ENHANCING SALT TOLERANCE OF WHEAT PLANT (*TRITICUM AESTIVUM L.*) BY APPLICATION OF PROLINE, ASCORBIC ACID, ARGININE, GLUTAMINE AND GLUTATHIONE

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ABSTRACT

Salinity is becoming nowadays one of the major abiotic stress issues worldwide. Wheat is considered as moderately salt tolerant crop and is adversely affected in response to the salt stress in terms of growth and yield. Pot experiment was conducted to determine the effect of exogenous proline, ascorbic acid, arginine, glutamine and glutathione application on some growth and biochemical parameters of wheat grown under salt stress conditions. The experiment was conducted under field conditions of wheat (*Triticum aestivum L.*) cultivar sakha 93. Three replicates, five grains per replicate were sown directly in plastic pots. Wheat plants were treated with 100 mg/l proline, 88 mg/l ascorbic acid, 100 mg/l arginine, 10 mg/l glutamine and 50 mg/l glutathione. Salinity treatments were established by adding 6000, 8000 and 10000 mg/l of sea salt. The results obtained from this experiment revealed that growth parameters such as shoot length, root weight and grain weight, in addition to biochemical compounds such as chlorophyll, starch, fiber, ash and fat were affected by both salt and treatments. These parameters were decreased in response to salinity stress compared to untreated plants. The decrement in the growth parameters and biochemical compounds were found to be increased with increasing salt concentrations, particularly at 6000 mg/l, 8000 mg/l and 10000 mg/l respectively. The selected compounds mitigate the negative effects of salt stress and improved growth parameters and

biochemical compounds compared with control plants under different salinity level.

Key words: abiotic stress, salt stress, salinity tolerance and wheat plant.

INTRODUCION

Salinity are major abiotic stress which leads to increasing yield losses in crops all over the world (Fulda *et al.,* 2011). High salinity is one of the most widespread abiotic stress factors in agriculture, causing problems in plant production both on naturally saline soils and on irrigated lands with unsuitable water management or exposure to high evaporation. Depending on the level of the stress and the stage of plant development, high salinity may induce various physiological malfunctions (Hossain *et al.,* 2015; Kranner and Seal, 2013). Salt stress, like many a biotic stress factors, reduces the ability of plants to take up water, leading to growth reduction as well as metabolic changes similar to those caused by the water stress (Munns, 2002). High salt concentration in root affects the growth and yield of many important crops (Taffouo *et al.,* 2004). The salinity may reduce the crop yield by upsetting water and nutritional balance of plant (Khan *et al.,* 2007; Taffouo *et al.,* 2009).

One of the biochemical changes occurring when plants are subjected to these harmful stress conditions such as salinity is the accumulation of reactive oxygen species (ROS) such as superoxide (O^{-2}), hydrogen peroxide (H_2O_2) and hydroxyl radicals (OH) (Xue and Liu, 2008). These ROS attack lipids, proteins and nucleic acids, causing lipid peroxidation, protein denaturing and DNA mutation. Plants possess several anti-oxidants enzyme systems that

protect their cells from the negative effects of ROS. Also reactive oxygen species cause chlorophyll degradation and damage the cellular components (Wang *et al.,* 2009; Van Breusegem and Dat, 2006; Verma and Mishra, 2005).

Salinity tolerance is defined as the ability of plants to continuously grow under salt stress conditions (Munns, 2002). This makes the uptake mechanisms and accumulation pattern of ions in different plant organs important factors to determine salt tolerance (Ashraf and Ahmad, 2000). Another major factor of salt tolerance mechanisms is the ability of plant cells to adjust osmotically and to accumulate organic solutes (proteins, sugar, amino acids, etc.). The accumulation of these compounds is not only important for cell osmo-regulation but also for the protection of subcellular structures (Munns, 2002) and maintenance of protein structures (Silveira *et al.,* 2001).

In response to salinity, plants have developed several strategies to cope with these challenges. One of the stress defense mechanisms is the antioxidant defense system, which includes antioxidants and antioxidant enzymes (Wang *et al.,* 2009). These include β-carotenes, ascorbate, α-tocopherol (α-toc), reduced glutathione (GSH) and enzymes including superoxide dismutase (SOD), peroxidase (POD), ascorbate peroxidase (APX), catalase (CAT), polyphenol oxidase (PPO) and glutathione reductase (GR) (Jaleel *et al.,* 2009). Most of the studies showed that increase in activity of SOD, APX, GR, CAT and POX in response to oxidative stress such as salt (Türkan *et al.,* 2005; Yasar *et al.,* 2006; Kusvuran *et al.,* 2012).

Wheat is the third largest cereal produced in the world and it supplies over 20% of calories in human food around the globe. Wheat production and productivity directly influence human survival in developing countries and quality of life in industrial countries (Shahzad *et al.,* 2013). Wheat is next to rice as a main source of food for consumers of developing countries and it is primary source of proteins (Braun *et al.,* 2010).

Mass and Hoffman (1977) in their classical work on salt tolerance classification had declared wheat (*Triticum aestivum L.*) as a moderately salt tolerant crop. Several approaches have been proposed to improve salt tolerance of wheat by introducing genes for salt tolerance into adapted cultivars (Munns, 2005), screening of large germplasm collections (Shahzad *et al.,* 2012), detailed field trials of selected cultivars (Munir *et al.,* 2011), conventional breeding methods (Salam *et al.,* 1999) and unconventional crosses with wild relatives (Colmer *et al.,* 2006).

Another approaches to improve salt tolerance in plants by application of some compounds. Exogenous amino acids have been shown to promote potassium and calcium uptake. Exogenous application of proline is known to induce abiotic stress tolerance in plants (Claussen, 2005). The most studied compound under salinity stress is proline. The amount of proline usually increases under salinity (Khatkar and Kuhad, 2000). Exogenous application of proline can play an important role in enhancing plant stress tolerance. This role can be in the form of either osmoprotection or cryoprotection (Ashraf and Foolad, 2007; Giri, 2011). Exogenous application of amino acids may reduce salt induced adverse effects and results in a significant increment of

growth and yield (Salama, 2009). Some experimental studies have shown that exogenous application of amino acids stimulates the germination percentage and early seedling growth of wheat (Afzal *et al.,* 2006; Kürşat and Göksel, 2015). The role of arginine in plant stress response has been reported (Zeid, 2009; Nasibi *et al.,* 2011; Zheng *et al.,* 2011). Also, glutathione (GSH) is considered the most important redox buffer of the cell during plant stress (Elie *et al.,* 2014).

