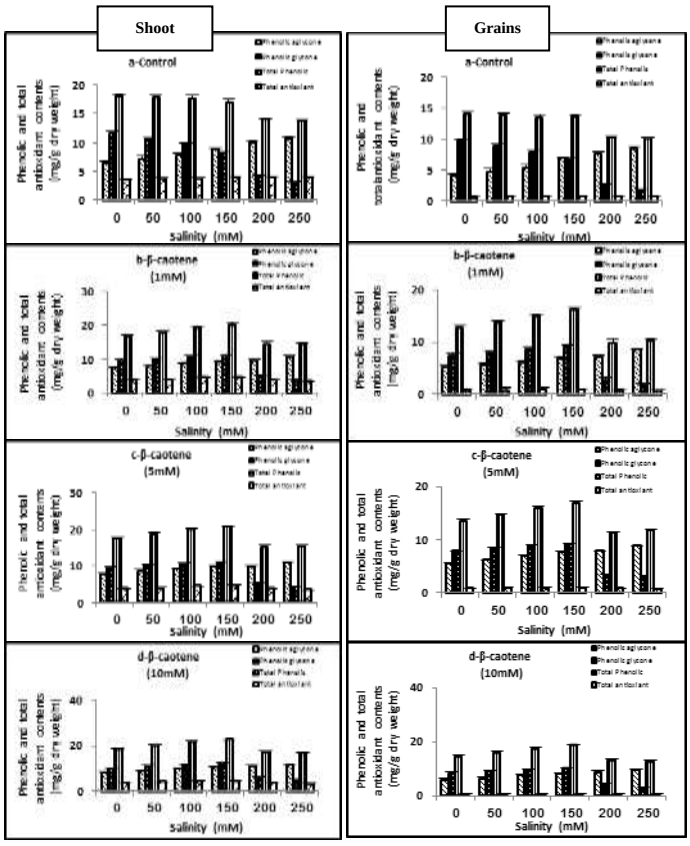
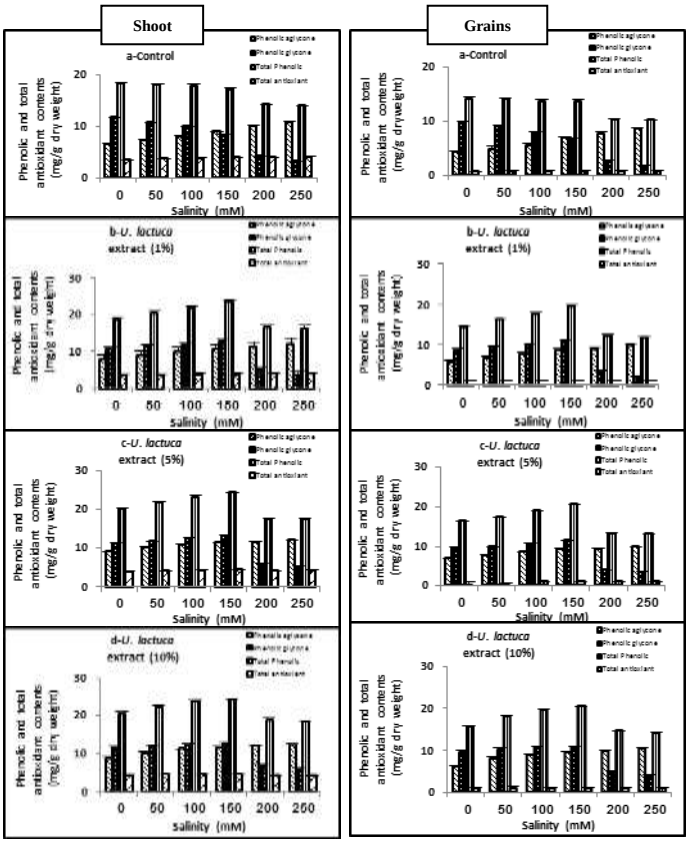


Figure (6): Effect of NaCl on phenolic and total antioxidants content (mg/g dry weight) of *Triticum aestivum* shoot and grains treated with different concentrations of *algal extract* (a) control, (b) algal extract (1%), (c) algal extract (5%) and (d) algal extract (10%). Data are the mean of three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

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