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Role of Plant Antioxidants (Ascorbic Acid, Glutathione, α –Tocopherol, Spermine) in Alleviating Salinity Stress on Growth and Yield of Wheat Plants

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A POT experiment was carried out to investigate the role of some plant antioxidants such as ascorbic acid, glutathione, α-tocopherol, spermine in alleviating the harmful effects caused by high salinity level (6g/l) in wheat plants. The seeds were pre-soaked and the plants sprayed with anyone of the antioxidants used. It was concluded that salinity stress depressed all growth parameters and yield components. The data also showed that the different antioxidants could partially alleviate the harmful effect of salinity stress on growth and yield of wheat plants.

The data showed that 6000 mg/l salinity level alone or in combination with any of antioxidants used increased the activity of total peroxidase, ascorbic peroxidase, superoxide dismutase and catalase in wheat leaves. In addition, salinity (6000 mg/l) alone or in combination with any of applied antioxidants increased the endogenous contents of ascorbic and glutathione and total phenols but decreased carotenoids.

Keywords: Wheat, Plant antioxidant, Salinity stress, Growth and yield.

Soil salinity is one of the major abiotic stresses affecting crop growth and productivity. Salt stress causes inhibition of growth and development, reduction in photosynthesis, respiration, and protein synthesis and disturbs nucleic acid metabolism. Decrease in uptake of K^+, Mg^{2+}, Ca^{2+}, and thereby reduced growth at higher sodium concentration have also been reported (Sairam & Srivastava, 2002). Yang *et. al.* (1990) reported that there are two ways that salinity could retard growth: (a) By damaging the growth of cells so that they can not perform their functions or (b) By limiting their supply of essential metabolites. Sakr (1996) indicated that salinity suppressed both cell division and cell enlargement proportionally in wheat plants. Regarding the effect of antioxidant on wheat under salinity stress, Shalata & Neumann (2001) found that ascorbic acid acts directly to neutralize superoxide radicals, single oxygen or superoxide and as a

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secondary antioxidant during reductive recycling of the oxidized form of α-tocopherole.

It is now widely accepted that reactive oxygen species (ROS) are responsible for various stress-induced damage to macromolecules and ultimately to cellular structure. Consequently the role of antioxidant enzymes viz. superoxide dismutase (SOD), ascorbate peroxidase (APX), glutathione reductase (GR) and catalase (CAT), as well as metabolites like ascorbic acid, glutalthione, α-tocopherol, flavonoids, carotenoids (CAR) are responsible for the quenching of ROS becomes very important (Sairam & Srivastava, 2002).

Ascorbic acid (AA) is an important antioxidant, which reacts not only with H_2O_2 but also with O_2, OH and lipid hydroperoxidases. On the other hand, AA has been associated with several types of biological activities in plants: (1) As an enzyme co-factor, (2) As an antioxidant and (3) As a donor/ acceptor in electron transport at the plasma membrane or in the chloroplasts, all of which are related to oxidative stress resistance (Conklin, 2001).

Regarding enzymatic antioxidants activity caused by salinity stress, Vaidyanathan *et. al.* (2003) reported that the non-enzymatic antioxidants (glutathione and ascorbate) showed an accumulation in root tissues in plants subjected to salt stress. Plants must possess efficient antioxidant system such as SOD, APX, GR, DIIAR, CAT and metabolites viz., ascorbic acid, glutathione, α-tocopherol, carotenoid, flavanoids etc. (Smirnoff, 1995).

Regarding the effect of polyamines, Smith (1975) established a specific role of polyamines in maintaining a cation-anion balance in plant tissues and in stabilizing membranes at high external salinity. Polyamines had an ameliorating effect on all morphological and physiological characters and prevented degradation of chlorophyll, however, polyamines enhanced accumulation of all organic compounds under salinity stress study, except phenols (Zhao & Qin, 2004).

The objective of this study was to investigate the role of selected antioxidants on mitigation or alleviation the harmful effect of salinity stress on wheat plant.

Materials and Methods

A pot experiment was carried out at the seed technology unit of Tag El-Ezz Research Station in Dakahlia Governorate, Agricultural Research Centre, Ministry of Agriculture, Egypt, during the 2006/2007 season. Wheat grains (var. Gemeza 9) were kindly supplied by the Plant Breeding Section of the Field Agricultural Research Centre, Ministry of Agriculture, Giza, Egypt. Uniform grains were soaked in antioxidants *i.e.* ascorbic acid (100 mg/l), reduced glutathione (100 mg/l), α –tocopherol (50 mg/l), spermine (10 mg/l) as well as tap water for 6 hr. Eight uniform grains were sown on November 15th, 2006, in plastic pots (30 cm diameter) under high salinity level (6000 mg/l NaCl). The plants were sprayed with the same antioxidant three times (30, 60 and 90 days from sowing).