The aim of the present study was to evaluate the response of wheat plant growth under salinity conditions and to study the role of proline, ascorbic acid, arginine, glutamine and glutathione in alleviating the harmful effects of salinity stress.

MATERIALS AND METHODS

Cultivation of wheat plant and treatment: Wheat plant cultivation was performed according to Tottman and Broad (1987). The experiment was conducted in Agricultural Genetic Engineering Research Institute (AGERI), Agricultural Research Center (ARC) Egypt. The experiment was conducted at field conditions during wheat seasons from November to the end of April, seasons 2012/2013 and 2013/2014. The grains were sown in plastic pots (30 cm diameter and 25 cm depth), each containing black soil, sand and peatmoss mixture in equal ratios (see Tables 1 and 2 for soil analysis) with ten seeds per pot, in three replicates for each treatment. All pots were watered with half-strength Hoagland's nutrient solution during the experiment. After germination, unwanted seedlings were removed to insure that there were 5 seedlings in each pot. Plants of similar size were divided into three groups as

follow: group one as a control, irrigated with tap water. Group two, subdivided into three sets, Each set treated with salt solution 6000, 8000 and 1000 mg/l. Group three subdivided into three sets, treated with salt solution as group two plus the addition of proline (100 mg/l), ascorbic acid (88 mg/l), arginine (100 mg/l), glutathione (50 mg/l) and glutamine (10 mg/l) for each salt concentration. Treatments of groups two and three were started when three leaves were fully expanded for each plant. The sea salt was prepared by dissolving the salt in distilled water at 6000, 8000 and 1000 mg/l (the major ions of sea water were: 487 mM Na^+, 10 mM K^+, 54 mM Mg^{2+}, 586 mM Cl, plus other less concentrated macro- and micro-nutrients). Leave tissue samples of treated and control plants were extracted for the evaluation of some antioxidant enzymes such as ascorbate peroxidase, peroxidase and catalase after two weeks of salt application. Dry root weight and shoot length were recorded after two months of sowing, while grains weight and biochemical analysis including total chlorophyll, total carbohydrate, starch, total protein, fiber, ash and fat content were recorded at the end of seasons (after four months of sowing).

Table 1. Chemical analysis of soil sample for wheat plant

O.M	**pH**	**EC.**	**Cations meq.L^{-1}**				**Anions meq.L^{-1}**		
			Na^+	**K^+**	**Ca^{2+}**	**Mg^{2+}**	**Cl^-**	**CO_3^{2-}**	**HCO_3^-**
2.91	7.07	2.30	6.35	1.44	13.92	1.30	2.485	0	1.281

Note: meq: Milliequivalent, K: Potassium, Na: Sodium, Ca: Calcium, Mg: Magnesium, Cl: Chloride, CO_3: Carbonate, HCO_3: Bicarbonate, EC: Electrical conductivity (ds.m^{-1}), O.M: Organic matter (%).

Table 2. Mechanical analysis of soil sample for wheat plant

The relative distribution of soil granules (%)				**Texture**
Coarse sand	**Fine sand**	**Silt**	**Clay**	
45.95	26.33	10.17	17.52	**Sandy loam**

Biochemical analyses

Determination of total chlorophyll content: Total chlorophyll was measured using chlorophyll meter SPAD-502 (Konica-Minolta, Osaka, Japan) according to manufacturer's instructions.

Determination of starch, fiber, ash and fat contents: Starch, fiber, ash and fat contents were measured using NIRS™ DA1650 Analyser (Foss Allé 1, Hilleroed, Denmark) according to manufacturer's instructions.

Assay of enzyme activities

Enzymes extraction:

Extraction of catalase and peroxidase enzymes: Extraction of catalase (CAT) and peroxidase (POX) enzymes were performed according to Kong-ngern *et al.,* (2012). Leaf samples (0.5 g) were homogenized in ice-cold 0.1 M phosphate buffer (pH 7.5) containing 0.5 mM EDTA, 1mM polyethyleneglycol, 1mM phenylmethelsulfonyl fluoride and 0.01% (v/v) triton x-100 with pre-chilled pestle and mortar. Centrifuge tubes were used for each homogenate and were centrifuged at 4°C for 15 min at 15,000 × g. The supernatants were used for enzyme activity assay.

Extraction of ascorbate peroxidase enzyme: Extraction of ascorbate peroxidase enzyme was performed according to Kong-ngern *et al.,* (2012). Leaf samples (0.5 g) were homogenized in ice-cold 0.1 M phosphate buffer (pH 7.5) containing 0.5 mM EDTA, 2 mM ascorbate, 1mM

polyethyleneglycol, 1mM phenylmethelsulfonyl fluoride, 5% polyvinylpyrrolidin and 0.01% (v/v) triton X-100 with pre-chilled pestle and mortar. The following stages were similar to the extraction of other enzymes

Activity of catalase enzyme: Catalase activity was measured according to Aebi (1984). About 3 ml reaction mixture containing 1.5 ml of 100 mM potassium phosphate buffer (pH 7), 0.5 ml of 75 mM H_2O_2, 0.05 ml enzyme extraction and distilled water to make up the volume to 3 ml. Reaction started by adding H_2O_2 and decrease in absorbance recorded at 240 nm (ε = 36 $mM^{-1}\cdot cm^{-1}$) for 1 min. Enzyme activity was computed by calculating the amount of H_2O_2 decomposed.

Activity of ascorbate peroxidase enzyme: Activity of ascorbate peroxidase was measured according to Yoshimura *et al.,* (2000) by monitoring the rate of ascorbate oxidation at 290 nm (ε =2.8 $mM^{-1}cm^{-1}$). The reaction mixture contained 25 mM phosphate buffer (pH 7), 0.1 mM EDTA, 1 mM H_2O_2, 0.25 mM ascorbic acid and 0.05 ml enzyme extraction. Enzyme activity was computed by calculating the amount of H_2O_2 decomposed.

