

Exogenous Application of Ascorbic Acid for Improve Germination, Growth, Water Relations, Organic and Inorganic Components in Tomato (*Lycopersicon esculentum* Mill.) Plant Under Salt-Stress

Hameda El Sayed Ahmed El Sayed

Department of Biology, Faculty of Applied Science, Umm Al Qura University, Makkah Al Mukaramah, K.S.A.
heelsayed@uqu.edu.sa & D.Hameda@hotmail.com

Abstract: A greenhouse experiment was carried out to study the response of presoaked tomato seeds (*Lycopersicon esculentum* Mill. var. Cerasiforme) in freshly prepared ascorbic acid (50 ppm ASC) or distilled water (control) for 12 h at natural environmental conditions, to reduced the effect of salinity stress. Generally, the tomato seeds germination occurred after 3 days, while, the germination rate (%) were more faster after soaking the seeds in ascorbic acid (+ASC) compared with control (soaked in distilled water). NaCl salt-stress treatments caused a reduction in all growth parameters (fresh and dry weights of plant, leaf area and number per plant) compared control, particularly at high NaCl level (8000 ppm) more reduced. In the mean time, ascorbic acid had reduced the effect NaCl salinity stress on all growth parameters. Photosynthetic pigments (chlorophyll a & b and carotenoids) and chloroplast efficiency were increasing with salinity stress, but the response was more pronounced at 8000 ppm NaCl whether alone or combined with ascorbic acid. Also, salinity stress treatments tended to increased all of the total available carbohydrates (Monosaccharide, Disaccharides & polysaccharides), nitrogenous components (protein, amino acids & proline), antioxidase, (catalase, peroxidase & superoxide dismutases) enzymes activities and inorganic mineral elements (Na⁺, K⁺, N⁺³, P⁺³, Ca⁺², Mg⁺² & Cl⁻) but after soaked the seeds in ascorbic acid (+ASC), these components tended to increased more. Application of NaCl salinity-stress on tomato plant induced the synthesis of nitrogenous components (protein, amino acids, proline), whereas, the tomato seeds soaked before planting in ascorbic acid (+ASC) which leads to remarkably increasing more for all nitrogenous components, antioxidase, carbohydrates and inorganic mineral elements content.

[Hameda El Sayed Ahmed El Sayed, **Exogenous Application of Ascorbic Acid for Improve Growth, Water Relations, Organic and Inorganic Components in Tomato (*Lycopersicon esculentum* Mill.) Plant Under Salt-Stress.** *N Y Sci J* 2013;6(10): 123-139]. (ISSN: 1554-0200). <http://www.sciencepub.net/newyork>. 20

Keywords: *Lycopersicon esculentum*; ascorbic acid; NaCl salinity; growth parameters; Pigments; Organic Components, antioxidase, minerals.

1. Introduction

Tomato plant one of the important and widespread crops in the world, is sensitive to moderate levels of salt in the soil. According to the FAO, tomato considered the second most cultivated vegetable in the world, after potato, the annual production of nearly 10⁸ ton of fresh tomato in 3.7×10⁶ ha worldwide, China, the USA and Turkey being the leading producers (FAO, 2004; Nuez et al., 2004; Matoo and Handa, 2008). Generally, salinity problems in the world are due to sodium salts, particularly sodium chloride. Unfortunately, water supplies are often of poor quality (drainage water) as a result of new irrigation projects consequently a high rate of evapotranspiration with low rainfall leads to increased the levels of soluble salts in the soil. The roles of potassium and sodium in plant nutrition have occupied numerous investigators, potassium is the only monovalent cation which is essential for all higher plants, although sodium can have beneficial effects on plant growth (Epstein, 1972). Much salinity resulted from NaCl cause osmotic pressure of external solution become more than osmotic pressure

of plant cells which is require to regulating osmotic pressure to preventing dehydration by plant cells. Uptake and transform of nutrition ions such as potassium and calcium, by excess sodium would make problems. High Na and Cl rates would cause to direct toxic effects on enzymatic and membranous systems (Nazarbeygi et al., 2011).

Ascorbic acids (ASC) is present in all living plant cells, the largest amounts being usually in the leaves and flowers, i.e., in actively growing parts (Smimoff et al., 2001; Ebrahim, 2005). The fact that it is very sensitive to reversible oxidation (ascorbic acid ↔ dehydroascorbic acid) suggests that it may be involved in cellular oxidation-reduction reactions, perhaps serving as a hydrogen-transport agent. Attempts have been made to employ active vitamins to overcome the drastic effects of salinity on seed germination and seedling growth as well as on some metabolic mechanisms (Khan and Zaidi, 1985; Ansari and Khan, 1986; Samiullah and Afridi, 1988). ASC is one of the most powerful antioxidants, the supply of ascorbic acid to tomato seedling might

decrease the synthesis of active oxygen species and thereby increase resistance to salt stress. ASC is an essential cofactor for α -ketoglutarate-dependent dioxygenases (e.g. prolyl hydroxylases) important for formation of covalent adducts with electrophilic secondary metabolites in plants (Lopez-Munguia et al. 2011; Traber and Stevens 2011). The exogenous application of ascorbic acid could mitigate reduce the harmful effects of salinity in different crops (McKersie, et al., 1999; Al-Hakimi and Hamada, 2001; Prochazkova et al., 2001).

Many studies have reported large variation among tomato genotypes in their response to salinity (Ben Ahamed et al., 2009). In addition to its economic importance, tomato consumption has recently been demonstrated to be beneficial to human health, because of its content of phytochemicals such as lycopene, β -carotene, flavonoids, vitamin C and many essential nutrients (Beutner et al., 2001; Palop et al., 2010). Many strategies used to combat salinity stress, exogenous application of plant growth regulators has received considerable attention (Afzal et al., 2005; Dolatabadian et al., 2008). Soaking seed before planting with growth regulators is beneficial in reducing negative effects of salinity on growth and physiological/biochemical responses of crops (Ashraf and Rauf, 2001; Afzal et al., 2005). Ascorbic acid also benefitted growth and may be due to the antioxidant activity of ascorbic acid protecting plants from damage due to abiotic stress (Beltagi, 2008). Priming typically affects germination time, leading to better growth and improved yield, especially in plants under stress (Halmer, 2004; Afzal et al., 2005; Piri et al., 2009).

The present work is an attempt to seeking means of improving the survival of horticulture tomato (*Lycopersicon esculentum* Mill. var. Cerasiforme) plant under experimental saline stress by soaking the seeds in Ascorbic acid (50 ppm ASC) for 12 h before. Applications of ASC for reduced the effects of NaCl salinity stress on germination, growth parameter, water relations, photosynthetic pigments and activity, antioxidant enzymes, organic and inorganic components.

1. Materials and Methods:

Plant material, germination and growth conditions:

A homogenous of clean-healthy tomato seeds (*Lycopersim esculentum*, Mill. var. Cerasiforme) were obtained from the Crop Institute, Agricultural Research Center, Giza, Egypt. The Seeds of tomato (*Lycopersim esculentum*, Mill. var. Cerasiforme) were surface sterilized with 2% sodium hypochlorite solution for 3minutes and then thoroughly rinsed with sterile deionized water. The

seeds were then soaked in freshly prepared ascorbic acid (50 ppm \approx 0.3 mM; El-Tayeb, 1995) or distilled water (control) for 12 h at natural environmental conditions. Batches of seeds were germinated in Petri dishes containing ash less filter paper moistened with a solution identical to that they had originally been soaked (distilled water and 50 ppm Ascorbic acid). The percent of germination was recorded after 3 days. Seedlings were selected on homogeneity of height and developmental stage. Transfer the seedling tomato plants to plastic pots (5 seedlings/pot) containing clay-sand soil ($\frac{1}{2} \approx$ v/v) was used, the soil was mixed thoroughly to assure complete and uniform distribution (40 cm diameter, 60 cm depth, 7.5Kg soil pot⁻¹) at 10°C/17°C, with supplementary lighting (red electric bulb, 20 w at night), at relative humidity of 75-80%. Two pots group experiments were conducted in the screen greenhouse condition during winter season (2011/2012) treated tomato plant seedling with NaCl concentrations (2000, 4000, 6000, 8000 ppm) and distilled water. The sand culture technique and nutrient solution were similar to those adopted by Hewitt (1952). One group from the pots were irrigated twice a week interval with normal tap water needed with full strength Hoagland nutrient solution (Hoagland and Arnon, 1950), while the other pots were irrigated with different concentrations from NaCl with full strength of Hoagland nutrient solution, and allowed to grow for about 70 days (flowering stage) post sowing. Plant have been harvested after 75 days for determined the all growth parameters, five replicates for each treatment, were used to calculate the mean of each growth parameters. The plants of each treatments were washed with distilled water, the length plants were measured (cm/plant), Number of leaves were recorded, Leaf area (cm²/leaf) assessed using the leaf No. 4 from the lower, by a Portable Area Meter Leaf Area Meter (*Laser Leaf Area Meters CI-202*), CID, Bio-Science, USA. The fresh after weighing, then dried at oven for drying at a temperature of 80- 85°C for 72 h (g/plant).