Growth parameters were measured in the vegetative phase (45 days after sowing). At physiological maturity yield and its components were recorded.

All the normal culture practices of growing wheat plants were applied as usual manner followed by the farmer in the district.

Leaves were collected after 75 days from sowing then frozen and stored till analysis. Each treatment was replicated 3 times and arranged in a complete randomized block design (Araus *et al.*, 2008).

Biochemical constituents

Non-enzymatic antioxidant contents

Ascorbic acid determination: 0.5 g of fresh leaves was ground in 50 ml of 2% (w/v) metaphosphoric acid using mortar and pastel and centrifuged for 30 min at 13000 rpm at 4°C. The ascorbate content (µ.mol / g FW) was measured in the supernatant at 25°C. The absorbance of red colour was measured at 520 nm according to Omaye *et al.* (1979) and Athar *et al.* (2008).

Total glutathione determination: The level of total glutathione (GSH) was determined in the fresh leaves with Ellman's reagent according to De Vos *et al.* (1992). 300 ul of sample buffer were mixed with 630 ul of 0.5 M K_2HPO_4 and 25 ul of mM 5, 5 -dithiobis (2-nitrobenzoic acid) (final pH 7). The absorbance at 412 nm was read after 2 min. GSH was used as a standard.

Total phenols determination: 1g of the fresh leaves were macerated in 5- 10 ml 80% ethanol for at least 24 hr at 0°C, the alcohol was clarified, the remained residue was re-extracted with 5-10 ml 80% ethanol 3 times. At the end, the clarified extract was completed to 50 ml using 80% ethanol. The colorimetric method of Folin-Denis as described by Daniel & George (1972) was employed for the chemical determination of phenolic compounds. Quantities were determined by reading the developed blue colour at 725 nm using 0.5 ml 80% ethanol and reagents only as a blank.

Carotenoids determination: Leaf samples (0.5gm from the third upper foliage leaf) were subjected to extraction by methanol for 24 hr at lab. temp. after adding a trace from sodium carbonate (Robinson *et al.*, 1983); then, carotenoids were determined spectrophotometrically (Spekol11) at wave-length 452 and calculated by the equation introduced by Mackinny (1941).

Enzymatic antioxidant activity determination

Ascorbate peroxidase activity determination: Ascorbate peroxidase (As-POD) was assayed spectrophotochemically according to Fielding (1978). The assay was carried out at 25°C in 1.0 cm light path cuvette and the reaction mixture consisted of 1500 ul phosphate buffer, 20 µl EDTA, 1000 µl sodium ascorbate and enzyme extract (20 µl). After mixing, the reaction was initiated by adding the 480 µl H_2O_2 and decreasing in optical density at 290 nm against blank (without extract) was continuously recorded every minute (for two minutes).

Total peroxidases activity determination: Peroxidase was assayed spectrophotochemically according to Amako *et al.* (1994).The assay was carried out at 25°C in 1.0 cm light path cuvette and the reaction mixture consisted of 1500 ul phosphate buffer, 1000 ul Pyrogallol and 480 ul H_2O_2 solution. After mixing the reaction was initiated by adding the enzyme extract (20 ul) and the increase in optical density at 430 nm against blank (without extract) was continuously recorded every minute (for three minutes).

Super oxide dismutase (SOD) activity determination: Leaf samples were collected in an ice bucket and brought to the laboratory. Leaves were then washed with distilled water and surface moisture was wiped out. Leaf samples (0.5g) were homogenized in ice cold 0.1M phosphate buffer (pH 7.5) containing 0.5Mm EDTA with pre-chilled pestle and mortar. The homogenate was transferred to centrifuge tubes and was centrifuged at 4°C in Beckman refrigerated centrifuge for 15 min at 15000 X g. The supernatant was transferred to 30 ml tubes and referred to enzyme extract.