Activity of peroxidase enzyme: Peroxidase activity was measured by following the change of absorbtion at 470 nm due to guaiacol oxidation. Peroxidase activity was measured according to Polle *et al.,* (1994), reaction mixture contained 100 mM potassium phosphate buffer (pH 7), 10 mM H_2O_2, 20 mM guaiacol, 0.05 ml enzyme extraction and distilled water to make up the volume to 3 ml. Reaction started by adding H_2O_2 and decrease in absorbance recorded at 470 nm (ε = 26.6 $mM^{-1}\cdot cm^{-1}$) for 1 min. Enzyme activity was computed by calculating the amount of H_2O_2 decomposed.

Statistical analysis: The data for all parameters were statistically analyzed using MSTAT software. Each treatment was analyzed in three replicates. Analysis of variance (ANOVA) showed significant treatment effects, Duncan's Multiple Range Test was applied to compare the means at $P<0.05$.

RESULTS AND DISCUSSION

The experiment was conducted under greenhouse conditions with wheat (*Triticum aestivum L.*) cultivar sakha 93. Three replicates, five grains per replicate were sown directly in plastic pots. This experiment was carried out to study the effect of adding different compounds on growth, yield and some physiological parameters of wheat plants under different salinity levels. Treatments were (i) control: plants receiving tap water, (ii) salinity treatment: plants receiving different concentrations from sea salt, (iii) salinity plus supplementary: plants receiving different concentrations from sea salt plus application of proline, ascorbic acid, arginine, glutathione and glutamine. The results obtained from this experiment revealed that growth parameters such as shoot length, root weight and grain weight, in addition to biochemical compounds such as chlorophyll, carbohydrate, starch, protein, fiber, ash and fat content were affected by both salt and treatments. These parameters were decreased in response to salinity stress compared to untreated plants. The decrement in the growth parameters and biochemical compounds were found to be increased with increasing salt concentrations, particularly at 6000 mg/l, 8000 mg/l and 10000 mg/l respectively. Five compounds represented in proline, ascorbic acid, arginine, glutathione and glutamine were used as exogenous application under different salinity level. Growth parameters and

biochemical compounds were improved when adding these compounds to plants compared with control plants. The data were recorded after 120 days of growing and were subjected to analysis of variance (ANOVA) and treatment means comparison using MSTAT statistical analysis program. Significance between treatments was compared at the 0.05 probability level.

Shoot length: Analysis of variance (ANOVA) of data presented in Table 3 shows that salt stress significantly decreases the shoot length compared with untreated plants, the decrement was found to be increased with increasing salt concentrations, particularly at 6000 mg/l, 8000 mg/l and 10000 mg/l respectively. Otherwise, addition of proline, ascorbic acid, arginine, glutathione and glutamine showed significant increases in shoot length compared with control plants under different salinity levels (Table 4). The increase of shoot length over control was 8.7, 10.9, 21.7, 23.9 and 19.6% when treated with proline, ascorbic acid, arginine, glutathione and glutamine respectively at 6000 mg/l, while the increase over control was 4.3, 8.6, 15.2, 19.6 and 13% at 8000 mg/l respectively. The increase of shoot length over control was 6.8, 9.1, 13.6, 16 and 11.4% at 10000 mg/l. Nevertheless the highest value of shoot length at 10000 mg/l was recorded when treated with glutathione compared with control plants followed by arginine, glutamine, ascorbic acid and proline respectively. These results are in agreement with those obtained by Hassanein *et al.,* (2009) who found that shoot height of Zea mays plants were significantly decreased with increasing the salinity level. Similar finding has been reported by Karima, (2005) who performed the experiment on sunflower plant under salt stress and the effect of application

of ascorbic acid that led to significant increase in shoot length. Other authers studied the effect of exogenous application of ascorbic acid on other plant species as Hassanein *et al.,* (2009) on *Zea mays*, who found that shoot height is increasing by the addition of ascorbic acid. Moreover, AL-Mayahi (2016) found a progressive increase in plant height, by increasing ascorbic acid level in date palm plant (*Phoenix dactylifera L.*). Also Athar *et al.,* (2008) suggested that ascorbic acid could accelerate cell division and improve the growth.

Table 3. Effect of salt stress on wheat growth parameters.

Parameter Treatmant	**Root dry weight (gm/plant)**	**Shoot length (cm)**	**Thousand grain weight (gm)**
control*	0.45 a	49.0 a	40 a
sea salt 6000 mg/l	0.43 ab	46.0 b	35 b
sea salt 8000 mg/l	0.41 ab	45.5 c	32 c
sea salt 10000 mg/l	0.37 b	44.0 d	29 d

Note: Values have the same letter in the same column are not significantly different at LSD≤0.05 level according to Duncan's Multiple Range Test (DMRT). *Wheat plants irrigated with tap water.

Table 4. Effect of proline, ascorbic acid, arginine, glutathione and glutamine on shoot length of wheat plants under different salinity levels.

Shoot length (cm)				
Treatments	**6000 mg/l**	**8000 mg/l**	**10000 mg/l**	**Mean**
Control*	46.00 i	46.00 i	44.00 j	45.33 d
Proline	50.00 ef	48.00 gh	47.00 hi	48.33 c
Ascorbic acid	51.00 de	50.00 ef	48.00 gh	49.67 c
Arginine	56.00 ab	53.00 c	50.00 ef	53.00 ab
Glutathione	57.00 a	55.00 b	51.00 DE	54.33 a
Glutamine	55.00 b	52.00 cd	49.00 fg	52.00 b
Mean	52.50 a	50.67 b	48.17 c	

Note: Values have the same letter in the same column are not significantly different at LSD≤0.05 level according to Duncan's Multiple Range Test (DMRT). *Wheat plants treated with sea salt only.

