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Full Length Research Paper

Algal extracts improve antioxidant defense abilities and salt tolerance of wheat plant irrigated with sea water

Hanaa H. Abd El-Baky[1*], Hussein M. M.[2] and Game S. El-Baroty[3]

[1]Plant Biochemistry Department, National Research Centre, Dokki, Cairo, Egypt.
[2]Water Relations and Irrigation Department, National Research Centre, Dokki, Cairo, Egypt.
[3]Department of Biochemistry, Faculty of Agriculture, Cairo University, Egypt.

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Effect of irrigation bread wheat plants (*Triticum aestivum L.,* cv. Giza 94) with sea water (10 and 20% v/v), spraying with microalgae extracts obtained from *Chlorella ellipoida* and *Spirulina maxima* (5 gL^{-1} dry weight in 0.1% Tween solution) cultivated under normal and stress conditions were studied. Some plant bioregulators (BRGs, ascorbic acid and benzyl adenine, at 200 ppm) at the vegetative growth stage on photosynthetic pigments, antioxidant components, activity of some antioxidant enzymes, lipid peroxidation products, growth parameters, mineral content and economic yield were estimated. Irrigation of wheat plants with sea water led to an increase in Na^+ ion, activities of antioxidant enzymes, superoxide dismutase, ascorbate peroxidase and total peroxidase, and TBARs components. In contrast, the contents of photosynthetic pigments and yield components were reduced. Furthermore, the overall growth of wheat plants was interrupted by irrigation with sea water (10 and 20%) and the effect was pronounced at higher level (20%). Application of BRGs had a slight effect on plant growth, antioxidant behavior and activity of antioxidant enzymes in plants irrigation with sea water compared with that in stressed wheat plants. Application of algal extracts significantly increased the contents of total chlorophyll and antioxidant phenomenon. In additional, application of algal extracts exhibited strong positive correlation with increase in fresh weight (FW), grain weight and yield components. It is concluded that productive purpose of wheat crop by mean of brackish water (at 20 v/v level) is possible under a level of economical value through its application of algal extracts.

Keywords: Microalgae, sea water, wheat, salinity stress, antioxidant systems

INTRODUCTION

Microalgae are one of the potential organisms, and useful to mankind in various ways. Microalgae constitute a vast potential resource in various applications such as marine culture, food, feed, fuel, medicine, industry and in com-bating pollution (Thajuddin and Subramanin, 2005). Also, microalgae are a rich source of several fine chemi-cals of economic value such as vitamins, carotenoids, phycobili-protein, polyols, polysaccharides, fatty acids, etc. with varied properties and possess anti-inflammatory, anti-cancer, anti-fungal, antioxidant and immune- modu-lator agents (El Baz et al., 2002; Abd El Baky et al., 2003, 2004) . In the area of agriculture and horticulture, micro-algae have been shown to stimulate the growth of plants, due to the presence of auxine, cytokinins, gibberellins and related growth regulators (Ördög et al., 2004; Molnar and Ördög, 2005).

Plants develop a plethora of biochemical and mole-cular mechanisms to cope with salt stress. Biochemical pathways lead to induce certain processes that improve salt tolerance are likely to act additively and probably synergistically (Parida and Das, 2005). These processes include: compartmentalization of compatible solutes, change in photosynthetic pathway, and alteration in membrane structure, induction of antioxidative enzymes and induction of plant hormones (Iyengar and Reddy, 1996; Abd El-Baky et al., 2004; Sairam and Tyagi, 2004).

Egypt is present in semiarid region of the world. In addition, human overpopulation becomes a serious con-straint for crop production, that crushed by pressure to produce more food per unit area of land. Sea water utili-

*Corresponding author. E-mail: abdelbakyh@hotmail.com

zation has been a recent effort to explore the possibility of obtaining reasonable yield and quality of products from crops. Salinity stress is usually causes a decrease in crop production. It inhibits the photosynthesis of plants, causes changes of chlorophyll contents and components and damage of photosynthetic apparatus (Iyengar and Reddy, 1996). It also inhibits the photochemical activities and decreases the activities of enzymes in the Calvin cycle (Sairam and Tyagi, 2004). It is well established that environmental stress inhibits the photosynthetic abilities of plants due to the breakdown of the balance between the production of reactive oxygen species (ROS) and the antioxidant defense causing accumulation of ROS such as superoxide radical (O_2^-), hydrogen peroxide (H_2O_2) and hydroxyl radical (OH). These radicals are produced during salinity stress, and are responsible for the damage to membranes and other essential macro-molecules such as photosynthetic pigments, proteins, DNA and lipids (Sairam and Tyagi, 2004).

To minimize the effects of oxidative stress, plant cells evolve a complex antioxidant systems, that is, low-molecular mass antioxidants [glutathione (GSH), ascorbate (AA) and carotenoids (CAR)] as well as ROS-scavenging enzymes, such as: superoxide dismutase (SOD), calatase (CAT), ascorbate peroxidase (APX), guaiacol peroxidase (GPX), and glutathione reductase (GR) (Alscher et al., 1997; Apel and Hirt, 2004). The generation of ROS and the increase the activity of many antioxidant enzyme activities during salt stress have been reported in cotton (Gosset et al., 1994), wheat (Sairam et al., 2001), tomato (Mittova et al., 2002), rice (Vaidyanathan et al., 2003), sugar beet (Bor et al., 2003) and maize (Azevedo et al., 2005). Thus, these data suggest the existence of correlation between salt stress tolerance and the presence of an efficient antioxidant system.

Very little work has been done on the influence of foliar algae extracts on reduction of the reactive oxygen species in wheat plant grown under normal and salt stress conditions. Hence, the present study shows the effect of spraying wheat plant irrigated with sea water with foliar algae on various antioxidant systems and their relevance to salinity stress and tolerance.

MATERIALS AND METHODS

Algal strains: Two algae pure strains, that is, *Chlorella ellipsoida* and *Spirulina maxim*a were obtained from the University of Texas Culture Collection, Austin, Texas, USA.

Growth conditions: *C. ellipsoida* was grown in NSI medium (3L) containing 140 (optimum level) and 10 (stressed level) ppm nitrogen concentrations, *S. maxim*a was cultivated on Zarrouk medium (Zarrouk, 1966) containing 410 (optimum level) and 51 ppm (stressed level) nitrogen concentrations. All cultivated flasks were illuminated by continuous cool white fluorescent lamps (Philips, 40 W) at 200 Wm^{-2}, regularly spaced around the culture flasks, (optimum light intensity) or and at 400 Wm^{-2} (stressed light intensity). The other cultivation conditions were mentioned by El-Baz et al. (2002) and Abd El-Baky (2003).

Preparation of algal extracts and plant bioregulator: Algal cells were harvested at the end of logarithmic phase by centrifugation at 5000 x g for 10 min. Algal cells (5 g) were homogenized in deionized water (100 ml) containing 1% Tween-20, and the volume was competed to one liter with deionized water (5 g cells L^{-1} in 0.1% Tween-20 solution). Plant bioregulators (BRGs; ascorbic acid (AA) and benzyl adenine (BA)) was prepared at 200 ppm in 0.1% Tween- 20 solutions. The algal extracts and BRGs were applied as a spray at the vegetative stage of wheat plants (40-days-old).

Wheat grains: The grains of wheat cultivar named Giza 94 were obtained from Wheat Department, Agriculture Research Centre, Ministry of Agriculture, Giza, Egypt. The wheat grains were surface-sterilized in 0.1% $HgCl_2$ solution for 3 min, washed thoroughly with distilled water before cultivation.