SOD activity was estimated by recording the decrease in absorbance of superoxide-nitro blue tetrazolium complex by the enzyme. About 3ml of reaction mixture containing 0.1ml of 1.5M sodium carbonate, 0.2ml of 200mM methionine, 0.1ml of 2.25Mm Nitro-blue tetrazolium, 0.1ml of 3mM EDTA, 1.5ml of 100mM potassium phosphate buffer, 1ml distilled water and 0.05 ml of enzyme were taken in test tubes in duplicate from each enzyme sample. Two tubes without enzyme extract were taken as control. The reaction was started by adding 0.1ml riboflavin (60mM) and placing the tubes below a light source of two 15 w florescent lamps for 15 min. Reaction was stopped by switching off the light and covering the tube with black cloth. Tubes without enzyme developed maximal colour. A non- irradiated complete reaction mixture which did not develop colour served as blank. Absorbance was recorded at 560nm and one unit of enzyme activity was taken as the quantity of enzyme which reduced the absorbance reading of samples 50% when comparison with types lacking enzymes (Dhindsa *et al.*, 1981).

Catalase activity was determined by measuring the rate of H_2O_2 conversion to O_2 at room temperature using a lipid-phase O_2 electrode (Hansatech, Norfolk, UK). Approximately 0.5g of plant tissue, consisting of the apical region of the shoot including the cotyledons, was extracted in 1.5 ml of 0.1mM Hepes/KOH - buffer (pH 7.4) and then centrifuged at 10.000g for 5 min. The rate of O_2 production was measured by adding 50 µl of the supernatant to 0.1M Hepes (pH 7.4) containing 530mM H_2O_2. Catalase activity was calculated on a fresh weight basis to keep the data uniform with the H_2O_2 measurements, and to reduce the chances of distribution as a result of protein synthesis alteration due to a heat-shock (Vierling, 1991 and Bettany, 1995).

The data of experiments were statistically analyzed as technique of the analysis of variance (ANOVA) according to Gomez & Gomez (1984). The treatment means were compared using the least significant differences (LSD).

Results

Plant growth

The data presented in Table 1 show that a high salinity level (6000 mg/l) alone or in combination with any of applied antioxidants (ascorbic acid, glutathione, α –tocopherol, spermine) decreased stem and leaf dry weights. Different applied antioxidant materials could partially counteract the harmful effect of high salinity stress level on growth of wheat plant.

TABLE 1. Effect of antioxidants on growth, yield, endogenous enzymatic and non-enzymatic antioxidants in wheat plants grown under a high salinity level (6 g/l). Combined analysis of the two growing seasons.

Parameters / Treatments	Stem DW (g)	Leaf DW (g)	Spike length (cm)	No. spikes/ plant	Spike weight/ plant (g)	No. grains/ spike	Grains weight/ spike (g)	Grains yield/ plant (g)
Tap water (control)	4.1	4.5	10.0	40	2.5	36	2.5	14.7
Salinity 6 g/l	2.4	3.1	7.3	23	1.6	28	1.6	10.2
S + ASA. 100mg/l	3.3	4.3	9.6	38	2.0	37	2.3	13.6
S + Glut. 100mg/l	3.1	4.3	8.7	35	1.9	35	2.2	13.6
S +Tocoph. 50mg/l	4.0	4.2	8.6	32	1.7	36	2.0	13.0
S + Sper. 10mg/l	3.2	4.0	8.3	33	1.6	35	2.2	13.0
LSD at 5%	1.9	0.43	0.31	0.99	0.16	2.6	0.16	0.13

	TPX activity	APX activity	Cat activity	SOD activity	Carot. content	ASA content	GSH content	Total phenols
Tap water (control)	152	125	53	307	0.48	7.9	220	129
Salinity 6 g/l	193	141	63	382	0.15	8.9	246	236
S + ASA 100 mg/l	386	218	163	380	0.28	10.2	291	286
S + Glut. 100 mg/l	341	154	165	396	0.21	10.1	206	274
S +Tocoph. 50 mg/l	223	157	106	366	0.17	9.6	278	233
S + Sper. 10 mg/l	276	218	102	360	0.32	9.1	235	287
LSD at 5%	1.78	2.67	1.52	1.62	0.076	0.18	3.04	1.36

TPX: total peroxidase activity (units /g fresh weight). APX: ascorbic peroxidase activity (units /g fresh weight). SOD: superoxide dismutase activity (units /mg protein/min). Cat: catalase activity umol H_2O_2 red/mg protein/min). Carot.: carotenoids content (mg/g fresh weight). ASA: ascorbic acid content (mg/g fresh weight). GSH: reduced glutathione (umol /g fresh weight). T. phenols: total phenols content (mg/g fresh weight). S: salinity stress at 6000mg/l. ASA: ascorbic acid. Glut.: glutathione. Tocoph: tocopherol. Sper.: spermine.