Grain weight: ANOVA Table 3 shows that salt stress significantly decreases the weight of thousand grains compared with untreated plants, the decrement was found to be increased with increasing salt concentrations, particularly at 6000 mg/l, 8000 mg/l and 10000 mg/l respectively. However, exogenous application of proline, ascorbic acid, arginine, glutathione and glutamine showed improvement and significant increases in this parameter compared with control plants under different salinity levels (Table 5). Grain weight increase over control was 20, 21.4, 25.7, 28.5 and 22.9% when treated with proline, ascorbic acid, arginine, glutathione and glutamine respectively at 6000 mg/l, while the increase of grain weight over control was 28.1, 28.1, 31.3, 37.5 and 31.3% respectively at 8000 mg/l. The increase over control at 10000 mg/l was 34.5, 38, 39.7, 41.4 and 38% respectively. Moreover the highest value of grain weight was recorded when treated with glutathione compared with control plants followed by arginine, ascorbic acid and

glutamine which have the same values while the lowest values were recorded with proline when using high salinity level (10000 mg/l). These findings are in accordance of the results obtained previously by Zadeh and Naeini, (2007); Hussein *et al*., (2008); Hussein *et al*., (2011); Hozayn *et al*., (2013), in which they reported that grain weight was reduced by increasing rate of salts application into soil. On the other hand, when treating with ascorbic acid under salinity conditions a significant increase in grain weight of grapevine and Soybean was evedent (Fayed, 2010; Sheteaw, 2007). Moreover, El-Bassiouny and Bekheta (2001) reported that treatment application of wheat with arginine increased significantly grain weight as compared with control plants under salinity condition**.** They also mentioned that arginine is intimately involved in salt treated wheat plant thereby regulating growth, and grain yield.

Table 5. Effect of proline, ascorbic acid, arginine, glutathione and glutamine on grain weight of wheat plants under different salinity levels.

Thousand grain weight (gm)				
Treatments	**6000 mg/l**	**8000 mg/l**	**10000 mg/l**	**Mean**
Control*	35.00 h	32.00 i	29.00 j	32.00 c
Proline	42.00 cde	41.00 def	39.00 g	40.67 b
Ascorbic acid	42.50 bcd	41.00 def	40.00 fg	41.17 b
Arginine	44.00 ab	42.00 cde	40.50 efg	42.17 ab
Glutathione	45.00 a	44.00 ab	41.00 def	43.33 a
Glutamine	43.00 bc	42.00 cde	40.00 fg	41.67 ab
Mean	41.92 a	40.33 a	38.25 b	

Note: Values have the same letter in the same column are not significantly different at LSD≤0.05 level according to Duncan’s Multiple Range Test (DMRT). *Wheat plants treated with sea salt only.

Root weight: Data presented in Table 3 shows that salt stress has a negative effect on root dry weight and significantly decreased it compared with untreated plants. Otherwise, the application of proline, ascorbic acid, arginine, glutathione and glutamine showed significant increases in root dry weight compared with control plants under different salinity levels (Table 6). However the increase of root weight over control was 16.3, 37.2, 34.9, 32.6 and 30.2% when treated with proline, ascorbic acid, arginine, glutathione and glutamine respectively at 6000 mg/l, while root dry weight increases over control were 14.6, 24.4, 22, 26.8 and 19.5% respectively at 8000 mg/l. The increase over control was 8.1, 29.7, 24.3, 27 and 21.6% respectively at 10000 mg/l. Nevertheless the highest value of root weight at 10000 mg/l was recorded when treated with ascorbic acid compared with control plants followed by arginine, glutathione, glutamine and proline respectively. These results were similar to that obtained by Khan *et al*., (2003) who reported that the effect of ascorbic acid on growth improvement comes from the fact that they act as an antioxidant under salinity. Our results are consistent with previous reports that different antioxidants such as ascorbic acid mitigated salinity effects and thus enhanced salt tolerance on various crop plants. These authors proposed that antioxidants ameliorate the damaging effect of salinity through interaction of antioxidant response and protection of membranes (Ekmekçi and Karaman, 2012). Furthermore, there are some reports which provide evidence that ascorbic acid accelerates cell division and cell enlargement as observed in different plants such as *Pisum* (Cabo *et al.,* 1996), and *Lupinus albus* (Citterio *et al.,* 1994). These findings suggest that growth

promoting effect of ascorbic acid under saline conditions may have been due to enhanced antioxidant capacity and increase in cell division and cell enlargement. Moreover, Couée *et al*., (2004) indicated that the stimulation of polyamines to root growth and development may be related to the high flexibility of polyamine metabolism and the metabolic link between polyamine and ethylene synthesis which strongly suggest that, polyamines may play a role in environmentally induced plasticity of root development. The increase in fresh and dry root weight of plants treated with arginine is a reflection to the increase in growth rate cell division and/or cell enlargement and differentiation (Lixiong *et al*., 2002). Also, these effects of arginine may be due to that polyamine have been implicated in a wide range of biological processes including growth development and abiotic stress responses and cell division and differentiation (Nasibi *et al.,* 2014).

Table 6. Effect of proline, ascorbic acid, arginine, glutathione and glutamine on root weight of wheat plants under different salinity levels.

Root dry weight (gm/plant)				
Treatments	**6000 mg/l**	**8000 mg/l**	**10000 mg/l**	**Mean**
Control*	0.43 ab	0.41 ab	0.37 b	0.4033 a
Proline	0.50 ab	0.46 ab	0.40 ab	0.4733 a
Ascorbic acid	0.59 a	0.51 ab	0.48 ab	0.5200 a
Arginine	0.58 a	0.50 ab	0.46 ab	0.5100 a
Glutathione	0.57 a	0.59 ab	0.45 ab	0.5100 a
Glutamine	0.56 a	0.47 ab	0.45 ab	0.4567 a
Mean	0.5267 a	0.4750 a	0.4350 a	

Note: Values have the same letter in the same column are not significantly different at LSD≤0.05 level according to Duncan's Multiple Range Test (DMRT). *Wheat plants treated with sea salt only.