Water Relations:

Succulence and Dry matter content (%):

The percentage of the Succulence content and dry matter content (DMC%) was determined after drying the shoot and root samples in air – circulation oven at 80°C after constant weight, and calculated as the following equation:

$$\text{Succulence} = \text{F. Wt.} / \text{Oven D. Wt.} \dots\dots\dots(1)$$

$$\text{D M C \%} = (\text{Oven D. Wt./F. Wt.}) \times 10 \dots\dots\dots(2)$$

Measurement of Relative Water Content:

The relative water content (RWC%) was measured according to a modification of the method of Weatherly (1950); Slatyer (1957); Weatherly and Barr (1962). Detached leaf samples were weight

immediately and floated on distilled water in a darkened refrigerator (5°C). Saturation of the leaves was attained after 24 h. and the leaves were rapidly and thoroughly blotted and weighed immediately. The leaves were then dried at 80°C to constant weight in an air – circulation oven to constant weight. The relative water content of leaves was expressed according to the following equation:

$$RWC\% (S. Wt. \%) = \frac{(F. wt. - Oven D. Wt.) \times 10}{(Saturated Wt. - Oven D. Wt.)} \dots\dots(3)$$

Physiological Studies

Photosynthetic Pigments:

Chlorophyll a, chlorophyll b and carotenoids of leaves were determined spectrophotometrically as the method described by Metzner et al. (1965). An 85% aqueous acetone extract of a known F.W. of leaf was assayed Spectrometrically (*LKB NOVASPEC*) at 664, 645, 420 nm. The following equations were used to determine the concentration of the pigment fractions as $\mu\text{g/ml}$.

$$\text{Chlorophyll a} = 10.3 E_{664} - 0.918 E_{645} \dots\dots\dots(4)$$

$$\text{Chlorophyll b} = 19.7 E_{645} - 3.870 E_{664} \dots\dots\dots(5)$$

$$\text{Carotenoids} = 403 E_{452} - (0.0264 \text{ Chl. a} + 0.426 \text{ Chl. b}) \dots\dots(6)$$

The pigment fractions were calculated as $\mu\text{g Chl./mg D.W.}$

Photosynthetic Activity:

Chloroplasts were prepared by the method of Aronoff (1949) and Osman, *et al.* (1982). Fresh leaves were shredded, ground for one min in a blender, using a buffered solution of 0.4 M sucrose, 20 mM HEPES-KOH (pH 7.8), 3 mM MgCl_2 , 4 mM sodium ascorbate and 0.1% bovine serum albumin (BSA). The much was strained through cheese-cloth, filtered and the suspension centrifuged (1 min at 8,000 X g). The pellet was re-suspended in the isolation medium, centrifuged (5 min at 300 X g) and the supernatant re-centrifuged (10 min at 1,000 X g). The sediment was re-suspended in a 2 ml buffer solution at pH 6.8 and the aggregates dispersed (Osman *et al.*, 1982). The levels of chlorophyll a & chlorophyll b were determined by the method described by Mackinney (1941). An aliquot of 0.2 ml of the chloroplast suspension was extracted with 3.8 ml of 85% cold aqueous acetone and the density of the extract measured at 652 nm. The chlorophyll content was calculated according to the following equation :

$$C = E_{652} \times 1,000 / 34.5 \text{ mg chl./L} \dots\dots\dots(7)$$

Where C = Chlorophyll a & b.

The photosynthetic activity of the isolated chloroplasts was measured using potassium ferricyanide ($5 \times 10^{-4}\text{M}$) as an electron acceptor. Reduction of ferricyanide was monitored spectrophotometrically (*LKB NOVASPEC*) at 420 nm

at room temperature. The reduction mixture contained 0.2 ml of chloroplast suspension, (0.2-0.8 mg chl. ml^{-1}), 3.8ml HEPES buffer (pH 7.8), and $5 \times 10^{-4}\text{M}$ potassium ferricyanide. The mixture was illuminated at 300 Wm^{-2} using a slide projector provided with a heat filter with a 24 v, 250 w quartz halide bulb, 15-45 cm from the well. The photosynthetic activity of the isolated chloroplasts was calculated from the standard curve and expressed as $\mu\text{mol ferricyanide mg chl}^{-1} \text{ h}^{-1}$ (Arnon and Shavit, 1963).

I- Organic Components:

Carbohydrate: 300 mg of oven dry plant material was extracted with 5 ml of borate buffer (28.63 g boric acid + 29.8 g KCl + 3.5 g NaOH in a liter of hot distilled water), left for 24 hr, then centrifuged and filtered. The filtrate was used for the determination of the direct reducing value (DRV-including all free monosaccharide) and total reducing value (TRV-including sucrose), while the residue was dried at 80°C for determination of polysaccharides (Naguib, 1963 & 1964).

Direct Reducing Value (DRV), was carried out by evaporation, 0.1 ml of extracted cleared borate buffer was reduced to dryness and then mixed with 1 ml of modified Nelson solution (Naguib, 1964). The mixture was maintained on a boiling water-bath for 15 min, after which it was cooled rapidly using running tap water. Thereafter 1 ml of arsenomolybdate (Nelson, 1944) was added, the mixture was diluted to a definite volume, and its intensity measured at 700 nm, using colorimeter (*LKB NOVASPEC Surplus Model 4049 Spectrophotometer*).

Total Reducing Value (TRV): For determination of total reducing value (TRV), 0.2 ml of cleared extract was mixed with deionized water up to 5 ml then 0.2 ml of the diluted extract was mixed with 0.1 ml of 1% invertase enzyme solution and the mixture maintained at 37°C for 0.5 hr. Thereafter, the reducing value was determined as described before for DRV (Naguib, 1963 & 1964). The difference between the value obtained from this step and that of the DRV is an estimated of **sucrose**, in terms of glucose made up to 3 ml left overnight at 28°C and then centrifuged.

Polysaccharides: 10 mg of the remaining residue was mixed with 0.2 ml of 1% taka diastase enzyme and 0.1 ml acetate enzyme and ml acetate buffer (6 ml acetic acid 0.2N+4 ml sodium acetate buffer 0.2 N). The reducing value of 1ml of filter was estimated as above (Naguib, 1963).

Proteins contents: Dry samples collected during the growth study were analyzed for protein content, after precipitating the protein with 15% TCA at 4°C according to Lowry et al. (1951).

Total Free Amino Acids contents: These were determined by the method described by Ya and Tunekazu (1966). An aliquot of 0.1 ml plant extract was heated in a test tube with 1.9 ml of ninhydrin citrate buffer-glycerol mixture in a boiling water bath for 12 min, and cooled at room temperature. Then the tube was well shaken and the optical density read at 570 nm. A blank was determined with 0.1 ml of distilled water and a standard curve obtained with 0.005 to 0.2 mM g Glycine.

Proline contents: This was estimated using the acid ninhydrin method described by Bates et al. (1973). Two ml of water extract were mixed 10 ml of 3% aqueous sulfosalicylic acid. Two ml of this mixture was allowed to react with 2 ml acid ninhydrin-reagent and 2 ml of glacial acetic acid in a test tube for 1 h at 100°C; the reaction was terminated by cooling the mixture in an ice bath. The reaction mixture was extracted with 4 ml toluene, and mixed vigorously for 15-20s. The chromatophore - containing toluene was aspirated from the aqueous phase, warmed to room temperature, and the absorbance read at 520 nm using toluene as a blank. The proline concentration was determined from a standard curve.

Total indole as described by Larsen et al. (1962) and **total phenol**, as described by Malik and Singh (1980) were estimated in the fresh shoots.

Determination of antioxidant enzyme activities: The **catalase** (CAT, EC 1.11.1.6) activity was assayed from the rate of H₂O₂ decomposition following the method of Cakmak and Horst (1991). **Peroxidase** (POD, EC 1.11.1.7) following the method of Macheix and Quessada (1984) and **superoxide dismutases** (SOD, EC 1.15.1.1) as described by Dhindsa et al. (1981).

II- Inorganic Components:

Mineral Elements:

Ions content measurements were carried out after extraction with 0.1 nitric acid of the ashed (powdered) milled samples at 500°C obtained after combustion in a muffle furnace, the milled samples were estimated following the "wet ashing procedure" (Richards, 1954); the acid digests of the oven dried samples were analysed. Oven dried plants were subjected to acid digestion and sodium (Na⁺) and calcium (Ca²⁺) estimated photo-metrically using a corning-400 flame photometer (Johnson and Ulrich, 1959; Allen et al., 1974). Phosphorous (P³⁺) was estimated using the method of Sekine et al. (1972) by the Molybdenum-blue method (Allen et al., 1974), while nitrogen was estimated by the Automatic MicroKjeldahl method (Allen et al., 1974). Determination of potassium (K⁺) content by Miller (1998) methods, the plant parts were dried in a ventilated oven for approximately 78 h at 60°C to a

constant weight and then ground, the samples were digested in a nitric-perchloric acid mixture and analyzed with Atomic Absorption Spectrometer (*Carl Zeiss Jena, Germany*). Chlorides were determined by the AgNO₃ titration method as described by Jackson and Thomas (1960).

Statistical Analysis:

All data were subjected to *F* test ANOVA and the means were compared using Duncan's multiple range ($P < 0.05$). Where relevant, the experimental data was subjected to analysis of variance. Percentage values were transformed into arcsines according to Bliss (1973) and analysis of variance was carried out according to Snedecor and Cochran (1967).

2. Results and discussion

Germination Rate:

Results presented in Table (1) indicated that the germination rate (%) of tomato seeds increased with seeds soaked in ascorbic acid (ASC) more than the seeds soaked in distilled water. Generally, the tomato seeds germination occurred after 3 days, but the germination rate were more faster after soaking the seeds in ascorbic acid (ASC) compared with control (distilled water). Germination is a crucial stage in seedling establishment and plays a key role in crop production. The germination process comprises two distinct phases the first is imbibitions, mainly dependent on the physical characteristics of the seeds and the second is a heterotrophic growth phase between imbibitions and emergence (Khajeh-hosseini et al, 2003; Akbari ghogdi et al., 2012). The ascorbic acid plays a remarkable role in case of seed germination, cell growth under salinity due to its antioxidants properties (Netondo et al., 2004).

The role of ascorbic acid (ASC) in seed germination and cell growth under salinity is remarkable its anti-oxidant activity, rather than its possible utility as an organic substrate for respiratory energy metabolism. So, after soaking the tomato seeds in Ascorbic acid (ASC), the rate of germination increased with times. The effect of ascorbic acid on plant survival is associated with the partial inhibition of a few interactions in active oxygen species production. An artificial increase in cellular level of an antioxidant such as ascorbic acid should be beneficial in improving stress tolerance at germination level (Shalata, and Neumann, 2001; Khan *et al.*, 2006a).

The similar results on seeds germination were reported also by treatment the seeds with exogenous ascorbic acid increasing the level of ascorbic acid uptake by different tissues under salinity (NaCl) stress (Shaddad *et al.*, 1990; Arrigoni and De Tullio, 2000; Arab and Ehsanpour, 2006).

Table (1): Impact of Ascorbic Acid (ASC) on germination rate of Tomato (*Lycopersicon esculentum* Mill. var. Cerasiforme) seeds .

Germination Rate (%) Ascorbic acid (50 ppm ASC)	Time / Days								
	1	2	3	4	5	6	7	8	9
-ASC	-----	-----	10	16	58	68	82	90	100
+ASC	-----	-----	38	46	83	96	100	100	100
<i>F</i> values	-----	-----	***	**	**	**	*	N.S.	N.S.

-ASC = Absence of ASC, Soaking Seeds in distilled water before germinated

+ASC= Presence of ASC, Soaking Seeds in Ascorbic Acid before germinated

Values are Expressed as the mean of five samples \pm Standard Deviation (\pm SD)

Statistical Analysis treatments, where relevant, the experimental data were subjected of One – Way analysis of variance (ANOVA). Note: *F* values * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$ and N.S. = Not Significant.