Yield and its components

A high salinity level (6000 mg/l) significantly decreased yield and its components as spike length, number of spikes/plant, spike weight, number of grain/spike, grain weight/spike and grains yield/plant. A high salinity level combined with any of applied antioxidants also decreased wheat yield and its components. The applied antioxidants mitigated the effects of salinity. It was noticed that the antioxidants can partially alleviate the harmful effects of salinity

stress on yield and its components. Glutathione and spermine showed the most beneficial effect in this respect.

Enzymatic antioxidants activity

The data show that 6000 mg/l salinity level alone or in combination with any of antioxidants used increased enzymatic activity of total peroxidase (TPX), ascorbic peroxidase (APX), super oxide dismutase (SOD) and catalase. High salinity level combined with ascorbate proved to be more effective in increasing all enzymatic antioxidants activity. Moreover the combination of glutathion is more effective in increasing catalase and superoxide dismutase activity, while spermine was more effective in increasing total peroxidase and ascorbic peroxidase activity.

Non enzymatic antioxidant contents

The data show that high salinity (6000mg/l) alone or combined with any of applied antioxidants decreased carotenoids content, while increasing each of the endogenous ascorbic, glutathione or total phenol contents. Ascorbic treatment was the most effective in improving endogenous non-enzymatic antioxidants.

It could be noticed that the combination between high salinity level and any of exogenous applied antioxidants caused synergistic effect on increasing endogenous non-enzymatic antioxidants such as ascorbic, glutathion and total phenols.

Discussion

Growth and yield

The inhibitory effect of salinity on wheat growth in the present investigation may be due to a decrease in water absorption, metabolic processes, meristematic activity and/or cell enlargement (Khadr *et al.*,1994 and Sakr, 1996). Yang *et. al.*, (1990) reported that there are two ways that salinity could retard growth: (a) By damaging growth cells so that they can not perform their functions, (b) By limiting their supply of essential metabolites. Perturbing the functioning of vital components of photosynthesis like PSI, PSII and Rubisco, (Chen & Murata, 2002 and Stepien & Klobus, 2005).

Salinity affected all stages of wheat growth and development, as well as yield of plants. The yield was much more depressed by salt than the vegetative growth which may be attributed to a decrease in the viability of pollen grain or in the receptivity of the stigmatic surface or both (Sakr *et al.*, 2004).

The reduction in seed yield is largely due to : (1) Reduction in pollen viability which has been related to decreased calcium mobilization from plant leaves treated with sodium chloride, which is important in pollen germination and pollen tube growth (Grattan *et al.*, 2002). (2) Abscission of flowers or young fruit due to ethylene induction by salinity (Roy *et al.*, 1995). (3) Decreasing production pollen grain, mean number of perfect flowers, and fruit set. (4) Decreasing the leaf area and number per plant, resulting reduction in the supply of carbon assimilate due to decreasing the net photosynthetic rate and biomass accumulation (Sakr *et al.*,2004).

As for, α-tocopherol, ascorbic acid and glutathione, it could be concluded that, these plant antioxidants can alleviate the harmful effect of ROS caused by salinity stress through several ways such as: (1) Inhibiting the lipid photoperoxidation (Michalski & Kaniuga, 1981). (2) Involving in both electron transport of PS II and antioxidizing system of chloroplasts (McKersie & Leshem, 1999). (3) Membrane stabilizers and multifaceted antioxidants, that scavenge oxygen free radicals, lipid peroxy radicals and singlet oxygen (Diplock *et al.*, 1989). (4) Reacting with peroxyl radicals formed in the bilayer as they diffuse to the aqueous phase (Hess, 1993). (5) Scavenging cytotoxic H_2O_2, and reacts non-enzymatically with other ROS: singlet oxygen, superoxide radical and hydroxyl radical (Larson, 1988). (6) Regenerating another powerful water-soluble antioxidant, ascorbic acid, via the ascorbate–glutathione cycle (Blokhina *et al.*, 2002). (7) Stabilize membrane structures (Blokhina, 2002). (8) Modulating membrane fluidity in a similar manner to cholesterol, and also membrane permeability to small ions and molecules (Foyer, 1992). (9) Decreasing the permeability of digalactosyl-diacyl-glycerol vesicles for glucose and protons (Berglund *et al.*, 1999).