Total chlorophyll content: Analysis of variance (ANOVA) of data presented in Table 7 shows that treatments significantly affected total chlorophyll content in leaves. This photosynthetic pigment tends to decrease with increasing salinity level. Treating salt-stressed wheat plant with proline, ascorbic acid, arginine, glutathione and glutamine significantly increased the production of photosynthetic pigment (chlorophyll) compared with control plants under different salinity levels (Table 8). However, increase of total chlorophyll over control was 1.7, 7.8, 9.5, 13.2 and 21.4% when treated with proline, ascorbic acid, arginine, glutathione and glutamine respectively at 6000 mg/l, while the increase over control was 2.5, 5, 6.2, 6.5 and 7.1% respectively at 8000 mg/l. The increase of total chlorophyll over control at 10000 mg/l was 2.9, 4.5, 6, 6.2, and 7.6% respectively. Nevertheless the highest value of total chlorophyll was recorded when treated with glutamine compared with control plants followed by glutathione, arginine, ascorbic acid and proline respectively when using high salinity level (10000 mg/l). These results are in agreement with those obtained by Ghassemi-Golezaniet *et al.*, (2012), Hellal *et al.*, (2012) and Bahari *et al.*, (2013), they stated that the reductions are due to the inhibitory effects of salinity on many metabolic processes including, activity of mitochondria and chloroplasts. Also, El-Bagoury *et al.*, (1999) suggested that, the biosynthesis of chlorophylls in generally might be inhibited by the depressive effect of stress conditions on the absorption of some ions involved in the chloroplast formation. Moreover, Fayed, (2010), Sheteaw, (2007) and Maksoud *et al.*, (2009) reported that the treatment with ascorbic acid mitigated salinity induced effect on chlorophyll

reductions. The beneficial effect of ascorbic acid as antioxidant on photosynthetic pigments may be due to its role in decreasing the rate of photochemical reduction, chloroplast structure, photosynthetic electron transfer as well as photosynthesis (Kumar *et al.*, 1988). Ali *et al.*, (2007) and Sharkey *et al.*, (2007) had treated wheat plant with proline under salinity stress. They found a considerable enhancement of total chlorophyll content. They suggested that the increase in chlorophyll content due to exogenous proline application primarily increased the rate of CO_2 diffusion and favored higher photosynthetic rate. Similar promoting effects of arginine on photosynthetic pigments had been observed by Nassar *et al.* (2003) and El-Bassiouny *et al.*, (2008). A possible explanation for the promoting effect of arginine on photosynthetic pigment of wheat plant in their work is that arginine might retard chlorophyll destruction and or increase their biosynthesis or stabilize the thylakoid membrane (Gonzalez *et al.,* 1997). They also demonstrated that arginine may retard senescence via altering the stability and permeability of such membranes and protecting membranes and prevent chloroplast from senescing and therefore retarding chlorophyll loss. The role of arginine in chlorophyll synthesis is supported by HuiGuo *et al.,* (2006) who found that exogenous application of arginine protects photosystem II (PSII) against water stress at both transcriptional and translational levels. Also, it allows PSII to retain a higher activity level during stress in wheat seedlings resulting in the increase in chlorophyll contents. Moreover, these increases could be attributed to the role of magnesium as a structural component of chlorophyll and reinforced the role of arginine in chlorophyll biosynthesis (Krishnamurthy, 1991).

Table 7. Effect of salt stress on some biochemical composition of wheat plants.

Parameter / Treatment	Total chlorophyll μg cm^{-2}	Starch (%)	Fiber (%)	Ash (%)	Fat (%)
control*	49.1 a	69.04 a	2 a	1.8 a	1.8
sea salt 6000 mg/l	46.3 b	64.93 b	1.5 b	1.6 b	1.7 b
sea salt 8000 mg/l	43.5 c	62 c	1.3 c	1.5 c	1.55 c
sea salt 10000 mg/l	42.3 d	59 d	1 d	1.45 d	1.35 d

Note: Effect of different salinity level on total chlorophyll in leaves and different contents of grains: total carbohydrate, starch, total protein, fiber, ash and fat. Values have the same letter in the same column are not significantly different at LSD≤0.05 level according to Duncan's Multiple Range Test (DMRT). *Wheat plants irrigated with tap water.

Table 8. Effect of proline, ascorbic acid, arginine, glutathione and glutamine on total chlorophyll of wheat plants under different salinity levels.

Total chlorophyll (μg cm^{-2})				
Treatments	**6000 mg/l**	**8000 mg/l**	**10000 mg/l**	**Mean**
Control*	46.3 hi	43.5 j	42.3 j	44.03 d
Proline	47.1 fgh	46.0 hi	45.2 i	46.10 c
Ascorbic acid	48.9 def	48.5 efg	46.8 ghi	48.07 b
Arginine	51.7 bc	49.7 de	48.3 efg	50.07 a
Glutathione	52.4 b	50.0 cde	48.5 efg	50.97 a
Glutamine	56.2 a	50.6 cd	49.9 de	51.40 a
Mean	50.43 a	48.05 b	46.83 b	

Note: Values have the same letter in the same column are not significantly different at LSD≤0.05 level according to Duncan's Multiple Range Test (DMRT). *Wheat plants treated with sea salt only.

Starch content: Analysis of variance (ANOVA) of data presented in Table 7 shows that salt stress significant decreases the starch content in grains compared with untreated plants. The results recorded in the same table indicated that, starch content was significantly decreased with increasing salt

concentrations, particularly at 6000 mg/l, 8000 mg/l and 10000 mg/l respectively. The results also showed that, application of proline, ascorbic acid, arginine, glutathione and glutamine increased the starch content compared with control plants under different salinity levels (Table 9). However, increase of starch content over control was 7.5, 6.3, 6.2, 7.4 and 4.7% respectively at 6000 mg/l, while increase was recorded when using the same treatments by 12, 10.8, 12.6, 12.1 and 10.5% respectively at 8000 mg/l. Whereas at 10000 mg/l the increase over control was 14.1, 12.4, 12.7, 12.9 and 10.2% respectively for the same treatments. Moreover the highest value of starch content was recorded when treated with proline compared with control plants followed by glutathione, arginine ascorbic acid and glutamine respectively when using the highest concentration of sea salt (10000 mg/l).

Table 9. Effect of proline, ascorbic acid, arginine, glutathione and glutamine on starch content of wheat plants under different salinity levels.

Starch (%)				
Treatments	**6000 mg/l**	**8000 mg/l**	**10000 mg/l**	**Mean**
Control*	64.93 d	62.00 e	59.00 f	61.98 b
Proline	69.82 a	69.41 a	67.30 bc	68.84 a
Ascorbic acid	68.99 ab	68.72 ab	66.32 cd	68.01 a
Arginine	68.98 ab	69.80 a	66.50 cd	68.43 a
Glutathione	69.78 a	69.53 a	66.60 cd	68.64 a
Glutamine	68.00 abc	68.50 ab	65.00 d	67.17 a
Mean	68.42 a	67.99 a	67.99 a	

Note: Values have the same letter in the same column are not significantly different at LSD≤0.05 level according to Duncan's Multiple Range Test (DMRT). *Wheat plants treated with sea salt only.