Wheat cultivation: A pot experiment was conducted in the greenhouse of National Research Centre, Dokki, Giza, Egypt, during the winter 2006/2007 in order to evaluate the effect of spraying of algal strains *C. ellipsoida* and *S. maxim*a extracts as source of plant bioregulators and of antioxidant compounds to enhance salinity tolerance of wheat plants irrigated with 10 and 20% (v/v) sea water and tap water as a control. The experiment included 21 treatments and repeated three times. The experimental was conducted in plastic pots (40 cm in diameter) filled with 20 kg soil (sand and clay 2:1 v/v), each one contained forty grains and irrigated with tap water as required. Also, the plants were received the optimum fertilizer levels (PO^{-4}, as super-phosphate; and N as ammonium nitrate) as recommended by Ministry of Agriculture in Egypt. After 40 days from cultivation (vegetation stage), the plants were sprayed (first treatment) with algal extracts (5g L^{-1} dry weight in 0.1% Tween solution) and bioregulators (AA and BA, 200 mg L^{-1}). Then, plants of each set were divided into three groups, which irrigated with 0, 10 and 20% sea water, respectively. Then, the plants were irrigated with sea water and tap water alternatively every 7 days until the end of experiments. After 14 days the second treatment with algal extracts and bioregulators were done. Samples from each treatment at 70 days-old were uprooted and washed with distilled water, dried with filter paper and used for chemical analysis.

Estimation of growth and grain yield: Nine plants from each treatment (three plants per replicate) were collected and immediately rinsed with iso-osmotic solution, blotted on filter paper and weighed to obtain the fresh weight (FW). For determination of dry weight (DW) the plant parts from each treatment dried to constant weights at 65^oC. Leaves and stems were separated and weighed. After harvest, the weight of 100 grains and grain yields were calculated.

Extraction and estimation of chlorophyll: Wheat leaves (1 g, FW) dried on filter paper were ground in acetone (5 ml, 80 %) and allowed to stand overnight in dark at 4°C followed by centrifugation at 10,000 x g for 5 min. The contents of total chlorophyll (T-Chl), chlorophyll a (Chl-a) and chlorophyll b (Chl-b) in the supernatant were determined according to Lichtenthaler (1987).

Determination of phycocyanin and carotenoid contents: The contents of total phycocyanin and catotenoids were spectrophotometrically determined by the method of Abd El-Baky (2003) and Lichtenthaler (1987), respectively.

Determination of total tocopherols and ascorbic acid: These substances were spectrophotometrically determined as outlined by AOAC (1995) and Augustin et al. (1985), respectively.

Determination of total phenolic content: Total phenolic content (TPC) was estimated as gallic acid equivalent using the Folin

Table 1. Antioxidants and chlorophyll contents of *Chlorella ellipsoida* and *Spirulina maxima* *grown under stress conditions*

Treatment	Carotenoids (mg-^{1}g D.W)	Phycocyanin (mg-^{1}g D.W)	Tecopherols (mg-^{1}g D.W)	Ascorbic acid (mg-^{1}g D.W)	Phenolic (mg-^{1}g D.W)	GSH (mg^{-1} g)	Chlorophylls (mg-^{1}g D.W)	Total antioxidants (mg-^{1} 5g D.W)
Chlorella ellipsoida Grown under control conditions	6.52^{a} ±0.58	0.0	1.51^{a} ±0.11	3.52^{a} ±0.22	0.921 ±0.11	2.9 ±0.41	19.21 ±1.21	174.5^{a} ±4.25
Chlorella ellipsoida grown under stress conditions	30.4 ^{d}±1.21	0.0	6.9^{f} ±0.35	11.1^{f} ±0.65	4.32 ±0.25	5.0 ±0.24	4.26 ±0.36	309.5^{d} ±5.12
Spirulina maxima Grown under control conditions	4.23 ^{a} ±0.23	86.3^{a} ±2.4	1.22^{a} ±0.15	2.36^{a} ±0.23	3.35 ±0.34	2.6 ±0.31	7.86 ±0.67	539.5^{f} ±7.8
Spirulina maxima Grown under stress conditions	18.63^{d} ±1.05	145.2^{d} ±3.5	3.36^{c} ±0.23	7.21^{d} ±0.51	15.36 ±1.21	5.7 ±0.25	2.26 ±0.22	984.5^{e} ±8.14
LSD at level (P< 0.05)	1.5	1.9	0.53	1.1	0.25	0.12	0.24	2.65

All values are significant at (P=< 0.05)
Values represent the mean of three replicates.

–Ciocalteu method (Li et al., 2007).

Extraction of cytosolic fraction: A plant material (ca. 5g) was excised and homogenized in 20 ml of ice-cold grinding buffer containing 0.4 M sucrose and 25 mM Tris (pH 7.2). The homogenate was passed through 4 layers of cheat cloth and centrifuged at 12,000 x g for 15 min at 4°C. The resulting supernatant was used for determination of enzyme activities, GSH, lipid oxidation products and protein contents.

Determination of glutathione (GSH) content: The GSH content of algal cells and wheat cytosolic extracts was measured by reaction with 5, 5`dithiobis-2-nitrobenzoic (DTNB) reagent according to Silber et al. (1992).

Enzyme assays: The activities of wheat cytosolic superoxide dismutase (SOD; EC, 1.15.1.1) and peroxidase (POX; EC, 1.11.1.7) were determined as described by Ginnopolitis and Ries (1977) and Chance and Maehly (1955), respectively. The activity of ascorbate peroxidase (APX), (EC, 1.11.1.11) was assayed according to Nakano and Asada (1981). The activity of each enzyme was expressed on protein basis.

Determination of lipid peroxidation products: The lipid peroxidation products in wheat cytosolic fraction were estimated by the formation of thiobarbaturic acid reactive substances (TBARS) and quantified in term of malonald- hyde (MDA) as described by Haraguchi et al. (1997) . The lipid peroxidation was expressed as micromoles of MDA calculated using the extinction coefficient of 1.56 x 10^{5} mM^{-1} cm^{-1}.

Determination of protein he totalprotein content in wheat cytosolic fraction was determined at 595 nm, using Comassein blue G 250 as mentioned by Bradford (1979). Bovine serum albumin (BSA) was used as a protein standard.

Determination of Na and Ca ions: The concentrations of Na^{+} and Ca^{2+} in wheat plants were determined with a flame photometer (Jenway-PFP7) according to Allen et al. (1986). Statistical analyses: Biochemical analyses were subjected to analysis of variance using the COSTAT computer package (Cohort Software, CA, USA). The mean values were compared with LSD test.

RESULTS AND DISCUSSION

Total antioxidant content of algal extracts

The algal antioxidants of *C*. *ellipsoida* and *S*. *maxima* extracts were obtained from the cells cultivated under optimal and stress conditions. The algal extracts had significant differences in antioxidant patterns. Table 1 shows the concentrations of total carotenoids (T-CAR), phycocyanin, tocopherols (TOC), ascorbic acid (AA), total phenolic compounds (TPCs) and glutathione (GSH) in algal *C*. *ellipsoida* and *S*. *maxim*a (in parentheses) grown under stress (high light intensity coupled with nitrogen deficient) were 30.4 (18.63), 0.0 (145.2), 6.9 (3.36), 11.1 (7.21), 4.32 (15.36) and 5.0 mg g^{-1} DW (5.7 mg g^{-1} DW), respectively. Whereas, the corresponding values 6.52 (4.23), 0.0 (86.3), 1.51 (1.22), 3.52 (2.36), 0.921 (3.35) and 2.9 mg g^{-1} DW (2.6 mg g^{-1} DW) were found in the two algal cells grow under optimum conditions. On contrary, the total chlorophyll content (T-Chl) in stressed cells was signify-cantly decreased to be about 4.26 (2.26) mg^{-1}g DW that in optimal cells 19.21 (7.8) mg g^{-1} DW. The total antioxidant present in algal extract at which may contribute greatly to the antioxidant