Regarding polyamines (PAs), it has been suggested that PAs may play role in anti-oxidative system and protect membrane from peroxidation. The alleviating effect of polyamines on plants grown under salinity stress may be due to one or more of the following factors: (1) Through activating anti-oxidative defense system (Chattopadhyay *et al.*, 2002). (2) Suppressed the level of superoxide and H_2O_2 in leaf stressed plants (Hernandes *et al.*, 1995). (3) Suppress H_2O_2 level and thereby membrane damage is being evaluated in terms of anti-oxidative system (Dionisio–Sese & TOBITA, 1998). (4) Caused reduction in ROS through quenching of singlet oxygen and excited chlorophyll by elevating level of CAR thereby maintained chloroplastic membrane (Velikova *et al.*, 2000). (5) reduce membrane leakage and lipid peroxidation and decreased MDA contents in sugarcane leaves (Zhang & Kirkham, 1996). (6) Stabilization of membrane damage may be due to its polycationic nature (Tiburcio *et al.*, 1994). (7) Increasing AXP and GR activity as well as CAR and GSH at all salinity levels (Tiburcio *et al.*, 1994). (8) Stimulation of chlorophylls synthesis and prevent chlorophyll degradation (Krishnamurthy, 1991). (9) Increasing all organic concentrations, that may be attributed to that polyamines that are involved in important biological processes, *e. g.* ionic balance and DNA, RNA and protein synthesis .

Generally speaking, the application of antioxidants proved to be more effective in alleviating the harmful effect of salinity on wheat plant. However, ascorbic and spermine showed the most beneficial effect in this respect (Sakr *et al.*, 2004).

Regarding enzymatic antioxidant activity, Dash & Panda (2001) reported that higher activity of antioxidant enzymes viz., SOD, GR and CAT caused lower H_2O_2 production, lipid peroxidation and higher membrane stability. Beneficial effect of higher osmolyte concentration (soluble sugars, glycine-betaine, proline and potassium) is reflected in stabilization of essential enzyme proteins such as SOD, GR and CAT resulting in higher activity under salinity stress.

Plants posses antioxidant systems in the form of enzymes such as SOD, APX, GR, DHAR, CAT and metabolites viz., ascorbic acid, glutathione, α-tocopherol, carotenoid, flavanoids, etc. These antioxidant enzymes and metabolites are reported to increase under various environmental stresses as well as comparatively higher activity has been reported in tolerant cultivars than the susceptible ones (Sairam & Srivastava, 2002).

As for non enzymatic antioxidants, ascorbic acid has been associated with several types of biological activities in plants: (1) As an enzyme co-factor, (2) As an antioxidant, and (3) As a donor/ acceptor in electron transport at the plasma membrane or in the chloroplasts, all of which are related to oxidative stress resistance (Conklin, 2001).

Total phenols in the wheat leaves tended to increase gradually with increasing salinity levels in soil as shown in Table 1. This increase showed some tendency of wheat to adjust osmotically against salt stress. Moreover, stress condition leads to an increase in phenolic compounds (Namiki, 1990). These phenolic compounds could be a cellular adaptive mechanism for scavenging oxygen free radicals during stress and this free radical scavenger and others such as ascorbate could be readily oxidized in the system of tissue representing subcellular damages.

Reducing glutathione (GSH) can function as an antioxidant in many ways. (1) It can react chemically with singlet oxygen, superoxide and hydroxyl radicals and therefore function directly as a free radical scavenger (Noctor & Foyer, 1998). (2) GSH may stabilise membrane structure by removing acyl-peroxides formed by lipid peroxidation reactions. (3) GSH is the reducing agent that recycles ascorbic acid from its oxidised to its reduced form by the enzyme dehydroascorbate reductase (Loewus, 1988).

The increase in GSH contents as reported in Table 1 may be due to the role of the enzymes ascorbate peroxidase, glutathione reductase, superoxide dismutase which involved in the regeneration of glutathione and ascorbate that are important in detoxification of ROS (Foyer *et al.*, 1994).