Growth Parameters:

Generally, salinity stress in plants retards all major growth processes that have been examined, the growth parameters decreased significantly with increasing NaCl salinity concentrations (2000, 4000, 6000 & 8000 ppm), whereas, the level of growth increased more in the presence of ascorbic acid (+ASC), compared as control (-ASC and +ASC) without salt-stress, as shown in Table (2). Tomato

plant tended to increased significantly the growth parameters (plant height, leaf number and area, fresh and dry weight) in the presence of ascorbic acid (+ASC) compared to tomato plant control. Overall, tomato plant height tended to decreased with increasing NaCl salinity concentrations while after soaked the seeds of tomato in ascorbic acid (+ASC) tended to increased plant height.

Table (2): Interactive effects of Ascorbic Acid (ASC) and NaCl Salinity Treatments on Plant Height, Leaf Area (cm^2) and Number, Fresh and Dry Weight of Tomato (*Lycopersicon esculentum* Mill. var. Cerasiforme) Plant.

The leaf area was estimated in the 5th leaves from the shoot top.

Plants were grown, under glasshouse conditions, for 75 days after germination and irrigated at 80% field capacity.

Growth Parameter Ascorbic Acids (50 ppm ASC)	NaCl Salinity Concentrations (ppm)				
	0.0	2000	4000	6000	8000
Plant Height (cm/plant)					
-ASC	39.37 \pm 0.25	33.23 \pm 0.35	29.33 \pm 0.38	25.08 \pm 0.50	20.07 \pm 0.35
+ASC	42.23 \pm 0.38	39.90 \pm 0.46	35.83 \pm 0.25	31.13 \pm 0.75	25.97 \pm 0.41
<i>F</i> values	N.S.	**	**	**	**
Leaf Number					
-ASC	9.33 \pm 0.18	8.44 \pm 0.01	7.01 \pm 0.06	5.67 \pm 0.58	4.33 \pm 0.18
+ASC	12.67 \pm 0.38	12.05 \pm 0.04	10.67 \pm 0.58	9.03 \pm 0.03	8.67 \pm 0.38
<i>F</i> values	*	**	**	**	**
Leaf Area (Cm^2 /Leaf)					
-ASC	38.37 \pm 0.24	30.57 \pm 0.58	24.88 \pm 0.25	20.70 \pm 0.51	18.27 \pm 0.36
+ASC	51.20 \pm 0.47	48.50 \pm 0.35	36.03 \pm 0.45	29.64 \pm 0.40	24.23 \pm 0.49
<i>F</i> values	**	**	**	**	**
Plant Fresh Weight (g/Plant)					
-ASC	16.75 \pm 0.19	14.67 \pm 0.07	13.38 \pm 0.21	12.40 \pm 0.06	11.69 \pm 0.02
+ASC	18.98 \pm 0.26	17.35 \pm 0.30	15.71 \pm 0.29	14.95 \pm 0.09	13.56 \pm 0.13
<i>F</i> values	N.S.	N.S.	*	*	*
Plant Dry Weight (g/Plant)					
-ASC	1.434 \pm 0.02	1.371 \pm 0.03	1.311 \pm 0.02	1.286 \pm 0.03	1.186 \pm 0.01
+ASC	1.670 \pm 0.03	1.611 \pm 0.04	1.381 \pm 0.03	1.319 \pm 0.02	1.292 \pm 0.02
<i>F</i> values	N.S.	*	N.S.	N.S.	N.S.

-ASC = Absence of ASC, Soaking Seeds in distilled water before germinated

+ASC= Presence of ASC, Soaking Seeds in Ascorbic Acid before germinated

Values are Expressed as the mean of five samples \pm Standard Deviation (\pm SD)

Statistical Analysis treatments, where relevant, the experimental data were subjected of One – Way analysis of variance (ANOVA). Note: *F* values * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$ and N.S. = Not Significant.

So, added ascorbic acid (+ASC) decreased the effects of salinity stress with all NaCl salinity concentration compared with control. The results agree with Ejaz et al. (2012) they found that the leaf area per plant was significantly reduced under salt stress, while ascorbic acid (ASC) applications markedly improved the inhibitory effects of salt on plants. The fresh weight in four tomato cultivars decreased with salinity stressed (Ashraf and Harris, 2004; Okhovatian-Ardakani *et al.*, 2010; Ali *et al.*, 2011).

Hence, it is assumed that exogenous ascorbic acid (ASC) improve seed tolerance to salinity significantly. Salinity reduces plant productivity first by reducing plant growth during the phase of osmotic stress and subsequently by inducing leaf senescence during the phase of toxicity when excessive salt is accumulated in transpiring leaves (Munns, 2002 b). Water deficits may exert their effects directly on cell extension and division (Greenway and Munns, 1983). Ascorbic acid (ASC) influence mitosis and cell growth in plants, affects phyto-hormone-mediated signaling processes during the transition from the vegetative to the reproductive phase as well as the final stage of development and senescence (Smirnov and Wheeler, 2000; Barth *et al.*, 2006). The beneficial effect of ascorbic acid (ASC) on plant height may be attributed to the fact that ascorbic acid (ASC) is involved in the regulation of shoot and root elongation, cell vacuole, Leaf area and cell expansion

(Smirnov, 1996; Sumalan and Carmen, 2002; El Hariri *et al.*, 2010; Farahat *et al.*, 2013). Although ascorbic acid is one of the most important and abundantly occurring water soluble antioxidants in plants, relatively little is known about its role in counteracting the adverse effects of salt stress on plant growth (Beltaji, 2008; Athar *et al.*, 2008).

Water Relations:

Data presented in Table (3) indicated that the succulence and relative water content (RWC %) decreased significantly with increasing NaCl salinity concentrations, especially in the absence of ascorbic acid (-ASC) more than in the presence of ascorbic acid (+ASC) in tomato plant. Whereas, dry matter content % (DMC%) increased significantly with increased NaCl salinity concentration in the absence of ascorbic acid (-ASC) more than in the presence of ascorbic acid (+ASC). So, increasing NaCl salinity concentration tended to reduced the absorption of water leading to a drop in water content, the inhibitory effect of NaCl on growth parameters could be attributed to the osmotic effect of NaCl salinity, in addition, the changes in water status under NaCl stress may cause a reduction in meristem activity as well as cell elongation (Salter *et al.*, 2007; Shah, 2007; Chookhampaeng, 2011). These suggestions are in a good agreement with present results, which showed that the increase of WC and RWC was associated with a decrease in transpiration rate.

Table (3): Interactive Effects of Ascorbic Acid (ASC) and NaCl Salinity on Water Relations and Dry Matter Content of Tomato (*Lycopersicon esculentum* Mill. var. Cerasiforme) Plant.

Plants were grown, under glasshouse conditions, for 75 days after germination and irrigated at 80% field capacity.

Water Relations Ascorbic Acids (50 mM ASC)	NaCl Salinity Concentrations (ppm)				
	0.0	2000	4000	6000	8000
Succulence (Fresh Weight / Oven Dry weight)					
-ASC	11.68±0.12	10.70±0.23	10.21±0.26	9.64±0.23	9.86±0.36
+ASC	11.37±0.21	12.01±0.31	11.38±0.18	11.33±0.38	10.50±0.43
F values	N.S.	*	N.S.	*	*
Dry Matter content (%)					
-ASC	8.56±0.09	9.36±0.31	9.79±0.23	10.37±0.61	10.16±0.56
+ASC	8.80±0.11	9.29±0.24	8.79±0.16	8.82±0.44	9.52±0.39
F values	N.S.	N.S.	*	**	*
Relative Water Content % (Saturated Weight)					
-ASC	88.45±0.19	86.93±0.21	85.72±0.25	84.78±0.34	84.03±0.75
+ASC	87.82±0.26	86.43±0.37	87.79±0.28	87.27±0.81	86.15±0.93
F values	N.S.	N.S.	*	**	*

Further, it could be suggested that the effectiveness of ASC depends on its mode of application, which may enhance the endogenous level of ASC and water status of treated plants (Azooz, 2004; Alqurainy, 2007; Athar *et al.*, 2008). Search

results consistent with the findings of the Sairam *et al.* (2002) and Ghoulam *et al.* (2002) they concluded that the salt treatment induced a reduction in leaves RWC. While, Mandhania *et al.* (2012) found relative water content (RWC) decreased progressively with

increasing duration and levels of NaCl in the leaves of both salt-sensitive WH-542 and salt-tolerant KRL-19 cultivars. The adverse effects of NaCl on *Silybum marianum* L. plants the growth parameters, water content (WC) and relative water content (RWC) were mitigated by seed 100 ppm vitamin C (Summart et al., 2010; Ekmekçi and Karaman, 2012).

Photosynthetic Pigments and Chloroplast efficiency:

Generally, the chloroplast pigments (chlorophyll *a*, chlorophyll *b*, and carotenoids) and photosynthetic efficiency exhibited marked significantly increased with salinity concentration, whereas more increased in the presence of ascorbic acid (+ASC) over control (-ASC) as presented in Table (4). Significant increases in the above mentioned characters were recorded from NaCl treatment plants compared to plants in the presence of ascorbic acid (+ASC). Salt stress (NaCl) has both osmotic (cell dehydration) and toxic (ion accumulation) effects on plants, impairing growth, ion homeostasis, photosynthesis and nitrogen fixation, among other key physiological processes (Zhu, 2001; Munns, 2002a; Tejera et al., 2004; Bartels and Sunkar, 2005). Chlorophylls (*a* & *b*) and

Carotenoids are main photosynthetic pigments and they play important role in photosynthesis, the changes in the amount of pigment were evaluated as the changes in photosynthesis. So, the changes of pigment contents under salt stress are used as parameter for selection of tolerant and sensitive cultivars in crop plants (Eryilmaz, 2007). Increasing sodium concentration in plant tissue can increase oxidative stress, which causes deterioration in chloroplast structure and an associate lose in chlorophyll. This leads to a decrease in chlorophyll, while increasing carotenoids content. (Khosravinejad and Farboondia, 2008; Pinheiro et al., 2008). The accumulation of soluble carbohydrates in plants has been widely reported as a response to salinity or drought, despite a significant decrease in net CO₂ assimilation rate (Crowe et al., 2002; Murakeozy et al., 2003). The reduced water potential could be explained by the fact that during stress carbon allocation, osmotic adjustment and accumulation of soluble sugars compete with other sinks and can affect growth (Shi and Tadashi 2001; Balibrea et al., 2003; Jiang et al., 2007; Gama et al., 2007).