activity were 984.5, 539.5, 309.5 mg 5 g L^{-1} (Table 1). Therefore, *S. maxim*a extract contained higher amounts of total antioxidant than that in *C*. *ellipsoida.* Also, in both stressed algal extracts had higher levels of total antioxidant substances than that in extract of algal cells grown under optimum condition. Total antioxidant levels in algal extract were in the decreasing order: stressed *S*. *maxim*a > normal *S*. *maxim*a > stressed *C*. *ellipsoida* > *C*. *ellipsoid*a, respectively. These results revealed that both algae strains accumulated large amounts of antioxidants associated with a marked reduction in total chlorophyll content when grown under high light intensity coupled with low nitrogen concentration. The effect of stress conditions on chemical composition of different algal strains have been previously reported by El Baz et al. (2002) , Abd El-Baky (2003) and Li et al. (2007). Under stress conditions, it was found that the amount of algae protein synthesis was decreased whereas the second metabolites' products included: T- CAR, TOC, AA, TPCs and GSH were increased. Abd El-Baky et al. (2004) observed that, in *Chorella* and *Spirulina* species grown under synergistic condition of low nitrogen and high light intensity, the T- CAR, phycocyanin, TOC and AA contents were increased in parallel to a decrease of T-Chl content. At N-limitation, accumulation of large amount of anti-oxidant molecules in *C*. *ellipsoida* and *S*. *maxim* a were observed (Rich and Bonner, 1978; Colla et al., 2007). However, under high light intensity, the energy absorbed in the algal photosynthetic system is not utilized suffi-ciently, and an excess of light energy accelerates chloro-phyll depletion through photo-oxidation. Therefore, accu-mulation of antioxidant molecules in algal cells grown at high light and low nitrogen may be protect the cellular bio-molecules against photo-oxidative damage by absorbing an excess of energy, quenching triplet state of photosynthetic pigment and scavenging singlet oxygen and other toxic oxygen species formed within the cells (Rise et al., 1994; El-Baz et al., 2002).

Effect of algal extracts on photosynthetic pigment contents of wheat plants cultivation under sea water stress.

The changes of photosynthetic pigment of wheat plants irrigated by sea water (10 and 20%, v/v) are shown in Table 2. Irrigation with sea water induced a significant decrease in the amount of T- Chl and Chl-a and Chl-b contents when compared with the values obtained from wheat plants irrigated normal water. The values of T-Chl contents in wheat plant irrigated 10 and 20% (v/v) sea water were 0.45 and 0.36 mg/g FW, respectively whereas these value was 0.62 mg g^{-1} FW, in wheat plants irrigated tap water. Thus, chlorophyll degradation was dependant on water salinity level. Application of algal *C*. *eliposies* and *S*. *maxim*a extracts to wheat plants irrigated sea water caused a significant increase in the concentration of T-Chl, Chl-a and Chl-b as compared with the values in plants irrigated sea water only. The T-Chl content was ranged from 0.68 to 0.81 and 0.69 to 1.05 mg/g FW in wheat plants irrigated by 10 % (v/v) sea water and treated with algal *C*. *eliposies* and *S* . *maxim*a extracts. Whereas, these values were ranged from 0.56 to 0.72 and 0.65 to 0.94 $mg^{-1}g$ (FW), respectively in wheat plants only irrigated with 20% (v/v) sea water. Also, the T-Chl, Chl-a and Chl-b contents were significantly increased in wheat plants sprayed with bioregulators (BA and AA). However, this increase was less pronounced, when com-pared with that induced in wheat stressed plant treated with algal extracts. Thus, the level of photosynthetic pigment was found to be restored when wheat plants irrigated sea water due to application of algal extracts. However, the increase of T-Chl, Chl-a and Chl-b, con-tents were obtained in treated wheat with algal anti-oxidant extracts containing higher amount of antioxidants. In the present study, the increasing of antioxidant con-tents in algal extracts was positive correlated with increaseed chlorophyll restored in wheat plants irrigated sea water. The magnitude order of restored chlorophyll in wheat plants influenced with algal extracts as follows: *S*. *maxima* contented high antioxidant (HAO) > *C. eliposies* with (HAO) > *S*. *maxima* contented normal antioxidant (NAO) > *C*. *eliposies* (NAO). Therefore, algal extracts might improve the salt tolerance of wheat plants by restoring the photosynthetic pigments.

Effect of algal extracts on antioxidant status of wheat plants cultivation under sea water stress

Irrigation of wheat plants with sea water (10 and 20% v/v) caused significant increase in the accumulation of T-CAR, TOC, AA, TPCs and GSH in wheat plants (Table 3). The concentration of those compounds being about 1.3 (1.5), 1.2 (1.5), 1.5 (2.2), 1.2 (1.4) and 1.1 (1.7) times greater than that of wheat plants irrigated tap water, respectively. Thus, irrigation with sea water increased the concentration of low-molecular mass antioxidant com-pounds in wheat plants.

Table 3 shows the antioxidants status of wheat plants irrigated with sea water (10 and 20%, v/v) due to applica-tion of algal extracts and BRGs. Wheat plants irrigated with 10% sea water, application of algal extracts of *S*. *maxima* and *C*. *eliposies* containing higher amount of antioxidants, caused significant increase in contents of antioxidant components including: T-CAR, TOC, AA, TPCs and GSH with values being about 2.3 (1.9), 3.3 (2.9), 2.7 (2.4), 1.4 (1.3) and 2.9 (2.3) times as greater as that in control plants irrigated with 10%, respectively. While, wheat plants irrigated with 20% sea water, these values were about 2.2 (1.9), 2.4 (2.0), 2.2 (1.8), 1.3 (1.3) and 2.32 (1.8) times as high as that in control plants irrigated with 20% sea water, respectively. Therefore, it is clear from the present results that the antioxidant con-tents of wheat plants irrigated with sea water were posi-tively correlated with the concentration of total antioxidant

Table 2. Effect of algal antioxidant extracts on photosynthetic pigment contents in wheat plants irrigated with sea water.