Generally, it was concluded that tocopherol, ascorbic and glutathione can help to alleviate the harmful effect of ROS may be through several ways such as: (1) Inhibiting the lipid photoperoxidation (Michalski & Kaniuga, 1981). (2) Affecting, both electron transport of PS II and the antioxidizing system of chloroplasts (McKersie & Leshman, 1999). (3) Stabilizing membranes (Thomas *et al.*, 1992). (4) Reacting with peroxyl radicals (Sairam & Srivastava, 2002). (5) Scavenging cytotoxic H_2O_2 and reacting non-enzymatically with other ROS (Larson, 1988). (6) Regenerating another powerful water-soluble antioxidant, ascorbic acid (Blokhina *et al.*, 2002). (7) Stabilizing membrane structures (Blokhina *et al.* 2002). (8) Modulating membrane fluidity in a similar manner to cholesterol and also membrane permeability to small ions and molecules (Foyer, 1992) and (9) Decreasing the permeability of digalactosyldiacylglycerol vesicles for glucose and protons (Berglund *et al.*, 1999).

As for the role of exogenous antioxidant polyamine on alleviating salinity stress in plants, the polyamines (PAs) including spermine (SPM, a tetramine), spermidine (SPD, a triamine) and their obligate precursor putrescine (a diamine) are implicated in induction of plant adaptation to stresses (Mishra *et al.*, 2003). It was suggested that PAs may play role in antioxidative system and protect membrane from peroxidation. The alleviating effect of polyamines on plants grown under salinity stress was previously discussed.

From the above-mentioned reported results: it could be noticed that the applied antioxidants could alleviate or minimize the harmful effect of NaCl salinity on wheat plant and ascorbic and spermine proved to be more effective in this respect.

References

Amako, K., Chen, G.X. and Asada, K. (1994) Separate assays specific for ascorbate peroxidase and guaiacol peroxidase and for the chloroplastic and cytosolic isozymes of ascorbate peroxidase in plants. *Plant and Cell Physiology*, **35**, 497-504.

Araus, J.L., Gustavo, R. and Dolores, M.S. (2008) Breeding for yield potential and stress adaptation in cereals. *Critical Reviews in Plant Science*, **27**, 377-412.

Athar, H.R., Khan, A. and Ashraf, M. (2008) Exogenous applied ascorbic acid alleviates salt-induces oxidative stress in wheat. *Environ. & Exp. Botany*, **63**, 224-231.

Berglund, A.H., Nilsson, R. and Liljenberg, C. (1999) Permeability of large unilamellar digalactosyldiacylglycerol vesicles for protons and glucose—influence of a-tocopherol, ß-carotene, zeaxanthin and cholesterol. *Plant Physiology and Biochemistry*, **37**, 179–186.

Bettany, A.J.E. (1995) Stress responses in cell cultures of *Lolium temulentum*. I. Transcriptional and post-transcriptional changes in gene expression during heat shock and recovery. *Journal of Plant Physiology*, **146**, 162-168.

Blokhina,O., Virolainen, E. and Fagersted, V. (2002) Antioxidants, oxidative damage and oxygen deprivation stress: A review. *Annals of Botany*, **91**, 179-194.

Chattopadhyay, M., Chattopadhyay, K., Tiwari, B.S., Chattopadhyay, G., Bose, A., Sengupta, D.N. and Ghosh, B. (2002) Protective role of exogenous polyamines on salinity-stressed rice (*Oryza sativa*) plants, *Physiologia Plantarum*, **116**, 192–199.

Chen, T.H. and Murata, N. (2002) Enhancement of tolerance of abiotic stress by metabolic engineering of betaines and other compatible solutes. *Curr. Opin. Plant Biol.* **5**, 250-257.

Conklin, P. (2001) Recent advances in the role and biosynthesis of ascorbic acid in plants. *Plant Cell Environment*, **24**, 383-94.

Daniel, H.D. and George, C.M. (1972) Peach seed dormancy in relation to indogenous inhibitors and applied growth substances. *Journal of American Society of Horticulture Science.*, **97**, 651-654.

Dash, M. and Panda, S.K. (2001) Salt stress induced changes in growth and enzyme activities in germinating *Phaseolus mungo* seeds. *Biologia Plantarum* **44**, 587-587.

De Vos, C.H., Vonk, M.J., Vooijs, R. and Hek, S. (1992) Glutathione depletion due to copper-induced phytochelation synthesis causes oxidative stress in *Silene cuchlus*. *Plant Physiology*, **98**, 859-858.

Dhindsa, R.S, Plumb-Dhindsa, P. and Thorn, T.A. (1981) Leaf senescence: correlation with increased levels of membrane permeability and lipid peroxidation and decreased superoxid dismutase and catalase. *Journal of Experimental Botany*, **32**, 93-101.

Dionisio-Sese, M.L. and Tobita, S. (1998) Antioxidant responses of rice seedlings to salinity stress. *Plant Science*, **135**, 1–9.