Table (4): Interactive Effects of Ascorbic Acid (ASC) and NaCl Salinity on Chloroplast Pigments ($\mu\text{g}/\text{mg}$ Leaf F.Wt.) and Photosynthetic Activity (μmol fericyanide $\text{mg chl}^{-1} \text{h}^{-1}$) of Tomato (*Lycopersicon esculentum* Mill. var. Cerasiforme) Plant.

The leaf was estimated in the 5th leaves from the shoot top.

Plants were grown, under glasshouse conditions, for 75 days after germination and irrigated at 80% field capacity.

Chloroplast Pigment & Photosynthetic Activity	NaCl Salinity Concentrations (ppm)				
	0.0	2000	4000	6000	8000
Ascorbic Acids (50 ppm ASC)					
Chlorophyll <i>a</i> ($\mu\text{g}/\text{mg}$ Leaf F. Wt.)					
-ASC	5.80±0.01	5.89±0.03	6.01±0.05	6.13±0.04	6.28±0.04
+ASC	7.33±0.01	7.42±0.04	7.49±0.01	7.58±0.02	7.64±0.03
<i>F</i> values	*	*	*	*	*
Chlorophyll <i>b</i> ($\mu\text{g}/\text{mg}$ Leaf F. Wt.)					
-ASC	2.24±0.042	2.35±0.015	2.39±0.032	2.52±0.035	2.76±0.021
+ASC	3.38±0.035	3.44±0.035	3.52±0.015	3.74±0.031	3.85±0.044
<i>F</i> values	*	*	*	*	*
Carotenoids ($\mu\text{g}/\text{mg}$ Leaf F. Wt.)					
-ASC	3.73±0.010	3.96±0.025	4.01±0.025	4.11±0.025	4.35±0.01
+ASC	4.51±0.025	4.72±0.006	4.84±0.006	4.94±0.006	5.05±0.047
<i>F</i> values	N.S.	N.S.	N.S.	N.S.	N.S.
Total Pigments ($\mu\text{g}/\text{mg}$ Leaf F. Wt.)					
-ASC	11.77±0.18	12.20±0.49	12.41±0.79	12.76±0.65	13.39±0.75
+ASC	15.22±0.74	15.58±0.54	15.85±0.63	16.26±0.48	16.54±0.81
<i>F</i> values	**	*	*	**	*
Photosynthetic Activity (μmol fericyanide $\text{mg chl}^{-1} \text{h}^{-1}$)					
-ASC	67.32±1.21	71.34±1.32	75.31±0.87	78.31±2.01	80.28±1.75
+ASC	73.83±2.14	79.26±2.42	83.86±2.61	86.87±2.09	89.27±2.65
<i>F</i> values	*	**	**	**	**

I- Organic Components:**Total Available Carbohydrates:**

In these investigation, the total available carbohydrates (Monosaccharide, Disaccharides, polysaccharides) contents in tomato plant increased significantly with salinity concentration at both in the presence (+ASC) or absence (-ASC) of ascorbic acid compared to control as shown in Table (5). The significantly increasing in total available carbohydrates, results from the close relationship between stomatal conductance and photosynthesis, thus lead to an increase in photosynthesis. The results of the study agrees with Farahat et al. (2013) they found that the combined treatment of ascorbic acid

(100 ppm and 200 ppm) with salinity level at water salinity (3000 and 6000 ppm) gave significantly increased chlorophyll a & b, carotenoids content and total carbohydrates % of shoots and roots compared with control plants. The highest values were recorded with 200 ppm ascorbic acid. The substantial increase in carbohydrate contents may be due to the activation of photosynthetic machinery, as a result of the stimulatory effects of the used plant growth bio stimulators on photosynthetic process. Chlorophyll a & b contents and total carbohydrates % of shoots and roots were reduced as external salinity in irrigation water increased.

Table (5): Interactive Effects of Ascorbic Acid (ASC) and NaCl Salinity on Total Available Carbohydrates (mg/100g D. Wt.) of Tomato (*Lycopersicon esculentum* Mill. var. Cerasiforme) Plant.

Plants were grown, under glasshouse conditions, for 75 days after germination and irrigated at 80% field capacity.

Carbohydrates Content (mg/100g D. Wt.) Ascorbic Acids (50 ppm ASC)	NaCl Salinity Concentrations (ppm)				
	0.0	2000	4000	6000	8000
Monosaccharide (DRV)					
-ASC	9.70±0.79	10.87±0.81	12.13±1.01	13.98±2.01	14.79±1.31
+ASC	12.30±0.70	13.71±0.72	15.81±1.91	16.31±0.69	16.81±1.98
F values	**	*	*	**	*
Disaccharide (Sucrose)					
-ASC	10.98±0.97	12.17±2.01	14.50±1.54	16.31±1.36	17.89±1.70
+ASC	13.01±1.03	18.80±1.80	19.70±0.79	20.31±1.03	21.31±1.31
F values	*	**	**	**	**
Polysaccharide					
-ASC	3.98±0.03	6.31±0.06	7.62±0.07	9.71±0.07	10.31±0.03
+ASC	7.31±0.03	8.91±0.09	9.81±0.08	10.82±0.08	11.09±0.09
F values	**	**	*	N.S.	N.S.
Total Available Carbohydrates					
-ASC	24.36±1.02	29.35±1.04	34.25±0.98	40.00±2.01	42.99±2.04
+ASC	32.62±1.71	41.42±1.27	45.31±1.09	47.44±1.69	49.21±1.83
F values	**	**	**	*	**

Nitrogenous Components:**Protein, Total Amino Acids, Proline Contents:**

Results presented in Table (6) indicated that the effect of salinity concentration on tomato plant in the presence (+ASC) or absence (-ASC) of ascorbic acid exhibited marked significant increase in total protein, total amino acids, proline contents compared with control tomato plants. Moreover, the protein, amino acids, proline contents increasing in tomato plants in the presence (+ASC) more than in the absence (-ASC) of ascorbic acid may be responsible for the

Campbell (1977) found that the chloroplasts have paramagnetic properties. Shabrangi and Majd (2009) concluded that, biomass increasing needs metabolic changes particularly increasing protein biosynthesis. Kapoor and Srivastava (2010) observed an increase in protein content when increasing salt concentration. Proline accumulation in response to lower salt concentration may contribute positively to

stimulation of growth. Accumulation of amino acids as well as proline was ascertained in different halophytes and non-halophytes under salinity conditions (Greenway and Munn 1980; Harborne 1988; Dubey and Rani 1989). It was suggested that build up of proline and other organic solutes in shoots and roots of salinized plants either contributes to osmotic balance in cells (Stewart and Lee 1974; Zidan, 1991) or helps to maintain enzymes activities (Pollard and Wyn Jones 1979; Greenway and Munns 1980).

salt tolerance, whereas the high concentration in leaf tissues under high salinity treatment may be partly due to leaf damage. The plants under salinity condition change their metabolism to overcome the changed environmental condition. According to Ebrahimian and Bybordi (2012) the soluble protein content decreased on account of salinity stress, one of the mechanisms affected by salt stress in plants was

protein synthesis. It is known that soluble protein content is an important indicator of physiological status of plants. Proline is synthesized in plants through two alternate pathways: L-ornithine and L-glutamate pathways (Parvaiz and Satyawati 2008).

Proline is a typical adaptive response in plants and it may be a part of stress signal (Maggio et al., 2002; Yang et al., 2009). Proline, which is an amino acid is one such organic solute that plays a major role in this osmotic adjustment (Chookhampaeng, 2011; Loukehaich et al., 2011). Likewise, in tomato salt tolerance was attributed to the degree of plant to accumulate osmoprotectants, like proline (Patel and Pandey, 2008; Dasgan et al.,

2009). Proline is one of well-known osmoprotectants and its accumulation is widely observed in various organisms under salt stress. The amino-acid may play a role in protecting membranes and proteins against adverse effects of higher concentrations of inorganic ions and temperature extremes. Chookhampaeng (2011) noted that the salinity treatments caused the increased proline content in pepper plant. One mechanisms utilized by the plants for overcoming the salt stress effects might be via accumulation of compatible osmolytes, such as proline and soluble sugar. Production and accumulation of free amino acids, especially proline by plant tissue during drought, salt and water stress is an adaptive response.

Table (6): Interactive effects of Ascorbic Acid (ASC) and NaCl Salinity on Total Proteins, Total Amino Acids, Proline (mg/100g D. Wt.), Catalase Enzyme (H_2O_2/g F. Wt. Protein/min) Peroxidase and Superoxide dismutase (units mg^{-1} protein) Contents of Tomato (*Lycopersicon esculentum* Mill. var. Cerasiforme) Plant.

Plants were grown, under glasshouse conditions, for 75 days after germination and irrigated at 80% field capacity.

Nitrogenous components & Antioxidant enzymes	NaCl Salinity Concentrations (ppm)				
	0.0	2000	4000	6000	8000
Ascorbic Acids (50 ppm ASC)					
Total Protein (mg/100g D.Wt.)					
-ASC	12.72±0.131	13.41±0.144	17.12±0.121	18.89±0.107	20.28±0.160
+ASC	15.48±0.067	17.19±0.185	20.17±0.172	22.24±0.110	26.39±0.175
F values	*	*	*	**	**
Total Amino Acids (mg/100g D.Wt.)					
-ASC	18.97±0.086	24.72±0.079	39.83±0.124	44.32±0.090	53.86±0.289
+ASC	23.41±0.125	33.97±0.162	47.42±0.191	58.14±0.242	76.50±0.210
F values	*	**	*	**	***
Proline (mg/100g D.Wt.)					
-ASC	10.13±0.98	11.91±1.01	13.38±1.19	15.19±0.81	16.89±0.38
+ASC	15.91±1.09	17.18±0.82	20.14±1.09	23.01±0.47	25.32±1.27
F values	*	*	*	**	**
Catalase enzyme (H_2O_2/g F. W. Protein/min)					
-ASC	13.54±1.195	17.85±0.953	20.63±1.064	24.71±1.121	27.54±1.075
+ASC	16.02±0.922	20.76±0.879	24.98±1.107	29.16±1.032	31.28±1.246
F values	*	*	*	**	**
Peroxidase (units mg^{-1} protein)					
-ASC	5.23±0.26	5.92±0.61	6.01±0.37	6.91±0.18	8.01±0.23
+ASC	9.72±0.47	10.13±0.52	11.38±0.51	12.13±0.22	13.70±0.53
F values	*	*	*	*	*
Superoxide dismutase (units mg^{-1} protein)					
-ASC	1.98±0.28	2.01±0.52	2.32±0.09	2.75±0.19	2.98±0.28
+ASC	5.08±0.71	5.21±0.38	5.76±0.04	5.98±0.38	6.13±0.52
F values	**	*	*	*	*

Total Phenols and Total Indole:

Also, the effect of salinity concentration on tomato seeds in the presence (+ASC) or absence (-ASC) of ascorbic acid exhibited marked significant increase in total phenols and total indole compared with control plants as shown in Table (6). Hassanein et al. (2009) and Abd-El Hamid (2009) suggested that the ascorbic acid increased IAA content, which

stimulates cell division and/cell enlargement and this in turn, improved plant growth.