Sea water stress	Treatment	Chl amg/ g f.w	Chl bmg/ g f.w	Total Chl.mg/ g f.w	Ratio a	Ratio b	Ratio c	Chl a/Chl b
Tap water	Sp.Rich antioxidant	0.867^{c} ±0.09	0.23^{b} ±0.04	1.1^{c} ±0.11	1.8	2.4	3.0	3.8
	Chl. Rich antioxidant	0.882^{c} ±0.09	0.22^{b} ±0.03	1.1^{c} ±0.14	1.8	2.4	3.0	3.5
	Sp.	0.997^{d} ±0.07	0.19^{a} ±0.02	1.1^{c} ±0.13	1.8	2.4	3.0	3.7
	Chl.	0.721^{b} ±0.09	0.17^{a} ±0.04	0.891^{b} ±0.16	1.4	2.0	2.4	3.1
	AA (Positive control)	0.539^{a} ±0.08	0.18^{a} ±0.04	0.719^{a} ±0.11	1.1	1.6	2.0	3.0
	BA (Positive control)	0.692^{b} ±0.06	0.20^{a} ±0.02	0.892^{a} ±0.17	1.4	2.0	2.4	3.5
	Nagative conntrol	0.452^{a} ±0.07	0.17^{a} ±0.04	0.622^{a} ±0.18	0.0			2.66
10% sea water	Sp.Rich antioxidant	0.836^{d} ±0.08	0.21^{b} ±0.03	1.05^{d} ±0.15	1.7	2.3	2.9	4.0
	Chl. Rich antioxidant	0.613^{c} ±0.06	0.21^{b} ±0.02	0.814^{c} ±0.17	1.3	1.8	2.2	2.9
	Sp.	0.53^{b} ±0.07	0.16^{a} ±0.09	0.69^{b} ±0.16	1.1	1.5	1.9	3.3
	Chl.	0.533^{b} ±0.09	0.15^{a} ±0.02	0.683^{b} ±0.17	1.1	1.5	1.9	5.4
	AA (Positive control)	0.491^{b} ±0.08	0.17^{a} ±0.±0.03	0.691^{b} ±0.08	1.1	1.5	1.9	2.9
	BA (Positive control)	0.585^{b} ±0.07	0.18^{b} ±0.04	0.765^{b} ±0.17	1.2	1.7	2.1	3.3
	Nagative conntrol	0.321^{a} ±0.06	0.13^{a} ±0.04	0.451^{a} ±0.07	0.7	0.0		2.5
20% sea water	Sp.Rich antioxidant	0.745^{c} ±0.08	0.2^{c} ±0.04	0.945^{d} ±0.08	1.5	2.1	2.6	3.8
	Chl. Rich antioxidant	0.531^{b} ±0.06	0.19^{b} ±0.03	0.721^{c} ±0.16	1.2	1.6	2.0	2.8
	Sp.	0.501^{b} ±0.07	0.15^{a} ±0.02	0.651^{b} ±0.08	1.0	1.4	1.8	3.3
	Chl.	0.495^{c} ±0.08	0.11^{c} ±0.02	0.56^{c} ±0.12	1.4	2.0	2.4	3.3
	AA (Positive control)	0.431^{b} ±0.09	0.17^{b} ±0.03	0.601^{b} ±0.14	1.0	1.3	1.6	2.5
	BA (Positive control)	0.511^{b} ±0.09	0.16^{b} ±0.04	0.671^{b} ±0.11	1.0	1.5	1.8	3.2
	Nagative conntrol	0.255^{a} ±0.08	0.11^{a} ±0.02	0.365^{a} ±0.08	0.6	0.8	0.0	2.3
LSD at level (P< 0.05)		0.11	0.06	0.13				

of algal extracts. The increase of the total antioxidant contents in sea water stressed wheat plants was in the decreasing order: *S. maxima* (HAO) > *C. eliposies* (HAO) > *S. maxima* (NAO) > *C. eliposies* (NAO). Also, application of AA and BA increased the T-CAR, TOC, AA, TPCs and GSH contents in wheat plant irrigated with sea water at 10 and 20%, but this increase was not significant when compared with the values obtained from un- irrigated wheat plants with sea water at 10 and 20% levels.

Our previous experiments show that the alleviation of low-molecular mass antioxidant compound levels are considered indexes for specific protective mechanisms against environmental stress conditions (Abd El-Baky et al., 2003, 2004). Looking at the concentrations of T- CAR, TOC, AA and TPCs in stressed wheat plants treated with algal extracts; one can deduce that the antioxidant components exerted positive influence against oxidative stress induced by the sea water. However, elevation of GSH and AA levels in treated plants was posi-tively associated with the increase of compounds act as free-radical traps (e.g. TOC and TPCs). These findings might indicate that the AA and GSH can act as hydrogen atom donors, regenerating of tocopherols (in reducing form) from inert tocopherol phenoxy radi-

Table 3. Influences of algal antioxidant spraying on antioxidant contents in wheat plants irrigated with sea water.

	Treatment	Carotenoids				Tecopherols				Ascorbic acid				Phenolic				GSH			
			Ratio a	Ratio b	Ratio c		Ratio a	Ratio b	Ratio c		Ratio a	Ratio b	Ratio c		Ratio a	Ratio b	Ratio c	m mol/ g F.W	Ratio a	Ratio b	Ratio c
Tap water	Sp.Rich antioxidant	0.685^{b} ±0.08	1.4	1.1	0.9	1.89^{b} ±0.26	2.4	2.4	1.2	0.589^{c} ±0.08	2.6	1.7	1.1	3.23^{e} ±0.29	2.4	2.1	1.7	0.823^{b} ±0.12	2.1	2.0	1.2
	Chl. Rich antioxidant	0.721^{b} ±0.05	1.5	1.2	1.0	2.01^{b} ±0.27	2.5	2.1	1.2	0.491^{b} ±0.18	2.1	1.4	0.9	2.31^{b} ±0.38	1.8	1.5	1.2	0.827^{b} ±0.18	2.1	2.0	1.2
	Sp.	0.58^{b} ±0.18	1.2	0.933	0.8	1.65^{b} ±0.23	2.1	1.7	1.0	0.452^{b} ±0.18	1.9	1.3	0.8	2.65^{d} ±0.44	2.0	1.7	1.4	0.774^{b} ±0.06	2.0	1.8	1.1
	Chl.	0.611^{b} ±0.07	1.2	1.0	0.8	1.45^{b} ±0.17	1.8	1.5	0.9	0.396^{b} ±0.08	1.7	1.1	0.7	1.98^{b} ±0.41	1.5	1.3	1.1	0.642^{b} ±0.12	1.6	1.5	0.9
	AA	0.535^{a} ±0.08	1.1	0.9	0.7	0.921^{a} ±0.24	1.2	1.2	0.6	0.314^{a} ±0.06	1.3	0.9	0.6	1.36^{b} ±0.28	1.03	0.9	0.7	0.521^{a} ±0.18	1.3	1.2	0.8
	BA	0.522^{a} ±0.18	1.1	0.8	0.7	0.882^{a} ±0.07	1.1	1.1	0.5	0.292^{a} ±0.05	1.2	0.8	0.5	1.21^{a} ±0.48	0.9	0.8		0.426^{a} ±0.06	1.1		0.6
	Negative control	0.492^{a} ±0.28	0.0			0.795^{a} ±0.17	0.0			0.239^{a} ±0.05	0.0			1.32^{a} ±0.33	0.0			0.392^{a} ±0.18	0.0		
10% sea water	Sp.Rich antioxidant	1.42^{e} ±0.08	2.9	2.3	1.9	2.65^{d} ±0.31	3.3	3.3	1.6	0.965^{e} ±0.11	4.0	2.7	1.8	2.21^{d} ±0.25	1.7	1.4	1.2	1.22^{d} ±0.18	3.1	2.9	18
	Chl. Rich antioxidant	1.19^{c} ±0.28	2.4	1.9	1.6	2.33^{d} ±0.33	2.9	2.9	1.4	0.859^{c} ±0.07	3.6	2.4	1.6	1.92^{c} ±0.26	1.6	1.3	1.0	0.986^{c} ±0.14	2.5	2.3	1.4
	Sp.	0.954^{b} ±0.28	1.9	1.5	1.3	1.86^{c} ±0.37	2.7	2.7	1.3	0.745^{c} ±0.08	3.1	2.1	1.4	1.97^{c} ±0.27	1.5	1.3	1.1	0.978^{c} ±0.07	2.5	2.3	1.4
	Chl.	0.921^{b} ±0.08	1.9	1.5	1.3	1.53^{b} ±0.38	1.9	1.6	1.3	0.651^{b} ±0.05	2.7	1.9	1.2	1.67^{b} ±0.16	1.3	1.1	0.9	0.897^{c} ±0.18	2.3	2.1	1.3
	AA	0.871^{b} ±0.18	1.8	1.4	1.2	1.21^{b} ±0.29	1.5	1.5	0.7	0.523^{b} ±0.07	2.2	1.5	1.0	1.64^{b} ±0.14	1.2	1.1	0.9	0.854^{a} ±0.09	2.2	2.0	1.3
	BA	0.853^{b} ±0.08	1.7	1.4	1.2	1.13^{a} ±0.37	1.4	1.4	0.7	0.459^{a} ±0.08	1.9	1.3	0.9	1.68^{a} ±0.17	1.3	1.1		0.754^{a} ±0.15	1.9		1.1
	Negative control	0.621^{a} ±0.07	1.3	0.0		0.974^{a} ±0.15	1.2	0.0		0.351^{a} ±0.05	1.5	0.0		1.53^{a} ±0.18	1.2	0.0		0.421^{a} ±0.08	1.1	0.0	
20% sea water	Sp.Rich antioxidant	1.63^{c} ±0.08	3.3	2.6	2.2	2.89^{e} ±0.33	3.6	3.6	2.4	1.15^{d} ±0.11	4.8	3.3	2.2	2.41^{d} ±0.13	1.8	1.6	1.3	1.58^{e} ±0.16	4.0	3.7	2.3
	Chl. Rich antioxidant	1.42c ±0.15	2.9	2.3	1.9	2.49^{c} ±0.33	3.1	3.1	2.0	0.953^{c} ±0.18	4.0	2.7	1.8	2.38^{d} ±0.36	1.8	1.6	1.3	1.23^{b} ±0.13	3.1	2.9	1.8
	Sp.	1.41^{c} ±0.18	2.9	2.3	1.9	2.11^{b} ±0.45	2.7	2.2	1.7	0.897^{c} ±0.18	3.8	2.6	1.7	2.22^{c} ±0.15	1.7	1.5	1.2	1.35^{c} ±0.11	3.4	3.2	2.0
	Chl.	1.31^{b} ±0.28	2.7	2.1	1.8	1.75^{b} ±0.38	2.2	1.8	1.4	0.784^{b} ±0.07	3.3	2.2	1.5	1.99^{b} ±0.25	1.5	1.3	1.1	0.989^{b} ±0.17	2.5	2.3	1.5