Fiber content: ANOVA Table 7 shows that salt stress significantly decreases the fiber content in wheat grains compared with untreated plants. However, fiber content decreased significantly and gradually with increasing salt concentrations, particularly at 6000 mg/l, 8000 mg/l and 10000 mg/l respectively. On the other hand, addition of proline, ascorbic acid, arginine, glutathione and glutamine increased significantly fiber content compared with control plants under different salinity levels (Table 10). The increase of fiber content over control was 40, 46.7, 33.3, 66.6 and 53.3% respectively at 6000 mg/l, while increase was recorded when using the same treatments by 69.2, 84.6, 61.5, 92.3 and 53.8% respectively at 8000 mg/l. Whereas at 10000 mg/l the increases over control were 90, 80, 91, 100 and 70% respectively. Moreover the highest value of fiber content was recorded when treated with glutathione compared with control plants followed by arginine, proline, ascorbic acid and glutamine respectively when using the highest concentration of sea salt (10000 mg/l). These results were similar to that obtained by Maqsood *et al.*, (2008) who reported that a decrease in fiber content accumulation in maize grain is associated with salt stress.

Table 10. Effect of proline, ascorbic acid, arginine, glutathione and glutamine on fiber content of wheat plants under different salinity levels.

Fiber content (%)				
Treatments	**6000 mg/l**	**8000 mg/l**	**10000 mg/l**	**Mean**
Control*	1.5 i	1.3 j	1.0 k	1.267 c
Proline	2.1 de	2.2 cd	1.9 fg	2.067 b
Ascorbic acid	2.2 cd	2.4 ab	1.8 gh	2.133 ab
Arginine	2.0 ef	2.1 de	1.9 fg	2.000 b
Glutathione	2.5 a	2.5 a	2.9 ef	2.267 a
Glutamine	2.3 bc	2.0 ef	1.7 h	2.067 b
Mean	2.100 a	2.083 a	1.717 b	

Note: Values have the same letter in the same column are not significantly different at LSD≤0.05 level according to Duncan's Multiple Range Test (DMRT). *Wheat plants treated with sea salt only.

Ash content: Analysis of variance (ANOVA) of data presented in Table 7 indicated that salt stress significant decreases the ash content in wheat grains compared with untreated plants. The data recorded in the same table indicated that the ash content was significantly decreased with increasing salinity level. Moreover the obtained data in table (11) clearly demonstrated that the application of proline, ascorbic acid, arginine, glutathione and glutamine showed significant increases in ash content compared with control plants under different salinity levels. Ash content increase over control was 15.6, 16.9, 17.5, 18.1 and 14.4% when treated with proline, ascorbic acid, arginine, glutathione and glutamine respectively at 6000 mg/l, while the increases over control were 34, 35, 36, 37 and 31% respectively at 8000 mg/l. However the increase in this parameter was 24.8, 26.2, 26.9, 27.6 and 24.1% respectively at 10000 mg/l for the same treatments. Moreover the highest value of ash content was recorded when treated with glutathione compared with control

plants followed by arginine, proline, ascorbic acid and glutamine respectively under high salinity condition (10000 mg/l).

Table 11. Effect of proline, ascorbic acid, arginine, glutathione and glutamine on ash content of wheat plants under different salinity levels.

Ash content (%)				
Treatments	**6000 mg/l**	**8000 mg/l**	**10000 mg/l**	**Mean**
Control*	1.60 b	1.50 b	1.45 b	1.517 b
Proline	1.85 a	1.84 a	1.81 a	1.833 a
Ascorbic acid	1.87 a	1.85 a	1.83 a	1.850 a
Arginine	1.88 a	1.86 a	1.84 a	1.863 a
Glutathione	1.89 a	1.87 a	1.85 a	1.867 a
Glutamine	1.83 a	1.81 a	1.80 a	1.813 a
Mean	1.820 a	1.788 a	1.763 a	

Note: Values have the same letter in the same column are not significantly different at LSD≤0.05 level according to Duncan's Multiple Range Test (DMRT). *Wheat plants treated with sea salt only.

Fat content: As show in ANOVA Table 7, salt stress significantly decreases the fat content in wheat grains compared with untreated plants. The results recorded in the same table indicated that, fat content was significantly decreased with increasing salt concentrations. The results also revealed that, application of proline, ascorbic acid, arginine, glutathione and glutamine showed significant increases in fat content compared with control plants under different salinity levels (Table 12). However, fat content increase over control was 14.7, 11.8, 10.6, 12.9 and 9.4% when treated with proline, ascorbic acid, arginine, glutathione and glutamine respectively at 6000 mg/l, while the increases over control were 18.1, 13.5, 17.4, 16.8 and 14.8% respectively at 8000 mg/l. The increase over control at 10000 mg/l was 32.6, 25.9, 27.4, 33.3 and 29.6% respectively for the same treatments.

Nevertheless, the highest value of fat content was recorded when treated with glutathione followed by proline, arginine, glutamine and ascorbic acid respectively when using high salinity level (10000 mg/l). These findings are in accordance of the results obtained previously by Parida and Das, (2005) who mentioned that during the onset and development of salt stress within a plant, all the major processes are affected such as photosynthesis, protein synthesis, energy production and lipid metabolism.

Table 12. Effect of proline, ascorbic acid, arginine, glutathione and glutamine on fat content of wheat plants under different salinity levels.

Fat content (%)				
Treatments	**6000 mg/l**	**8000 mg/l**	**10000 mg/l**	**Mean**
Control*	1.70 cd	1.55 d	1.35 e	1.533 b
Proline	1.95 a	1.83 abc	1.79 abc	1.857 a
Ascorbic acid	1.90 ab	1.76 abc	1.70 cd	1.787 a
Arginine	1.88 abc	1.82 abc	1.72 bcd	1.807 a
Glutathione	1.92 ab	1.81 abc	1.80 abc	1.837 a
Glutamine	1.86 abc	1.78 abc	1.75 bc	1.797 a
Mean	1.868 a	1.758 abc	1.685 abc	

Note: Values have the same letter in the same column are not significantly different at LSD≤0.05 level according to Duncan's Multiple Range Test (DMRT). *Wheat plants treated with sea salt only.