Diplock, A.T., Machlin, L.J., Packer, L. and Pryor, W.A. (1989) Ed., "*Vitamin E: Biochemistry and Health Implications*". Ann. N.Y. Acad. Sci., **570**, 555-559.

Fielding, J. (1978) A biochemical and cytochemical study of peroxidase activity in root of *Pisum sativum*. *Journal of Experimental Botany*, **29**, 969-981

Foyer, M.J. (1992) The antioxidant effects of thylakoid vitamin E (α-tocopherol). *Plant Cell and Environment*, **15**, 381–392.

Foyer, C.H., Lelandais, M. and Kunert, K.J. (1994) Photooxidative stress in plants. *Physiologia Plantarum*, **92**, 696-717.

Gomez, K.A. and Gomez, A.A. (1984) "*Statistical Procedures for Agric. Research*". Jon Wiley and Sons, Inc., New York.

Grattan, S.R., Zeng, L.H., Shannon, M.C. and Roberts, S.R. (2002) Rice is more sensitive to salinity than previously thought. *California-Agriculture*, **56** (6), 189-195.

Hernandez, J.A., Corpas, O.E, Sevila, F. and Gel, L.A., (1995) Salt-induced oxidative stress in chloroplast of pea plants. *Plant Science*, **105**, 151-167.

Hess, J.L. (1993) Vitamin E, α - tocopherol. In: "*Antioxidants in Higher Plants*" R.G.Alscher and J.L. Hess (Ed.). pp. 111-134. CRC Press, Inc. Boca Rarton, Florida.

Khadr, I.F., Nyireda, F., Nielsen, C. and Andria, R. (1994) Ethephon alters corn growth under drought stress. *Agronomy Journals*, **86**, 283-288.

Krishnamurthy, R. (1991) Amelioration of salinity effect in sail tolerant rice (*Oryza sativa* L.) by foliar application of putrescine. *Plant Cell Physiolgy*, **35** (5), 699-703.

Larson, R.A. (1988) The antioxidants of higher plants. *Phytochemistry*, **27**, 969–978.

Loewus, F.A. (1988) Ascorbic acid and its metabolic products. In: "*The Biochemistry of Plants*", Vol. 14. Preiss, J. (Ed) p. 85- 107. Academic Press, New York,

Mackinny, G. (1941) Absrption of light by chlorophyll solution. *Journal of Biological Chemistry*, **140**, 315-322.

McKersie, B.D. and Leshem, Y.Y. (1999) Salt stress In. "*Stress and Stress Coping in Cultivated Plants*", pp. 55-78. Kluwer Academic Pub. London.

Michalski, W.P. and Kaniuga, Z. (1981) Photosynthetic apparatus of chilling-sensitive plants. IX. The involvement of alpha-tocopherol in the electron transport chain and the anti-oxidizing system in chloroplasts of tomato leaves. *Biochimica et Biophysica Acta*, **12**, 25-37.

Mishra, N.M., Makkar, K. and Verma, S. (2003) Polyamines in plant growth and development. In: "*Advances in Plant Physiology*," A. Hemantranjan (Ed.) pp. 155–224, Scientific Publishers, India.

Namiki, M. (1990) Antioxidants/antimutagens in food. *CRC Critical Reviews in Food Science and Nutrition*, **29**, 273–300.

Noctor, G. and Foyer, C.H. (1998) Ascorbate and glutathione: keeping active oxygen under control. *Annual Review of Plant Physiology and Plant Molecular Biology*, **49**, 249–279.

Omaye, S.T., Turnbm, J.D. and Saubermch, H. E. (1979) Selected methods for the determination of ascorbic acid in animal cells. tissues and fluids. "*Methods in Enzymology*". 62. pp 1-8. Academic Press. New York.

Robinson, S.P., Dowenton, J.W., John, D. and Millhouse, J.A. (1983) Photosynthesis and ion contents of leaves and isolated chloroplasts of salt stressed spinach. *Plant Physiology*, **73**, 238-242.

Roy, D., Basu, N., Marik, R. and Banerjee, S.K. (1995) Changes in levels of endogenous gibberellins and Abscisic acid in salt-sensitive germinating rice by NaCl-Salinity. *Plant Physiology. & Biochemistry*, New Delhi, **22**(2), 200-202.

Sairam, R.K. and Srivastava, G.C. (2002) Changes in antioxidant activity in sub-cellular fractions of tolerant and susceptible wheat genotypes in response to long term salt stress. *Plant Science*, **162**, 897–904.