Table 3 continue.

	AA	1.21[b] ±0.15	2.5	1.9	1.7	1.35[b] ±0.33	1.7	1.7	1.1	0.698[b] ±0.09	2.9	2.0	1.3	1.93[a] ±0.26	1.5	1.3	1.0	0.978[a] ±0.09	2.5	2.3	1.4
	BA	1.11[b] ±0.28	2.3	1.9	1.5	1.39[a] ±0.33	1.7	1.8	1.2	0.573[a] ±0.07	2.4	1.6	1.1	1.96[a] ±0.24	1.5	1.3		0.984[a] ±0.07	2.5		1.4
	Negative control	0.731[a] ±0.05	1.5	1.2	0.0	1.22[a] ±0.33	1.5	1.3	0.0	0.532[a] ±0.18	2.2	1.5	0.0	1.86[a] ±0.17	1.4	0.9	0.0	0.681[a] ±0.12	1.7	1.6	0.0
LSD at level (P< 0.05)		0.22				0.28				0.23				0.31				0.28			

cal (oxidized form) during oxidative stress. Here again, the increase of antioxidant production in treated wheat plants with algal extracts may be able to provide protection against oxidative stress. Thus, one can suggest that the treatment of wheat plants with algal extracts can tolerate its resistance against salinity stress.

Effect of algal extracts on lipid peroxidation in wheat plants cultivation under sea water stress

As shown in Table 4 and Figure 1, TBARS concentration in irrigated wheat plants with sea water (10 and 20%, v/v) was significantly increased over than that in wheat plants irrigated with tap water. The increase of TBARS levels was generally thought to be the consequence of increased production of peroxides under sea water stress. The application of algal extracts of *C*. *eliposies* and *S*. *maxima* (in parentheses) reduced TBARS content by 64.3 (78.6%) and 42.1 (61.9%) than that of irrigated wheat plants with 10 and 20% (v/v) sea water, respectively. Application of AA and BA reduced TBARS levels of wheat plants irrigated sea water at both levels (10 and 20% v/v). However, reduction level of TBARS in treated plants with BRGs was less than that of plants treated with algal extracts. Hence, application of algal extracts caused a significant reduction in lipid peroxidation products as TBARS in stressed plants due to scavenge lipid peroxy radicals and inhibit the degradation products of lipid hydroperoxide. The present results suggest that the application of algal extracts and BRGs increased all non- enzymatic antioxidant that protect the cells against attack by free radicals. Also, these results show a strong correlation between sea water tole-rance and the levels of antioxidants of the algal extracts. In addition, the balance between the antioxidant status and reduction of oxidative stressed played an important role in providing wheat defense mechanism against salt-induced oxidative damage. In other words, algal extracts may enhance the biosynthesis of non-enzymatic compounds. There are several reports suggested that the rate of ROS production is less than the scavenging mechanism. Changes in antioxidant level in many crops and oxidized products as TBARs have been observed under salt stress. Also, the correlation between the antioxidant capacity and salt tolerance has been documented (Gossett et al., 1994; D'Amico et al., 2004; Parida and Das, 2005; Raza et al., 2007).

Effect of algal extracts on antioxidant enzymes of wheat plants cultivation under sea water stress.

The activity of SOD of wheat plants was significantly (P 0.05) affected by irrigation with sea water at both levels (10 and 20% v/v) (Table 5) as compared with the level of wheat plants irrigated with normal water. At 10% (v/v) sea water, the activity of SOD of wheat plants treated with algae (*C. ellipsoida* and *S. maxima*) extracts containing high antioxidant levels being about 1.3 (1.4) and 1.1 (1.2) as high as that found in wheat plant irrigated with 10 and 20% (v/v) sea water, respectively. Thus, SOD activity in irrigated-wheat plants showed the remarkable changes when wheat plants treated with algal extracts. Application of BRGs substances (AA and BA) did not cause any significant (P 0.05) increase in SOD activity in irrigated wheat plants with low and high levels of sea water. However, the increase in SOD activities of wheat plants treated with algae extracts was correlated with the levels of antioxidants present in algal extracts. A POX and APX activities were significantly increased (P 0.05) in wheat plants irrigated with 10 and 20% (v/v) sea water. The increase in POX and APX activities was correlated with the increase of sea water levels (Table 5). Application of algal extracts on wheat plants by sea water caused significant increase in POX and APX activities. As compared with values of POX and APX activities of wheat plant irrigated with 20% sea water. The application of algal *Chl. eliposies* and *Sp. maxima* (in parenthesis) extracts contained high antioxidan

Table 4. Effect of algal antioxidant extracts on lipid peroxidation in wheat plants irrigated with sea water

Sea water stress	Treatment	TBARs MDA mmol/ mg protein	Inhibition %
Tap water	Sp.Rich antioxidant	8.00 ± 1.36	94.20
	Chl. Rich antioxidant	14.00 ± 1.44	90.00
	Sp.	10.3 0 ±0.98	92.60
	Chl.	20.00 ±2.36	85.70
	AA (Positive control)	25.00 ± 2.33	82.10
	BA (Positive control)	27.00 ± 2.75	80.70
	Normal control	30.00 ± 2.65	78.60
10% sea water	Sp.Rich antioxidant	30.00 ± 2.71	78.60
	Chl. Rich antioxidant	50.00 ± 3.11	64.30
	Sp.	60.30 ± 3.33	56.90
	Chl.	74.40 ± 3.58	46.90
	AA (Positive control)	82.9 ± 4.33	40.80
	BA (Positive control)	85.50 ± 4.54	38.90
	Negative control	95.9 ± 3.35	31.50
20% sea water	Sp.Rich antioxidant	53.3 ± 3.64	61.90
	Chl. Rich antioxidant	81.1 ± 3.54	42.10
	Sp.	92.1 ± 2.33	34.20
	Chl.	102.6 ± 3.11	26.70
	AA (Positive control)	127.5 ± 4.98	9.20
	BA (Positive control)	134.1 ± 3.11	4.20
	Nagative conntrol	140.0 ± 4.88	0.00
LSD at level (P< 0.05).		5.26	

All values are significant at (P= 0.05)
Data are present the mean ± of three experiment with replicated measurements.