Antioxidant Enzymes:

Catalase, Peroxidase, and Superoxide Dismutase:

Data presented in Table (6) showed that the effect of salinity concentration on tomato plant in the presence (+ASC) or absence (-ASC) of ascorbic acid increased significantly in the activities of the

antioxidant enzymes (catalase, peroxidase, and superoxide dismutase), but the activity increased more in the presence of ascorbic acid (+ASC) compared with control. The plants defend against these reactive oxygen species by induction of activities of certain anti-oxidative enzymes such as catalase, peroxidase, glutathione reductase, and superoxide dismutase, which scavenge reactive oxygen species (Mittova et al., 2003). Among them, ion homeostasis, osmotic adjustment, enhancement of antioxidant defense systems and increase of the photosynthetic ability are the most important ones (Zhu, 2000; Xiong and Zhu, 2002). It is newly clear that the antioxidant defense systems are very important for the determination of plant salt tolerance (Sairam et al., 2005; Gossett et al., 1994; Mittler, 2002). Several studies demonstrated that salt-tolerant species increase their antioxidant enzyme activities and antioxidant content in response to salt stress, while salt-sensitive species do not (Ashraf and Harris 2004; Khan et al., 2002; Shalata et al., 2001). While, Mandhania et al. (2012) found that the activities of Catalase (CAT) and Ascorbate Peroxidase (APX) increased with increasing the salt stress in both salt tolerant and salt sensitive wheat cultivars. Dolatabadian et al. (2008) they found the exogenous application of ascorbic acid induces activation of antioxidant enzyme system in canola (*Brassica napus* L.) resulting in reduction of detrimental effects of salinity. Antioxidative enzyme such as superoxide dismutase, ascorbate peroxidase and catalase play an important role against oxidative stress. Plants containing high activities of antioxidant enzymes have shown considerable resistance to oxidative damage caused by Reactive Oxygen Species (ROS) (Apel and Hirt, 2004; Khan et al., 2007; Gapinska et al., 2008; Frary et al., 2010).

Indeed, several studies have shown that ASC plays an important role in improving plant tolerance to abiotic stress (Shalata and Neumann, 2001; Al-Hakimi and Hamada, 2001; Athar et al., 2008). Application of ascorbic acid can reduce the harmful effects of salt stress and may have stimulatory effects on plants; ascorbic acid is synthesized in the higher plants and improves plant growth. It is a product of D-glucose metabolism which affects some nutritional cycle activities in higher plants and plays an important role in the electron transport system (El-Kobisy et al., 2005).

II- Inorganic Components

Mineral Elements:

Data presented in Table (7), indicated that the increasing NaCl salinity concentration tended to increase the inorganic mineral elements (potassium, Nitrogen, phosphorous, sodium, calcium, magnesium

and chloride) contents compared with control plant. Whereas, mineral elements increased significantly more in the presence (+ASC) than in the absence (-ASC) of ascorbic acid. The deleterious effects of salinity on plant growth are associated with low water potential of the root medium which causes a water deficit within the plant; toxic effects of ions mainly Na^+ and Cl^- ; nutritional imbalance caused by reduced nutrient (K^+ , Ca^{2+} , Mg^{2+}) uptake and/or transport to the shoot. Salinity mainly causes both hyper-osmotic stress and hyper-ionic toxic effects and the consequence can be plant demise (Munns and Termaat, 1986; Ashraf, 1994; Marschner, 1995; Serrano et al., 1999; Hasegawa et al., 2000).

Babu et al. (2012) found that the Potassium content was found in leaves and tomato fruits to be decreasing with increase in salt stress. Also, Khafagy et al. (2009) found that the significant decrease in K^+ concentration occurred with increasing salinity levels in sweet pepper plants. In this respect, high salinity level (6000 ppm) NaCl (11.88 dSm^{-1}) was the most effective in this concern as compared to control. However, K^+ concentration decreased in the root system more than shoot. Whereas, K^+ concentration increased with application of ASC compared to non-salinized plants. In most cases, pre-soaking with ASC at 100 ppm was the most effective in increasing K^+ concentration in the shoots and roots. Moreover, all interactions between salinity and ASC increased K^+ concentration as compared to salinity stress. Potassium may play a role on the synthesis of endogenous plant hormones (Haeder et al., 1981). Sucrose played a main role in the regulation of the root osmotic potential followed by K, glucose and Na this agree with the results (Eisa and Ali, 2003). However, exogenous application of ASC with varying levels (0, 50, 100 mg L^{-1}) in hydroponics also increased the accumulation of Na^+ in the leaves of both wheat cultivars (Khan et al., 2006b). Despite its obvious importance, the low mobility of Ca^{2+} make the rates of its uptake and distribution limiting processes for many key plant functions. Furthermore, the general lack of recognition of the limiting role of Ca^{2+} is due in part to the fact that some important plant functions are controlled by changes in very small physiologically active pools of Ca^{2+} within the cytoplasm. As such, whole-leaf Ca^{2+} levels might not reflect any potential limitations (McLaughlin and Wimmer, 1999).

Ascorbic acid has effects on many physiological processes including the regulation of growth and metabolism of plants under saline conditions and increasing physiological availability of water and nutrient (Barakat, 2003). The accumulation of nitrogen-containing compatible solutes including proline is known to function in

osmotic adjustment, protection of cellular macromolecules from damage by salts, storage of nitrogen and scavenging of free radicals. The results of the present study agree with Farahat et al. (2013) where found the highest nitrogen in shoot resulted from ascorbic acid (200 ppm) and salinity (3000 ppm) level.

In the present data the results finding from Farahat et al. (2013) agree with they reported that the all of nitrogen (N); phosphorus (P) and potassium (K) contents in both shoots and roots increased gradually with increasing the levels of ascorbic acid. So, ascorbic acid (ASC) protect metabolic processes against H₂O₂ and other toxic derivatives of oxygen affected many enzyme activities, minimize the damage caused by oxidative processes through synergistic function with other antioxidants and stabilize membranes (Shao et al., 2008). Also,

Bassuony et al. (2008) found the content of K⁺; Ca⁺² and Mg⁺² in *Zea mays* plant decreased significantly under salinity stress, compared with control. While, application of 100 ppm from vitamins C (ascorbic acid) resulted significantly increases of K⁺, Ca⁺² and Mg⁺² contents compared with controls. While, Flores et al., (2001) found that the salt stress inhibits the uptake and transport of potassium, calcium and phosphorus, we predict that sodium chloride will inhibit growth in our tomato plants. Soltani Nezhad et al. (2011) found that the phosphorus content in tomato (*Lycopersicon peruvianum* L) plant decreased was increased NaCl at 150 mM. Whereas, with increasing salinity levels, the phosphorus content of roots decreased in all NaCl concentrations. In contrast, by increasing of salt concentration in the culture medium, phosphorus content decreased significantly in roots compared to untreated plants.

Table (7): Interactive effects of Ascorbic Acid (ASC) and NaCl Salinity on Inorganic Minerals (mg/100g D.Wt.) of Tomato (*Lycopersicon esculentum* Mill. var. Cerasiforme) Plant.

Plants were grown, under glasshouse conditions, for 75 days after germination and irrigated at 80% field capacity.

Mineral Elements (mg/100g D. Wt.) Ascorbic Acids (50 ppm ASC)	NaCl Salinity Concentrations (ppm)				
	0.0	2000	4000	6000	8000
Potassium (K ⁺)					
-ASC	7.78±0.08	9.39±0.09	10.31±0.03	11.73±0.04	13.78±0.07
+ASC	10.21±0.11	14.81±0.13	15.83±0.09	18.20±0.08	20.13±0.06
F values	*	**	*	**	**
Nitrogen (N ⁺³)					
-ASC	13.10±1.21	14.70±1.17	15.78±1.17	17.10±1.84	19.01±1.91
+ASC	14.80±1.41	18.30±1.81	19.99±1.91	20.33±0.91	23.01±2.30
F values	N.S.	*	**	*	**
Phosphorous(P ⁺³)					
-ASC	4.78±0.08	6.57±0.05	7.92±0.07	9.01±0.09	10.91±0.01
+ASC	7.61±0.06	9.11±0.07	12.01±0.09	15.30±0.13	17.80±0.08
F values	**	*	**	**	***
Sodium (Na ⁺)					
-ASC	75.38±3.81	79.18±1.17	84.98±2.13	86.13±3.61	99.17±4.75
+ASC	86.19±2.83	92.13±4.18	96.81±3.31	105.01±1.21	112.81±1.83
F values	*	***	*	***	**
Calcium (Ca ⁺²)					
-ASC	24.05±1.01	26.31±1.18	29.13±1.01	32.10±1.01	36.31±1.06
+ASC	26.57±0.87	28.09±1.01	32.01±0.91	35.09±1.07	39.01±1.21
F values	*	*	**	*	*
Magnesium (Mg ⁺²)					
-ASC	28.30±1.13	30.98±2.07	33.98±1.48	36.01±0.91	39.12±1.03
+ASC	39.10±2.01	40.89±2.01	46.01±1.61	47.01±1.24	48.98±1.81
F values	**	**	**	**	**
Chloride (Cl)					
-ASC	26.98±2.01	34.21±2.01	37.33±1.03	42.13±1.27	44.10±1.08
+ASC	29.33±0.97	34.88±2.01	39.01±2.13	45.84±1.83	47.17±1.07
F values	*	N.S.	*	**	**

Abd El-Aziz et al. (2007) found that the N, P and K contents in *Syngonium podophyllum*, (L) increased gradually by increasing the concentration of ASC to 100 ppm compared with the untreated plants. The increment in N concentration due to ASC treatments could be explained by the findings of Talaat (2003) who showed that accumulation of nitrate by ASC foliar application may be due to the positive effect of ASC on root growth which consequently increased nitrate absorption. In this context the increase in P concentration by thiamine and ASC treatments may be attributed to the postulation. So, the present results not consistent with Farouk (2011) he reported that potassium, magnesium and calcium contents decreased with increasing salinity levels up to 11 dsm⁻¹. While, the present results agreed with where he found application of antioxidants, especially ascorbic acid, significantly increased potassium, calcium and magnesium wheat flag leaf. The application of antioxidants, especially ascorbic acid, partially reversed the negative effects of salinity in this respect. While, our current findings agree with Taffouo et al. (2010a & b) they found that the effect of NaCl salinity on tomato he showed that the salt treatments increased significantly Na⁺ contents in roots, stems and leaves of plants. While no agree with K⁺ and Ca²⁺ concentrations of plants were decreased in all tomato cultivars. These results agree with Hussein et al. (2011) they reported that the interaction effect between ascorbic acid (ASC) and rates of salinity water on nutrients uptake. Under water salinity irrigation, increasing the ascorbic acid spraying rate from 0 to 200 ppm increased the uptake of essential nutrients N, P, K, Ca and Mg of wheat, but did not agree with where observed decreased the Na and Cl uptake so the ascorbic acid played an important role of decreasing effects of saline conditions.