Antioxidant enzymes: Analysis of variance (ANOVA) of data presented in Table 13 shows that salt stress significantly increases the catalase activity in leaves of wheat plant compared with untreated plants. The data recorded in the same table indicated that the catalase activity was gradually increased with increasing salinity level. As shown in Table 14 the application of proline, ascorbic acid, arginine, glutathione and glutamine caused a further increase in catalase activity compared with control plants under different salinity level. However the increase of catalase activity over control was 6.3,

12.5, 25, 56.3 and 25% when treated with proline, ascorbic acid, arginine, glutathione and glutamine respectively at 6000 mg/l, while the increase over control for the same treatments was 5.6, 8.3, 22.2, 44.4 and 16.7% respectively at 8000 mg/l. Whereas the increase of catalase activity over control was 2.5, 5, 15, 37.5 and 20% respectively at 10000 mg/l when using the same treatments. Moreover the highest value of catalase activity was recorded when treated with glutathione followed by glutamine, arginine, ascorbic acid and proline respectively when using the highest concentration of sea salt (10000 mg/l).

Table 13. Effect of salt stress on some antioxidant enzymes of wheat plants.

Parameter / Treatmant	APX activity	POX activity	CAT activity
control*	470 a	95 d	23 d
sea salt 6000 mg/l	620 b	140 c	32 c
sea salt 8000 mg/l	675 c	156 b	36 b
sea salt 10000 mg/l	735 d	171 a	40 a

Note: Effect of different salinity level on Ascorbate peroxidase (APX), peroxidase (POX) and catalase (CAT) activity (unit $min^{-1} \cdot g^{-1}$ FW). Values have the same letter in the same column are not significantly different at LSD≤0.05 level according to Duncan's Multiple Range Test (DMRT). *Wheat plants irrigated with tap water.

Table 14. Effect of proline, ascorbic acid, arginine, glutathione and glutamine on Catalase activity of wheat plants under different salinity levels.

Catalase activity (unit $min^{-1} \cdot g^{-1}$ FW)				
Treatments	**6000 mg/l**	**8000 mg/l**	**10000 mg/l**	**Mean**
Control*	32 n	36 l	40 I	36.00 e
Proline	34 m	38 k	41 h	37.67 d
Ascorbic acid	36 l	39 j	42 g	39.00 c
Arginine	40 i	44 f	46 e	43.33 b
Glutathione	50 c	52 b	55 a	52.33 a
Glutamine	40 i	42 g	48 d	43.33 b
Mean	38.67 c	41.83 b	45.33 a	

Note: Values have the same letter in the same column are not significantly different at LSD≤0.05 level according to Duncan's Multiple Range Test (DMRT). *Wheat plants treated with sea salt only.

It has been found in the present investigation that, salt stress significantly increases the peroxidase activity in leaves of wheat plant compared with untreated plants. However, peroxidase activity was increased significantly and gradually with increasing salinity levels as shown in Table (13). Moreover, addition of proline, ascorbic acid, arginine, glutathione and glutamine showed significant increases in peroxidase activity compared with control plants under different salinity levels (Table 15). However, peroxidase activity increase over control was 35.7, 23.6, 40, 37.1 and 42.9% when treated with proline, ascorbic acid, arginine, glutathione and glutamine respectively at 6000 mg/l, while the increase over control was 26.9, 15.4, 28.2, 29.5 and 33.3% respectively at 8000 mg/l. Whereas the increase over control was 20, 5.9, 18.8, 22.9 and 23.5% respectively for the same treatments at 10000 mg/l. Nevertheless, the highest value of peroxidase activity was recorded when treated with glutamine compared with control

plants followed by glutathione, arginine, proline and ascorbic acid respectively under high salinity condition (10000 mg/l).

Table 15. Effect of proline, ascorbic acid, arginine, glutathione and glutamine on Peroxidase activity of wheat plants under different salinity levels.

Peroxidase activity (unit $min^{-1} \cdot g^{-1}$ FW)				
Treatments	**6000 mg/l**	**8000 mg/l**	**10000 mg/l**	**Mean**
Control*	140 o	156 n	170 m	155.3 f
Proline	190 j	198 g	204 d	197.3 d
Ascorbic acid	173 l	180 k	180 k	177.7 e
Arginine	196 h	200 f	202 e	199.3 c
Glutathione	192 i	202 e	209 b	201.0 b
Glutamine	200 f	208 c	210 a	206.0 a
Mean	181.8 c	190.7 b	195.8 a	

Note: Values have the same letter in the same column are not significantly different at LSD≤0.05 level according to Duncan's Multiple Range Test (DMRT).

Analysis of variance (ANOVA) of data presented in Table 13 shows that salt stress significantly increases the ascorbate peroxidase activity compared with untreated plants, the increment was found to be increased with increasing salt concentrations, particularly at 6000 mg/l, 8000 mg/l and 10000 mg/l respectively. However the effect caused by different treatments on ascorbate peroxidase activity was observed by comparing the different treatments with the control as shown in Table 16. The increase over control was 40.3, 3.8, 11.3, 62.6 and 55.2% when treated with proline, ascorbic acid, arginine, glutathione and glutamine respectively at 6000 mg/l, while the increase over control was 32.1, 1.2, 1.1, 63.1 and 47.3 % respectively at 8000 mg/l. Whereas the increase over control was 25.3, 5.2, 75.8 and 44.6% when treated with proline, arginine, glutathione and glutamine respectively at 10000 mg/l while there was a decrease in ascorbate peroxidase activity for

treatment with ascorbic acid comparing with control plants. Moreover the highest value of ascorbate peroxidase activity was recorded when treated with glutathione compared with control plants followed by glutamine, proline, arginine and ascorbic acid respectively when using high salinity level (10000 mg/l).

Table 16. Effect of proline, ascorbic acid, arginine, glutathione and glutamine on Ascorbate Peroxidase activity of wheat plants under different salinity levels.