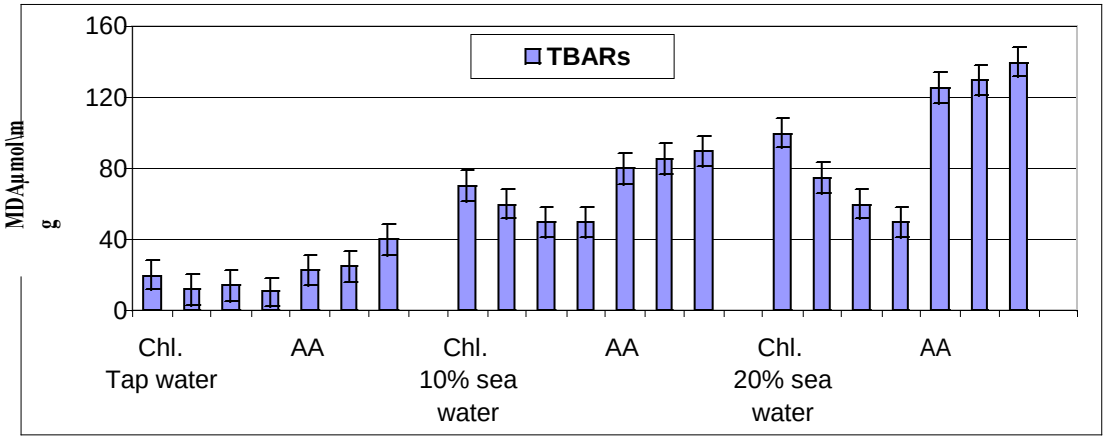

Figure 1. Effect of algal antioxidant extracts on lipid peroxidation in wheat plants under sea water salinity stress.

levels increased POX and APX activities, values being about 1.2 (1.5) and 1.3 (1.5) - fold, respectively. Also, SOD, POX and APX showed similar trend in increasing these enzyme activities. Antioxidant enzyme activities of wheat plants stressed by sea water were increased due to treatment with AA and BA bioregulator. The increase in SOD, POX and APX enzyme activities of wheat plants under sea water salinity stress sprayed with algal extracts followed the order: *S. maxima* (HAO) > *C. eliposies* (HAO) > *S. maxima* (NAO) > *C. eliposies* (NAO). According to these finding, it seems possible that the antioxidants present in algal extracts might be required for

Table 5. Effect of algal antioxidant extracts on antioxidant enzymes in wheat plants irrigated with sea water

Sea water	treatment	SOD	Ratio [a]	Ratio [b]	Ratio [c]	POX	Ratio [a]	Ratio [b]	Ratio [c]	APX	Ratio [a]	Ratio [b]	Ratio [c]
stress		U/mg protein /min				U/mg protein /min				U/mg protein /min			
Tap water	Sp.Rich antioxidant	49.52^{e} ± 1.55	1.5	1.2	1.0	45.29^{e} ± 1.54	1.4	1.2	1.0	397.2^{e} ±4.54	1.4	1.3	1.2
	Chl. Rich antioxidant	44.25d ± 1.54	1.3	1.1	0.9	40.21^{d} ± 1.74	1.2	1.0	0.9	377.3^{c} ± 4.79	1.3	1.2	1.1
	Sp.	45.74^{e} ± 1.65	1.3	1.1	1.0	39.15^{c} ± 1.39	1.2	1.0	0.9	375.2^{c} ± 4.52	1.3	1.2	1.1
	Chl.	40.51^{c} ± 1.74	1.2	1.0	0.8	38.45^{c} ± 1.99	1.2	1.0	0.9	352.5^{c} ± 4.71	1.2	1.1	1.1
	AA	38.23^{c} ± 1.87	1.1	0.9	0.8	37.78^{b} ± 1.74	1.2	1.0	0.8	330.9^{b} ± 4.54	1.2	1.1	1.0
	BA	35.28^{b} ± 1.39	1.0	0.9	0.7	35.21^{b} ± 1.54	1.1	0.9	0.8	310.2^{b} ± 4.71	1.1	1.0	0.9
	Negative control	34.22^{a} ± 1.69	0.0			32.41^{a} ± 1.99	0.0			282.3^{a} ± 4.45	0.0		
10% sea water	Sp.Rich antioxidant	58.31^{e} ± 1.54	1.7	1.4	1.2	54.82^{e} ± 1.99	1.7	1.4	1.2	482.3^{c} ± 1.54	1.7	1.6	1.4
	Chl. Rich antioxidant	53.48^{d} ± 1.74	1.6	1.3	1.1	48.27^{d} ± 1.54	1.5	1.2	1.1	475.2^{c} ± 4.52	1.9	1.5	1.4
	Sp.	51.52^{c} ± 1.39	1.5	1.3	1.0	46.37^{c} ± 1.79	1.4	1.2	1.0	437.5^{c} ± 4.71	1.5	1.4	1.3
	Chl.	49.31^{c} ± 1.99	1.4	1.2	1.0	43.32^{c} ± 1.74	1.3	1.1	1.0	391.4^{b} ± 4.52	1.4	1.3	1.2
	AA	45.72^{b} ± 1.54	1.3	1.1	0.9	43.25^{b} ± 1.99	1.3	1.1	1.0	369.2^{b} ± 4.54	1.3	1.2	1.1
	BA	42.19^{a} ± 1.99	1.2	1.0	0.9	42.01^{a} ± 1.54	1.3	1.1	0.9	333.7^{a} ± 4.84	1.2	1.1	1.0
	Negative control	41.21^{a} ± 1.74	1.2	0.0		39.23^{a} ± 1.39	1.2	0.0		310.2^{a} ± 4.84	1.1	0.0	

the induction of antioxidant enzyme of wheat plants irrigated with sea water. Also, the results revealed that levels of antioxidant enzyme activities were in positively correlated with the level of antioxidants occurring in algal extracts.

One can suggest that the antioxidant content in algal ex-tracts may causes a significant role in controlling the activities of antioxidant enzymes of wheat plants stressed by sea water. Therefore, the proactive effect of algal extracts might be attributed to the induction of high antioxidant enzyme activities (SOD, POD and APX) required for decreasing of ROS levels of treated plants.

Effect of algal extracts on dry weight and yield of wheat plants cultivation under sea water stress: The adverse effect of irrigation with sea water at 10 and 20% (v/v) on the overall growth of wheat plants was already evident. The irrigation with sea water caused significant reduction of plant height, shoot fresh, spike length and spikelet's/ spike (data not showed). The whole plant dry weigh (PDW) and grain yield (GY) of wheat plants were significantly reduced to 58 - 66% and 50 - 61%, respectively of that of plants irrigated tap water (Table 6 and Figure 2). Application of algal extracts was markedly increased either PDW or GY of sea water stressed-wheat plants. As com-pared with those levels of wheat plants irrigated 20% sea water, application of algal *C. eliposies* and *S. maxima* extracts containing low and high level of antioxidants, significantly increased PDW with values being about 1.62(1.78) - and 1.8(2.01)-fold, respectively. Whereas, the increase of grain yield GY was being about 1.53(1.71)- and 1.88(2.11)-fold, respectively. The improvement in growth parameter and grain yield and components of stressed wheat plants with algal extracts was in the decreasing order: *S. maxima* (HAO) > *C. eliposies* (HAO) > *S. maxima* (NAO) > *C. eliposies* (NAO). The PDW and GY caused non-significant elevation of irrigated wheat plants with 10 and 20 % seawater when treated with BRGs. However, the increase values of GY and PDW were posi-tively correlated with the concentration of antioxidant content in algal extracts. The decreasing in FW or DW levels was also reported in salt-treated plants of wheat and barley Sairam and Srivastava, 2002). Francois et al. (1984) reported that irrigation of wheat plants with 10 or 25% sea water generally decreased grain yield and their reduction was attributed to a reduction of grain weight per (spike and individual weight. However, the increase of