Conclusion

Results of the current study showed the positive impacts of ascorbic acid (ASC) on growth of tomato plant as well as salinity treatment than control. So as a simple and safe method, soaking the seeds of tomato plant before planting can be used to improvement plant growth and water efficiency. It appears that utilization of ASC can led to improve quantity and quality of tomato (*Lycopersicon esculentum* Mill var. Cerasiforme) plant by accumulated the organic and inorganic components. It suggests that ASC could stimulate defense system for salt-stress. Generally, using ASC treatment could be a promising technique for agricultural improvements but extensive research is required on different crops.

Corresponding Author:

Dr. Hameda El Sayed Ahmed El Sayed, Department of Biology, Faculty of Applied Science, Umm Al Qura University, Makkah Al Mukaramah, K.S.A.
heelsayed@uqu.edu.sa & D.Hameda@hotmail.com.

References

1. Abd El- Aziz, N. G.; El-Quesni, F. E. M.; Farahat, M. M. (2007). Response of vegetative growth and some chemical constituents of *Syngonium Podpphyllum* L. to foliar application of Thiamine, Ascorbic acid and Kinetin at nubaria. World Journal of Agricultural Sciences. Vol. 3(3): 301-305.
2. Abd-El Hamid, E. K. (2009). Physiological effects of some phytohormones on growth, productivity and yield of wheat plant cultivated in new reclaimed soil. Ph. D. thesis, Girls College, Ain Shams Univ. Cairo, Egypt.
3. Afzal, I.; Basra, S. M. A.; Ahmad, N.; Farooq, M. (2005). Optimization of hormonal priming techniques for alleviation of salinity stress in wheat (*Triticum aestivum* L.). Caderno de Pesquisa Ser. Bio., Santa Cruz. do Sul. Vol. 17: 95-109.
4. Akbari ghogdi, E.; Izadi-Darbandi, A.; Borzouei, A. (2012). Effects of salinity on some physiological traits in wheat (*Triticum aestivum* L.) cultivars. Indian J. Sci. Technol. Vol.5 (1).
5. Al-Hakimi, A.M.; Hamada, A.M. (2001). Counteraction of salinity stress on wheat plants by grain soaking in ascorbic acid, thiamine or sodium salicylate. Biol. Plant. Vol. 44(2): 253-261.
6. Ali, S. G.; Khan, A. N. U.; Nawab, K. (2011). Enhanced proline synthesis may determine resistance to salt stress in tomato cultivars. Pak. J. Bot. Vol. 43(6): 2707-2710.
7. Allen, S.; Grimshay, H. M.; Parkinson, J. A.; Quarmby, C. (1974). Chemical Analysis of ecological materials Black well Scientific Publications, Osney . Oxford, London, 565.
8. Alqurainy, F. (2007). Responses of bean and pea to vitamin C under salinity stress. Res. J. Agric. Biol. Sci. Vol. 3: 714-722.
9. Ansari, S. A. and F. A. Khan. (1986). Effect of pre-sowing seed treatment with pyridoxine on growth and yield performance of summer moong. Indian Bot. Soc. 65: 316-322.
10. Apel, K.; Hirt, H. (2004). Reactive oxygen species metabolism, oxidative stress, and signal transduction. Ann. Rev. Plant Biol. Vol.55: 373-399.
11. Arab, L. and Ehsanpour, A. A. (2006): The effects of ascorbic acid on salt induced alfalfa (*Medicago sativa* L.) *in vitro* culture. Biokemistr. Vol. 18(2): pp 63-69
12. Arnon, M.M.; Shavit, N. (1963). A sensitive and simple methods for determination of ferricyanide. Anal. Biochem., 6 : 549 – 554.
13. Aronoff, S. (1949). Photochemical reduction of chloroplast grana. Journals of Plant Physiology, 21: 393 – 409.
14. Arrigoni, O. and De Tullio, M. C. (2000): The role of ascorbic acid in cell metabolism: between gene-directed functions and unpredictable chemical reactions. Journal of Plant Physiology. Vol. 157: pp 481–488.

15. Ashraf, M. (1994). Breeding for salinity tolerance in plants. *Crit. Rev. Plant Sci.*, Vol. 13: 17-42.
16. Ashraf, M.; Harris, P. J. C. (2004). Potential biochemical indicators of salinity tolerance in plants. *Plant Sci.* Vol. 166: 3-16.
17. Athar, H. R.; Khan, A.; Ashraf, M. (2008). Exogenously applied ascorbic acid alleviates salt induced oxidative stress in wheat. *Original Research Article. Environmental and Experimental Botany.* Vol. 63. (1-3): 224-231.
18. Azooz, M. M. (2004). Proteins, sugars and ion leakage as a selection criterion for the salt tolerance of three sorghum cultivars at seedling stage grow under NaCl and nicotinamide. *International Journal of Agriculture & Biology.* Vol. 6(1): 27-35.
19. Babu, M. A.; Singh, D.; Gothandam, K. M. (2012). The effect of salinity on growth, hormones and mineral elements in leaf and fruit of tomato cultivar pkm1. *The Journal of Animal & Plant Sciences.* Vol. 22(1): 159-164.
20. Balibrea, M. E.; Cuartero, J.; Bolarín, M. C.; Pérez-Alfocea, F. (2003). Sucrolytic activities during fruit development of *Lycopersicon* genotypes differing in tolerance to salinity. *Physiol. Plant.* Vol. 118: 38-46.
21. Barakat, H. (2003). Interactive effects of salinity and certain vitamin on gene expression and cell division. *Int. J. Agric. Biol.* Vol. 3: 219-225.
22. Bartels, D.; Sunkar, R. (2005). Drought and salt tolerance in plants. *Critical Reviews in Plant Sciences.* Vol.24: 23-58.
23. Barth, C. ; De Tullio, M.; Conklin, P.L. (2006). The role of ascorbic acid in the control of flowering time and the onset of senescence. *Journal of Experimental Botany.* Vol. 57: 1657-1665.
24. Bassuony, F. M. ; Hassanein, R. A.; Baraka, D. M.; Khalil, R. R. (2008). Physiological Effects of Nicotinamide and Ascorbic Acid on *Zea mays* Plant Grown Under Salinity Stress II- Changes in Nitrogen Constituents, Protein Profiles, Protease Enzyme and Certain Inorganic Cations. *Australian Journal of Basic and Applied Sciences.* Vol.2(3): 350-359.
25. Bates, I. S.; Waldern, R. P.; Teare, I. D. (1973). Rapid determination of free proline for water stress studies. *Plant and Soil.* Vol. 39: 205-207.
26. Beltagi, M. S. (2008). Exogenous ascorbic acid (vitamin C) induced anabolic changes for salt tolerance in chick pea (*Cicer arietinum* L.) plants. *African Journal of Plant Science.* Vol. 2 (10): 118-123.
27. Ben Ahamed, H.; Sayeh, I.; Manna, A.; Ghidhaoui, J.; Zid, E. (2009). Varietal differences in salinity tolerance and mineral nutrition in tomatoes (*Solanum lycopersicum*). UC Davis: The Proceedings of the International Plant Nutrition Colloquium XVI. <http://escholarship.org/uc/item/65b1n6h9>.
28. Beutner, S.; Bloedorn, B.; Frixel, S.; Blanco, I. H.; Hoffman, T.; Martin, H. (2001). Quantitative assessment of antioxidant properties of natural colorants and phytochemicals: carotenoids, flavonoids, phenols and indigoids. The role of β -carotene in antioxidant functions. *Journal of Science and Food Agric.* Vol. 81: 559-568.
29. Bliss, C. I. (1973). *Plant Protection.* No. 12. Leningrad.
30. Cakmak, I.; Horst, W.J. (1991). Effect of aluminum on lipid peroxidation, superoxide dismutase, catalase and peroxidase activities in root tips of soybean (*Glycine max*). *Plant Physiol.*, 83: 463-468.
31. Campbell, G.S. (1977). An introduction to environmental biophysics. Springer-Verlag, N.Y., USA.
32. Chookhampaeng, S. (2011). The effect of salt stress on growth, chlorophyll content proline content and antioxidative enzymes of pepper (*Capsicum Annuum* L.) seedling. *European Journal of Scientific.* Vol.49, No.1: 103-109.
33. Crowe, J. H.; Carpenter, J. F.; Crowe, L. M.; Anchordouguy, T. J. (2002). Are freezing and dehydration similar stress vectors A comparison of models interaction of stabilizing solutes with biomolecules. *Cryobiol.* Vol. 27: 219- 231.
34. Dasgan, H. Y.; Kusvuran, S.; Abak, K.; Leport, L.; Larher, F.; Bouchereau, A. (2009). The relationship between citrulline accumulation and salt tolerance during the vegetative growth of melon (*Cucumis melo* L.). *Plant, Soil and Environment.* Vol. 55: 51-57.
35. Dhindsa, R.A.; Dhindsa, P.P.; Thorpe, T.A. (1981). Leaf senescence correlated with increased permeability and lipid peroxidation and decreased levels of superoxide dismutase and catalase. *J. Exp. Bot.*, 126: 93-101.
36. Dolatabadian, A.; Sanavy, S. A. M. M.; Chashmi, N. A. (2008). The effects of foliar application of ascorbic acid (vitamin C) on antioxidant enzymes activities, lipid peroxidation and proline accumulation of canola (*Brassica napus* L.) under conditions of salt stress. *J. Agron. Crop Sci.* Vol.194(3): 206-213.
37. Dubey, R. S. And Rani, M. (1989). Influence of NaCl salinity on growth and metabolic status of protein amino acids in rice seedling. *J. Agron and Crop Sci.* 162: PP: [97-106].
38. Ebrahim, M. K. (2005). 'Amelioration of sucrose-metabolism and yield changes, in storage roots of NaCl-stressed sugarbeet, by ascorbic acid'. *Agrochimica.* XLIX (3 - 4): 93-103.
39. Ebrahimian, E.; Bybordi, A. (2012). Effect of salinity, salicylic acid, silicium and ascorbic acid on lipid peroxidation, antioxidant enzyme activity and fatty acid content of sunflower. *African Journal of Agricultural Research.* Vol. 7(25): 3685-3694.
40. Eisa, S. S.; Ali, S. H. (2003). Biochemical, physiological and morphological responses of sugar beet to salinization [online]. Zu finden in <http://geb.uni-giessen.de/geb/volltexte/2003/1235/>
41. Ejaz, B.; Sajid, Z. A.; Aftab, F. (2012). Effect of exogenous application of ascorbic acid on antioxidant enzyme activities, proline contents, and growth