Ascorbate peroxidase activity (unit $min^{-1} \cdot g^{-1}$ FW)				
Treatments	6000 mg/l	**8000 mg/l**	**10000 mg/l**	**Mean**
Control*	620 h	675 h	735 fgh	676.7 d
Proline	870 defg	892 cdef	921 bcde	894.3 bc
Ascorbic acid	650 h	683 efgh	720 fgh	706.6 d
Arginine	690 gh	747 efgh	773 efgh	736.7 cd
Glutathione	1008 bcd	1101 b	1292 a	1211 a
Glutamine	962 bcd	994 bcd	1063 bc	1006 b
Mean	800 a	859.2 a	956.2 a	

Note: Values have the same letter in the same column are not significantly different at LSD≤0.05 level according to Duncan's Multiple Range Test (DMRT). *Wheat plants treated with sea salt only

The higher activities of ascorbate peroxidase of wheat plants were in agreement with those reported by Mandhania *et al*., (2006), who concluded that under salinity stress, the stimulation of ascorbate peroxidase activity was much higher in salt-tolerant than those of salt-sensitive cultivar. These results could interpret that salt tolerance of cultivar sakha 93 seems to be linked with increase in the activity of antioxidant enzymes. The salt-induced enhancement of catalase activity in wheat plants indicated that it had a higher capacity for the decomposition of H_2O_2 generated by SOD. Thus, catalase activity coordinated with SOD activity can represent a central protective role

in the O_2^- and H_2O_2 scavenging process, also catalase is the main scavenger of strong oxidant H_2O_2 in peroxisomes and it converts H_2O_2 to water and molecular oxygen (Bahari *et al*., 2013). Plants have several antioxidant strategies like ROS-scavenging enzymes such as peroxidases and catalases response to salt stress (Rea *et al.,* 2004; Aronova *et al.,* 2005). Generally, the increases of catalase activity are a strategy for improving salt tolerance (Vaidyanathan *et al*., 2003). The active involvement of these enzymes is related, at least in part, to salt-induced oxidative stress tolerance in wheat plant. Also like catalase the peroxidase plays a vital role in plant defense against oxidative stress by scavenging H_2O_2 in chloroplast, cytosol, mitochondria and peroxisome of plant cells (Asada, 2006). Similar finding were reported by Bartels and Hussain, (2008) who found that peroxidase and catalase activity were increased in response to salinity. Moreover, Treatment application of ascorbic acid had a stimulating effect on some oxidative enzymes of wheat plants. These increases were concurrently with increasing protein levels indicating that vitamins could alleviate the inhibitory effects of salt stress by enhancing protein synthesis, as vitamins might act as activators for protein synthesis. Also, Padh, (1990) reported that ascorbic acid plays an important role in preserving the activities of enzymes that contain prosthetic transition metal ions. Ascorbic acid acts as a primary substrate in cyclic pathway for enzyme detoxification of hydrogen peroxide (Shalata and Neumann 2001). In conclusion, it can be concluded that treatment application of ascorbic acid can reduce the harmful effects of ROS and improves plant resistant under salt stress conditions. However, In our study, application of

ascorbic acid significantly increase activity of antioxidant enzymes such as catalase, peroxidase and ascorbate peroxidase of wheat plant compared with control under different salinity levels. These results are in good agreement with those of Shalata and Neumann (2001) who found that catalase and peroxidase activities increased in tomato plants treated with ascorbic acid under salt stress. Similar responses were reported by Yan *et al.,* (2000) and Hua and Guo, (2002), they observed that Proline application lead to the increase production of ascorbate peroxidase and peroxidase in salt stressed *Glycine max* plants. Also Seki *et al.,* (2007), Sharma and Dietz, (2006) and Simon-Sarkadi *et al.,* (2005, 2006) stated that exogenous polyamine increases activity of peroxidase and catalase, along with proline production. In conclusion, several studies have shown that polyamine accumulation occurs under salinity stress (Groppa and Benavides, 2008; Pang *et al.,* 2007). Hence, high cellular levels of polyamines (PAs) correlate with plant tolerance in a wide array of environmental stresses. However, the physiological significance of polyamine (PA) accumulation remains elusive and must be revealed whether these responses are due to stress-induced injury or a protective response to abiotic stress. Alternative approaches also include the use of PA as an external application which can also be administered for increasing tolerance to various stresses. In the long run, PA can be exploited in the same way as farm chemicals to mitigate stress-induced injury for crop protection. However, evidence from field level studies is still lacking. Some striking evidences of exogenous application of PA to counteract environment stresses are expected to promote its extended application to other crops (Hussein *et al.,* 2011).

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رفع كفاءة نبات القمح لتحمل الإجهاد الملحي عن طريق إضافة البرولين، حمض الأسكوربيك، الأرجنين، الجلوتامين، الجلوتاثيون.

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المستخلص

اشتملت هذه الدراسه على بعض المركبات التي لها دور في تحسين تحمل النبات للاجهاد الملحى.

وقد استهدفت الدراسة تطبيق استخدام المركبات تحت الدراسة لمعرفة تأثيرها في تخفيف التأثير السلبى للملوحة على نباتات القمح.

حيث تم تصميم تجربة لمعرفة تأثير بعض المركبات متمثلة فى البرولين، الأرجنين، الجلوتاثيون، الجلوتامين، حمض الأسكوربيك على رفع كفاءة تحمل نبات القمح صنف سخا ٩٣ للملوحة.

حيث تم معاملة نباتات القمح بثلاث مستويات مختلفة من الملح (٦٠٠٠، ٨٠٠٠،١٠٠٠٠ مجم/ لتر) وفى كل مستوى تم إضافة المركبات تحت الدراسة بتركيزات كالتالى: البرولين ١٠٠ مجم/لتر، الأرجنين١٠٠ مجم/لتر، الجلوتاثيون٥٠ مجم/لتر، الجلوتامين١٠ مجم/لتر، حمض الأسكوربيك ٨٨ مجم/لتر.

وكانت أهم النتائج المتحصل عليها كالتالى:

- تم إثبات التأثيرات السلبية للتركيزات المرتفعة من ملح البحر على نباتات القمح حيث أدت إلى تدهور عوامل إنتاجية النباتات.
- تم إثبات التأثيرات الإيجابية للمركبات تحت الدراسة حيث أدت إلى تحسين عوامل إنتاجية النباتات تحت الظروف الملحية وبالتالى تعمل على تقليل التأثيرات السلبية للتركيزات المرتفعة من ملح البحر.