Table 6. Effect of algal antioxidant extracts on dry weight and yield in wheat plants irrigated with sea water

Sea water stress	Treatment	Whole plant dry weight /g	Ratio [c]	100 grain weight g	Ratio [c]
Tap water	Sp.Rich antioxidant	3.54 ±0.36	2.62	5.3 ±0.45	3.11
	Chl. Rich antioxidant	2.88 ±0.27	2.13	4.9 ±0.36	2.88
	Sp.	2.73 ± 0.21	2.02	4.5 ±0.27	2.60
	Chl.	2.63 ±0.33	1.94	4.3 ±0.27	2.50
	AA (Positive control)	2.45 ±0.24	1.81	3.9 ±0.34	2.30
	BA (Positive control)	2.31 ±0.28	1.71	3.8 ±0.26	2.20
	Normal conntrol	2.32 ± 0.14	1.72	3.4 ±0.22	2.00
10% sea water	Sp.Rich antioxidant	2.84 ±0.16	2.10	4.5 ±0.36	2.60
	Chl. Rich antioxidant	2.56 ±0.26	1.90	3.9 ±0.41	2.88
	Sp.	2.16 ±0.31	1.60	3.4 ±0.28	2.30
	Chl.	2.01 ±0.24	1.48	2.9 ±0.37	1.70
	AA (Positive control)	2.11 ±0.21	1.66	2.6 ±0.36	1.50
	BA (Positive control)	2.0 ±0.17	1.48	2.3 ±0.26	1.35
	Negative control	1.55 ±0.11	1.15	2.1 ±0.15	1.23
20% sea water	Sp.Rich antioxidant	2.71 ±0.21	2.01	3.6 ±0.25	2.11
	Chl. Rich antioxidant	2.45 ±0.17	1.80	3.2 ±0.24	1.88
	Sp.	2.4 ±0.14	1.78	2.9 ±0.17	1.71
	Chl.	2.2 ±0.16	1.62	2.6 ±0.18	1.53
	AA (Positive control)	2.15 ±0.13	1.59	2.2 ±0.19	1.29
	BA (Positive control)	2.03 ±0.12	1.50	1.9 ±0.16	1.11
	Negative conntrol	1.35 ±0.09	0.00	1.7 ±0.14	0.00
LSD at level (P< 0.05).		0.15		0.12	

Ratio [c]: Treatment / Negative conntrol (with out any spraying and irrgated by 20% sea water).
All values are significant at (P=< 0.05)
Data are present the mean ± of three experiment with replicated measurements

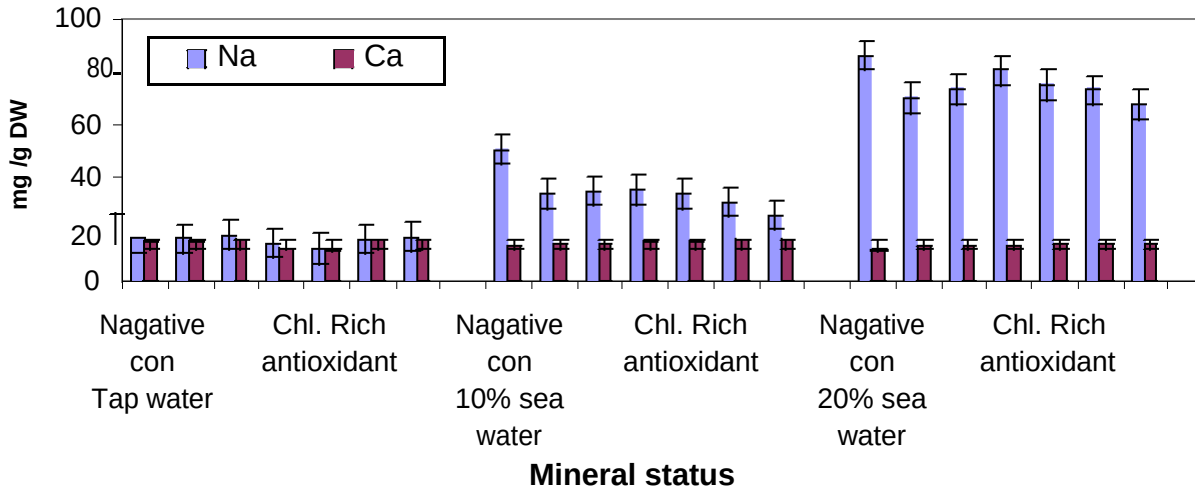

Figure 2. Effect of algal antioxidant extracts on mineral status in wheat plants under sea water salinity stress.

PDW and SY as one of the parameters of salt tolerance in crop plants indicated that metabolic and photosynthetic processes was found to be restored by application of algal extracts.

Effect of algal extracts on mineral contents of wheat plants cultivation under sea water stress

The accumulation of Na^+ in the wheat plants was signifi-

Table 7. Effect of algal antioxidant extracts on mineral status in wheat plants irrigated with sea water.

Sea water stress	Treatment	Na (mg g^{-1} D W)	Ca (mg g^{-1} D W)	Ca^{2+} / Na^{+} (mg g^{-1} D W)
Tap water	Sp.Rich antioxidant	16.93 ± 1.36	16.18 ± 1.36	0.95
	Chl. Rich antioxidant	12.25 ± 1.86	11.56 ± 1.58	0.94
	Sp.	16.22 ± 1.57	16.21 ± 1.69	0.94
	Chl.	14.55 ± 1.66	12.53 ± 1.31	0.86
	AA (Positive control)	16.26 ± 1.87	15.22 ± 1.55	0.94
	BA (Positive control)	17.82 ± 2.11	15.59 ± 1.36	0.87
	Normal conntrol	16.47 ± 1.32	15.31 ± 1.36	0.93
10% sea water	Sp.Rich antioxidant	25.27 ± 1.36	16.21 ± 1.31	0.64
	Chl. Rich antioxidant	33.25 ± 1.57	15.35 ± 1.68	0.46
	Sp.	30.26 ± 1.34	15.92 ± 1.55	0.53
	Chl.	35.25 ± 1.37	15.23 ± 1.69	0.43
	AA (Positive control)	33.45 ± 1.87	14.25 ± 1.14	0.43
	BA (Positive control)	34.52 ± 1.87	14.11 ± 1.17	0.41
	Negative conntrol	50.33 ± 2.36	13.21 ± 1.31	0.33
20% sea water	Sp.Rich antioxidant	67.52 ± 2.54	14.32 ± 1.87	0.21
	Chl. Rich antioxidant	75.23 ± 1.57	13.98 ± 1.14	0.18
	Sp.	72.98 ± 2.68	13.85 ± 1.57	0.19
	Chl.	80.45 ± 2.36	13.42 ± 1.28	0.17
	AA (Positive control)	70.21 ± 2.34	13.57 ± 1.69	0.19
	BA (Positive control)	73.22 ± 2.44	13.21 ± 1.73	0.18
	Negative control	86.25 ± 2.79	11.52 ± 1.19	0.13
LSD at level (P< 0.05)		3.26	1.60	

Data are present the mean ± of three experiment with replicated measurements.
All values are significant at (P = 0.05).