- parameters of *Saccharum* spp. hybrid cv. HSF-240 under salt stress. *Turk. J. Biol.* Vol.36: 630-640
42. Ekmekçi, B. A.; Karaman, M. (2012). Exogenous ascorbic acid increases resistance to salt of *Silybum marianum* (L.). *African Journal of Biotechnology.* Vol. 11(42): 9932-9940.
 43. El-Hariri, D. M.; Sadak, M. S.; El-Bassiouny, H. M. S. (2010). Response of flax cultivars to ascorbic acid and A-Tocopherol under salinity stress conditions. *International Journal of Academic Research.* Vol. 2(6): 101-109.
 44. El-Kobisy, D. S, K. A. Kady, R. A. Medani and R. A. Agamy. (2005). Response of pea plant *pisum sativum* L. to treatment with ascorbic acid. *Egypt. J. Appl. Sci.* 20: 36-50.
 45. El-Tayeb, M. A. (1995). 'Effect of thiamin seed presoaking on the physiology of *Sorghum bicolor* plants grown under salinity stress'. *Egypt Journal of Bot.* 35(2): 201-214.
 46. Epestein, E. (1972). Mineral nutrition of Plant. Principles and Perspectives. John Wiley and Son. Inc. London, 412pp.
 47. Eryilmaz, F. (2007). The relationships between salt stress and anthocyanin content in higher plants. *Biotechnol. Biotechnol., Eq.* Vol. 20 (1): 47-52.
 48. FAO (2004). *Crop Description and Climate*. Available descript [24 January 2005]. <http://www.fao.org/ag/agl/aglw/cropwater/tomato.stm#>
 49. Farahat, M. M.; Azza, A. M.; Mona, H. M.; Sahar, M. Z. (2013). Salt Tolerance in *Grevillea robusta* Seedlings via Foliar Application of Ascorbic Acid. *Middle-East Journal of Scientific Research.* Vol. 14 (1): 09-15.
 50. Farouk, S. (2011). Ascorbic Acid and α -Tocopherol Minimize Salt-Induced Wheat Leaf Senescence. *Journal of Stress Physiology & Biochemistry.* Vol. 7 No. (3): 58-79.
 51. Flores, P.; Carvajal, M.; Cerda, A.; Marinez, V. (2001). Salinity and Ammonium/Nitrate Interactions on Tomato Plant Development, Nutrition and Metabolites. *Journal of Plant Nutrition.* Vol. 24: 1561-1573.
 52. Frary, A.; Gol, D.; Keles, D.; Okmen, B.; Pinar, H.; Sigva, H. O. (2010). Salt tolerance in *Solanum pennellii*: antioxidant response and related QTL. *BMC. Plant Biol.* 10:58.
 53. Gama P. B. S.; Inanaga, S.; Tanaka, K.; Nakazawa, R. (2007). Physiological response of common bean (*Phaseolus Vulg. L.*) seedlings to salinity stress. *African J. of Biotechnology.* Vol.6 (2): 79-88.
 54. Gapinska, M.; Sklodowska, M.; Gabara, B. (2008). Effect of short and long –term salinity on the activities of antioxidative enzymes and lipid peroxidation in tomato roots. *Acta Physiol Plant.* Vol. 30: 11-18.
 55. Ghoulam, C.; Foursy, A.; Fares, K. (2002). Effects of salt stress on growth, inorganic ions and proline accumulation in relation to osmotic adjustment in five sugar beet cultivars. *Environmental and Experimental Botany.* Vol. 47: 39–50.
 56. Gossett, D. R.; Millhollon, E. P.; Cran-Lucas, M. (1994). Antioxidant response to NaCl stress in salt-tolerant and salt-sensitive cultivars of cotton. *Crop Science.*, Vol.34: 706–714.
 57. Greenway, H. And Munns, R. (1980). Mechanisms of salt tolerance in nonhalophytes. *Ann Rev. Plant Physiol* 31: PP: [149-190].
 58. Greenway, H. And Munns, R. (1983). Interaction between growth, uptake of Cl and Na and water relations of plants in saline environment. II. Highly vacuolated cells. *Plant Cell Environ* 6: PP: [575-589].
 59. Haeder, H.E.; Beringer, H. (1981). Influence of potassium nutrition and water stress on the content of abscisic acid in grains and flag leaves of wheat during grain development. *J. Sci. Food Agric.*, 32: 552–556.
 60. Halmer, P. (2004). Methods to improve seed performance in the field. In: *Handbook of seed physiology; Application to agriculture.* (Eds.): R.L. Benech-Arnold and R.A. Sanchez. The Haworth Press. New York. pp 125-165.
 61. Harborne, J. B. (1988). Introduction to Ecological Biochemistry. Academic Press, San Diego, USA PP: [26-31].
 62. Hasegawa, P. M.; Bressan, R. A.; Zhu, J. K.; Bohnert, H. J. (2000). Plant cellular and molecular response to high salinity. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* Vol. 51: 463- 499.
 63. Hassanein, R. A.; Bassuony, F. M.; Baraka, D. M.; Khalil, R. R. (2009). Physiological effects of nicotinamide and ascorbic acid on *Zea mays L.* plant grown under salinity stress. i-changes in growth, some relevant metabolic activities and oxidative defense systems. *Research Journal of Agriculture and Biological Sciences.* Vol.5(1): 72-81.
 64. Hewitt, E. J. (1952). Sand and water culture methods used in the study of plant nutrition. Commonwealth. Bureau of Horticulture and Plantation Crop. Tech. Communications. No. 22, England.
 65. Hogland, D. R.; Arnon, I. I. (1950). The water culture method for growing plants without soil. *Journal Circular. California Agricultural Experiment Station.* Vol. 347 No. 2nd edit pp. 32.
 66. Hussein, M. M.; Abd El-Rheem, K. H. M.; Khaled, S. M.; Youssef, R. A. (2011). Growth and Nutrients Status of Wheat as Affected by Ascorbic Acid and Water Salinity. *Nature and Science.* Vol. 9 (10): 64-69 <http://www.sciencepub.net/nature>.
 67. Jackson, W. A. and Thomas, G. W. (1960): Effect of KCl and Dolomitic limestone on growth and ion up take of sweet potato. *Soil. Science.* Vol. 89: 347-352.
 68. Jiang, J.; Li, T.; Lu, S., Liu, S. (2007). The relationship between the added NaCl of different concentration and the development of tomato fruits under the soilless culture. *Northern Hortic.* Vol. 7: 49-51.