Saker, M.T., El-Hadidy, M., Abo El-Kheer, A.M. and Farouk, S. (2004) Physiological studies of some osmoregulator on canola. *International Conversation Microbiology and Biotechnology in Africa and Arab Reagan*, 27^{th} to 29^{th}. pp. 295- 321.

Sakr, M.T. (1996) Physiological studies on the role of GA_3. kinetin and Ethrel inducing salt tolerance of wheat seedlings. *Journal of Agricultural Science, Mansoura University*, **21**, 633-642.

Shalata, A. and Neumann, P.M. (2001) Exogenous ascorbic acid (vitamin c) increases resistance to salt reduces lipid peroxidation. *Journal of Experimental Botany*, **52** (364), 2207-2211.

Smirnoff, N. (1995) Antioxidant system and plant responses. In: "*Environment and Plant Metabolism: Flexibility and Acclimation*", N. Smirnoff, (Ed.) pp. 217–243, Bios Scientific Publishers, Oxford.

Smith, T.A. (1975) Recent advances in the biochemistry of plant amines. *Phytochemistry*, **14**, 865-890.

Stepien, P. and Klobus, G. (2005) Antioxydant defense in the leaves of C3 and C4 plants undersalinity stress. *Physiologia Plantarum*, **125**, 31-40

Thomas, C.E., McLean, L.R., Parker, R.A. and Ohlweiler, D.F. (1992) Ascorbate and phenolic antioxidant interactions in prevention of liposomal oxidation. *Lipids*, **27**, 543–550.

Tiburcio, A.F., Besford, T., Capell, A., Borrell, P.S, Testillano, S. and Risueño, S. (1994) Mechanisms of polyamine action during senescence responses induced by osmotic stress, *Journals of Experimental Botany*, **45**, 1789–1800.

Vaidyanathan, H., Sivakumar, P., Chakrabarty, R. and Thomas, G. (2003) Scavenging of reactive oxygen species in sodium chloride -stressed rice (*Oryza sativa* L.) differential response in salt-tolerant sensitive varieties. *Plant Science*, **165**, 1411–1418.

Velikova.,V., Velikova, I., Yordanov, M. and Edreva, E. (2000) Oxidative stress and some antioxidant systems in acid rain-treated bean plants protective roles of exogenous polyamines. *Plant Science*, **151**, 59–66.

Vierling, E. (1991) The roles of heat shock proteins in plants. *Annual Review of Plant Molecular Biology*, **42**, 579-620.

Yang, Y., Newton, R. and Miler, F. (1990) Salinity tolerance in sorghum. *Crop Science*, **30**, 781-85.

Zhang, J. X. and Kirkham, M. B. (1996) Lipid peroxidation in sorghum and sunflower seedlings as affected by ascorbic acid, benzoic acid and propyl gallate. *Journal of Plant Physiology*, **149**, 489–493.

Zhao, F.G. and Qin, P. (2004) Protective effect of exogenous polyamines on root tonoplast function against salt stress in barley seedlings. *Plant Growth Regulation*, **2**, 97–103.

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دور منظمات النمو (حمض الأسكوربيك والجلاتثيون والتوكوفيرول والإسبرمين) فى تخفيف الإجهاد الملحى على نمو ومحصول نبات القمح

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أجريت تجربة أصص لدراسة دور بعض مضادات الأكسدة مثل حمض الأسكوربيك والجلاتثيون والتوكوفيرول والإسبرمين لتخفيف الأثر الضار المتسبب عن مستوى الملوحة المرتفع (٦جم/لتر) لنباتات القمح. تم نقع البذور ورش النباتات بمضادات الأكسدة وتبين أن الملوحة أدت إلى تقليل القياسات الخضرية والمحصولية. وكذلك أوضحت النتائج دور مضادات الأكسدة فى تقليل الأثر الضار للملوحة فى كل من القياسات الخضرية والمحصولية لنبات القمح.

أوضحت البيانات أن تركيز الملوحة ٦٠٠٠ مليجرام/لتر منفردا أو مع أى من مضادات الأكسدة زاد من نشاط البيروكسيديز الكلى وأنزيم الأسكوربيك بيروكسيديز والسوبر أكسيديز ديسميتيز والكتاليز فى أوراق القمح. بالإضافة إلى زيادة المحتوى الداخلى لكل من الأسكوربيك والجلاتثيون والفينولات الكلية، لكن أنقصت الكاروتينيدات.