significantly increased (P<0.05) due to irrigation with sea water at 10 and 20% levels, whereas, Ca^{++} was slightly decreased the accumulation of Na^{+}, whereas Ca^{++} was slightly reduced, compared with that in stress plants. The ratio of Ca^{++}/ Na^{+} was used as a marker for mask the influence of application algal extracts on stressed wheat plants (Table 7 and Figure 2) . The Ca^{++}/ Na^{+} values in treated wheat plants with algal extracts were ranged from 0.86 to 0.95 whereas, these values in stressed plants by sea water at 10 and 20% were 0.33 and 0.13, respectively. Thus, the decrease of Ca^{++} and increase of Na^{+} and Ca^{++}/ Na^{+} ratio can be taken as indicators for improvement the growth of sprayed wheat plants with algal extracts. These effects might be due to discrimination of Na^{+} against Ca^{++}. Ashraf and Harris (2004) and Raza et al. (2007) postulated that Ca^{++}/ Na^{+} ratio might valid selection criteria for assessing salinity tolerance of different crop species. Thus, algal extracts led to better maintenance of reduced the accumulation of Na^{+} and increase Ca^{+}/ Na^{+} ratio to induce improvement of wheat plant growth under sea water stress. Also, D' Amico et al. (2004) reported that 20% (v/v) sea water inhibited growth of wheat plants causing a significantly decrease in biomass production caused by Na^{+}. However, applied of algal extracts was modulated the level of Ca^{++} to reach approximately that level of wheat plants decreased (Table 7 and Figure 2). Application of algal extracts and bioregulators (AA and BA) significantly irrigated with normal water (negative control). However, the role of Ca^{++} in antioxidant enzyme signal transduction was reported by Agarwal et al. (2005) who reported that Ca^{++} acts as a second messenger and causes a transient increase in H_2O_2, which in turn induces antioxidant enzyme activity leading to a decrease in ROS on long- time basis.

Finally, the findings of present study show that application of algal extracts increased the contents of photosynthetic pigments, non- enzymatic antioxidant including: TCO, T- CAR, GSH and TPCs and enhanced the activities of most antioxidant enzyme systems (POX, APX and SOD). The increase of antioxidant defense system was associated with the decrease of TBARs contents. Thus, algal extracts had multitude of functions as preserving the activity of enzymes, especially those of antioxidant enzyme and protecting against oxidative damage and may be reacted directly with ROS radicals. In addition, the algal extracts could be contain some bioactive components act as growth regulator substances such as auxine and cytokinins which lead to mitigate the effect of sea water salinity stress on wheat metabolic activity. However the results of Ördög et al. (2004) and Molnar and Ördög (2005) suggest that some plant growth

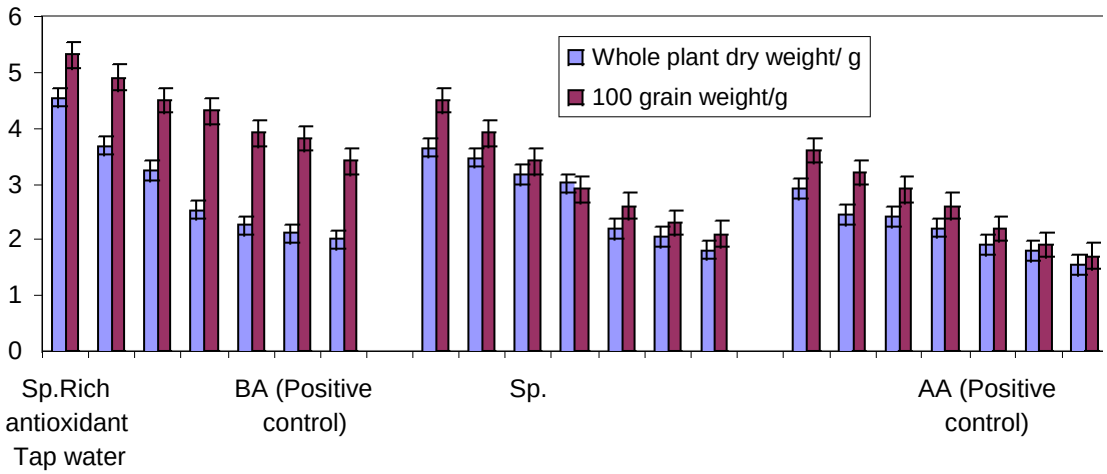

Figure 3. Effect of algal antioxidant extracts on dry weight and yield in wheat plants under sea water salinity stress

regulators found in microalgae possessed beneficial effects on tissue cultures of recalcitrant plants.

However, several plants have defense systems against ROS induced under salt stress, including either limiting the generation or quenching the ROS radicals. Also, Plants detoxify ROS by up-regulating antioxidant enzymes, such as POX, APX and SOD. The ROS which are by-products of hyperosmotic and ionic stresses, cause membrane dysfunction and cell death (Asada, 1992; Bohnert and Jensen, 1996). The plants defend against these reactive oxygen species by induction of activities of certain antioxidative enzymes such as CAT, PEX, GR, and SOD, which scavenge their reactive oxygen species. In view of several researchers, salt tolerance is often correlated with increasing the activity of antioxidative enzymes such as APX, GR and SOD, in wheat grown under sea water stress (Gossett et al., 1994; Meneguzzo and Navarilzzo, 1999; Hernandez et al., 2000). Also, the higher antioxidant enzyme activities such as SOD, POD, APX, GR and GST in tomato, barley, maize and sunflower plants were observed under salt stress (Liang, 1999; Rodriguez- Rosales et al., 1999; Muthukumarasamy et al., 2000; Rios-Gonzalez et al., 2002). Kennedy and De Fillippis (1999) reported that activities of CAT, polyphenol oxidase, SOD, and lipoxygenase were significantly increased as a result of NaCl treatment. Most of the aforementioned data suggest a correlation between stress tolerance and the presence of an efficient antioxidant system. Salt stress causes an oxidative stress because high amount of ROS are generated such as superoxides (O_2^-) and hydroxy (OH) and peroxy radicals (OOH) (Elstner, 1991). Their enhanced in amount of ROS need to be scavenged to maintain the normal cellular function and to avoid damage. However, the scavenging of O_2^- by SOD results in the production of H_2O_2, which is removed by APX (Asada, 1992) or CAT (Scandalias, 1990). The POX also was in detoxification of peroxy radicals. However, both O_2^- and H_2O_2 are not as toxic as the ($^{\cdot}$OH), which is formed by the combination of O_2^- and H_2O_2 in the presence of trace amounts of Fe^{2+} and Fe^{3+} by the Haber–Weiss reaction. Hydroxyl radical can damage chlorophyll, proteins, DNA, lipids and other important macromolecules. Thus, fatally affect plant metabolism and ultimately growth and yield (Frankel, 1985; Farr and Kogama, 1991). Therefore, salt tolerance is correlated with a more efficient antioxidative system (Raza et al., 2007).

Application of algal extracts seemed to reduce sea water salinity stress of wheat plants by decreasing Na^+ level and at same time increase the contents of photosynthetic pigments. Furthermore, the improvement of algal extracts on salinized wheat plants was associated with the increase of antioxidant defense abilities included non-enzymatic and enzymatic antioxidant systems, which led to alleviation of oxidative damage of functional molecules and maintenance of many physiological processes of wheat plants such as photosynthetic activity and productivity. However, the improvement of algal extracts on salinized wheat plants were decreased in the following orders: *S. maxima* (HAO) > *C. eliposies* (HAO) > *S. maxima* (NAO) > *C. eliposies* (NAO).

In conclusion, our results indicate that, even if oxidative stress is induced in wheat plants irrigated with 10 and 20% (v/v) sea water, 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. Therefore, the irrigation of wheat plants by mean of brackish water at 20% (v/v) is possible when treated with algal extracts.

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