69. Johnson, C. M.; Ulrich, A. (1959). Analytical methods for use in plant analysis. U.S. Dept. Agric. Calif., Univ., Agric. Inform. Bull, 766.
70. Kapoor, K.; Srivastava, A. (2010). Assessment of salinity tolerance of *Vinga mungo* var. Pu-19 using ex vitro and in vitro methods. *Asian J. Biotechnol.* Vol. 2 (2): 73–85.
71. Khafagy, M. A.; Arafa, A. A.; El-Banna, M. F. (2009). Glycine betaine and ascorbic acid can alleviate the harmful effects of NaCl salinity in sweet pepper. *Australian Journal of Crop Science.* Vol. 3(5): 257-267
72. Khajeh-hosseini, M.; Powell, A. A.; Bingham, I. J. (2003). The interaction between salinity stress and seed vigour during germination of soybean seeds. *Seed Sci. & Technol.* Vol. 31: 715-725.
73. Khan, F. A.; A. K. Zaidi. (1985). On optimizing growth and yield of mustard by pre-sowing seed treatment with pyridoxine. *Indian Bot. Soc.* 64: 91-94.
74. Khan, M. H.; Singha, K. L. B.; Panda, S. K. (2002). Changes in antioxidant levels in *Oryza sativa* L. roots subjected to NaCl-salinity stress. *Acta Physiol Plant.* Vol. 24: 145–148.
75. Khan, M. A.; Ahmed, M. Z. and Hameed, A. (2006a): Effect of sea salt and L-ascorbic acid on the seed germination of halophytes. *J. Aird Environ.* Vol. 67:pp 535-540.
76. Khan, M. A.; Ahmad, M. S. A.; Habib-Ur-Rehman, A. and Ashraf, M. (2006b). Interactive Effect Of Foliar Applied Ascorbic Acid And Salt Stress On Wheat (*Triticum Aestivum* L.) At The Seedling Stage. *Pak. J. Bot.* Vol.38(5): 1407-1414.
77. Khan, N. A.; Samiullah, S. S.; Nazar, R. (2007). Activities of antioxidative enzymes, sulphur assimilation, photosynthetic activity and growth of wheat (*Triticum aestivum*) cultivars differing in yield potential under cadmium stress. *J. Agron Crop Sci.* Vol.193: 435-444.
78. Khosravinejad, F.; Heydari, R.; Farboodnia, T. (2008). Effect of salinity on photosynthetic pigments, respiration and water content in barley varieties. *Pakistan Journal of Biological Sciences.* Vol. 11: 2438-2442.
79. Larsen, P.; Harbo, A.; Klungron, S.; Ashein, T.A. (1962). On the biosynthesis of some indole compounds in *Acetobacter xylinum*. *Physiol. Plant.*, 15: 552-565.
80. Lopez-Munguia, A.; Hernandez-Romero, Y.; Pedraza-Chaverri, J.; Miranda-Molina, A.; Regla, I.; Martinez, A.; Castillo, E. (2011). study of their free radical scavenger mechanism. *PLoS One* Vol. 6 (6): 201-215.
81. Loukehaich, R. ; Elyachioui, M.; Belhabib, N.; Douira, A. (2011). Identifying multiple physiological responses associated with salinity tolerance for evaluating three tomato cultivars selected from Moroccan territory. *J. Anim. Plant Sci.* Vol. 21(1) : 1219-1231.
82. Lowry, O. H.; Rosebrough, N. J.; Farr, A. L.; Randall, R. J. (1951). Protein measurement with the Folin phenol reagent. *Journal of Biol. Chem.* Vol. 193: 265–275.
83. Macheix, J. J.; Quessada, M. P. (1984). Caractérisation d'une peroxydase impliquée spécifiquement dans la lignification, en relation avec l'incompatibilité au greffage chez l'abricotier. *Physiologie Végétale*, 22: 533–540.
84. Mackinney, G. (1941). Absorption of light by chlorophyll solutions. *Journal of Biological Chemistry*, 140: 315 – 322.
85. Maggio, A.; Miyazaki, S.; Veronese, P.; Fujita, T.; Ibeas, J. I.; Damsz, B.; Narasiman, M. L.; Hasegawa, P. M.; Joly, R. J.; Bressan, R. A. (2002): Does praline accumulation play an active role in stress induced growth reduction. *Plant J.* Vol. 31: 699-712.
86. Malik, C.P.; Singh, M.B. (1980). *Plant Enzymology and Histoe-nzymology*. Kalyani Publishers. New Delhi. Marinkovic B., Ilin Z., Marinkovic J., Culibrk M., Jacimovic electromagnetic field. *Biophysics in agriculture production*. University of Novi Sad, Tomograf.
87. Mandhania, S.; Madan,S.; Sawhney,V. (2012). Antioxidant defense mechanism under salt stress in wheat seedlings. *Biol Plant.* Vol. 50: 227–231.
88. Marschner, H. (1995). *Mineral Nutrition of Higher Plants*. Academic Press, San Diego, p. 889.
89. Matoo, A.; Handa, A. (2008). Higher polyamines restore and invigorate metabolic memory in ripening fruit. *Plant Sci.* Vol. 174: 386-393.
90. McKersie, B. D.; Bowley, S. R.; Jones, K. S. (1999). Winter survival of transgenic alfalfa over expressing superoxide dismutase. *Plant Physiology.* Vol. 119: 839–847.
91. McLaughlin, S. B.; Wimmer, R. (1999). *Transley Review No.104- Calcium physiology terrestrial ecosystem processes*. *New Phytol.* Vol.142: 373–417.
92. Metzner, H.; Rau, H.; Senger, H. (1965). Untersuchungen zur synchronisier-barkeit ein zelner pigmentmangei Mutanten von Chlorella. *Planta.* Vol. 65: 186-194
93. Miller, R.O. (1998). Nitric-perchloric acid wet digestion in an open vessel, *Handbook of Reference Methods for Plant Analysis*, Kalra, Y.P., Ed., Boca Raton: CRC, 57–61.
94. Mittler, R. (2002). Oxidative stress, antioxidants and stress tolerance. *Trends in Plant Science.* Vol.7 (9): 405-410.
95. Mittova, V.; Ta, M.; Volokita, M.; Guy, M. (2003). Upregulation of the leaf mitochondrial and peroxisomal antioxidative systems in response to salt-induced oxidative stress in the wild salt-tolerant tomato species *Lycopersicon pennellii*. *Plant Cell Environ.* Vol. 26: 845–856.
96. Munns, R. (2002a). Comparative physiology of salt and water stress. *Plant, Cell and Environment.* Vol. 25: 239-250.
97. Munns R. (2002b). Salinity, growth and phytohormones. In: Läubli A, Lüttge U. (eds.)

- Salinity: Environment - Plants - Molecules. Kluwer, Academic Publishers Dordrecht. PP 271-290.
98. Munns, R.; Termaat, A. (1986). Whole-plant responses to salinity. *Aust. J. Plant Physiol.* Vol. 13: 143–160.
 99. Murakeozy, E. P.; Nagy, Z.; Duhaze, C.; Bouchereau, A.; Tuba, Z. (2003). Seasonal changes in the levels of compatible osmolytes in three halophytic species of inland saline vegetation in Hungary. *J. Plant Physiol.* Vol.160: 395–401.
 100. Naguib, M. I. (1963). Colorimetric estimation of plant polysaccharides. *Zucker.* Vol. 16 : 15-18.
 101. Naguib, M. I. (1964). Effect of sevin on the carbohydrate and nitrogen metabolism during germination of cotton seeds. *Ind. Journal Exp. Biol.* Vol. 2: 149-152.
 102. Nazarbeygi, E.; Yazdi, H. L. ; Naseri, R.; Soleimani, R. (2011). The effects of different levels of salinity on proline and A- B - chlorophylls in Canola. *American-Eurasian J. Agric. & Environ. Sci.* Vol. 10 (1): 70-74.
 103. Nelson, N. (1944). Aphotometric adaptation for somagi method for determination of glucose. *Journal of Biol. Chem.* Vol.153: 375-380.
 104. Netondo, G. W.; Onyango, J. C.; Beck, E. (2004). Sorghum and Salinity: II. Gas Exchange and Chlorophyll Fluorescence of Sorghum under Salt Stress. *Crop Science* Vol. 44(2): 806-811
 105. Nuez, F.; Prohens, J.; Blanca, J. M. (2004). Relationships, origin, and diversity of Galapagos tomatoes: implications for the conservation of natural populations. *American Journal of Botany.* Vol. 91: 86–99.
 106. Okhovatian-Ardakani, A. R. ; Mehrabian, M.; Dehghani, F.; Akbarzadeh, A. (2010). Salt tolerance evaluation and relative comparison in cuttings of different pomegranate cultivars. *Plant, Soil and Environment.* Vol. 56: 176–185.
 107. Osman, M. E. H., Metzner, H.; Fisher, K. (1982). Effect of nitrate on thylakoid reaction. I- Influence on photosynthetic electron transport. *Phosynthetica*, 16: (1), 7–12.
 108. Palop, S.; Özdikicierler, O.; Köstekli, M.; Escriva, M.; Esteve, M. J.; Frígola, A. (2010). Ascorbic acid in tomatoes during refrigeration storage with absorbing sheet of ethylene. *International Conference on Food Innovation.* 1-4.
 109. Parvaiz, A.; Satyawati, S. (2008). Salt stress and phyto-biochemical responses of plants– a review. *Plant, Soil and Environment.* Vol. 54: 89–99.
 110. Patel, A. D.; Pandey, A. N. (2008). Growth, water status and nutrient accumulation of seedlings of *Holoptelea integrifolia* (Roxb.) Planch in response to soil salinity. *Plant, Soil and Environment.* Vol. 54: 367–373.
 111. Pinheiro, H. A.; Silva, J. V.; Endres, L.; Ferreira, V. M.; Camara, C. A.; Cabral, F. F.; Oliveira, J. F.; De Carvalho, L. W. T.; Santos, J. M.; Santos Filho, B. G. (2008). Leaf gas exchange, chloroplastic pigments and dry matter accumulation in castor bean (*Ricinus communis* L) seedlings subjected to salt stress conditions. *Ind. Crop Prod.* Vol. 27: 385-392.
 112. Piri, M. ; Mahdieh, M. B.; Olfati, J. A.; Peyvast, G. (2009). Germination and seedling development of cucumber are enhanced by priming at low temperature. *Int. J. Veg. Sci.* Vol. 15: 285-292.
 113. Pollard, A. and Wyn Jone, R. G. (1979). Enzymes activities in concentrated solutions of glycinebetaine and other solutes. *Planta* 144: PP: [291-298].
 114. Prochazkova, D.; Sairam, R. K.; Srivastava, G. C.; Singh, D. V. (2001). Oxidative stress and antioxidant activity as the basis of senescence in maize leaves. *Plant Sci.* Vol.161: 765-771.
 115. Richards, L. A. (1954). *Diagnosis and Improvement of saline and alkali soils* U. S. Dept. of Agriculture. Hand book No. 60.
 116. Sairam, R. K.; Rao, K.V.; Srivastava, G. C. (2002). Differential response of wheat genotypes to long term salinity stress in relation to oxidative stress, antioxidant activity and osmolyte concentration. *Plant Science.* Vol.163: 1037-1046.
 117. Sairam, R. K.; Srivastava, G.C.; Agarwal, S.; Meena, R. C. (2005). Differences in antioxidant activity in response to salinity stress in tolerant and susceptible wheat genotypes. *Biologia Plantarum.* Vol. 49: 85-91.
 118. Salter, J.; Morris, K.; Bailey, P. C. E.; Boon, P. I. (2007). Interactive effects of salinity and water depth on the growth of *Melaleuca ericifolia* Sm. (*Swamp paperbark*) seedlings. *Aquat. Bot.* Vol. 86: 213-222.
 119. Samiullah, S. A.; Afridi, M. M. (1988). B- vitamins in relation to crop productivity. *Indian Rev. Life Sci.* 8: 51-74.
 120. Sekine, T. Sasakawa, Morita, S., Kimara, T.; Kuratomi, K. (1972). Cf. *Laboratory Manual for Physiological studies of Rice* Eds. Yashida, S.; Feno, D.; Cook, J. N.; Gomez, K. A. Publ. by International Rice Research Institute Manila.
 121. Serrano, R.; Mulet, J. M.; Rios, G.; Marquez, J. A.; De Larriona, I. F.; Leube, M. P.; Mendizabal, I.; Pascual-Ahuir, A.; Proft, M.; Ros, R.; Montesinos, C. (1999). A glimpse of the mechanisms of ion homeostasis during salt stress. *J. Exp. Bot.* Vol. 50: 1023– 1036.
 122. Shabrangi, A.; Majd, A. (2009). Effect of magnetic fields on growth and antioxidant systems in agricultural plants. *PIERS Proceedings, Beijing, China, March, 23-27.*
 123. Shaddad, M. A.; Radi, A. F.; Abdel-Rahman, A. M. and Azooz, M. M. (1990): Response of seeds of *lupines termis* and *Vicia faba* to the interactive effect of salinity and ascorbic acid or pyridoxine. *Plant and Soil.* Vol. 122: pp177-183.
 124. Shah, S. H. (2007). Effects of salt stress on mustard as affected by gibberellic acid application. *Gen Appl Plant Physiol.* Vol.33: 97–106.
 125. Shalata, A. and Neumann, P. M. (2001): Exogenous ascorbic acid (vitamin C) increases resistance to salt stress reduces lipid peroxidation. *Journal of Experimental Botany.* Vol. 52: pp 2207-2211.

126. Shalata, A.; Neumann, P. M. (2001). Exogenous ascorbic acid (vitamin C) increases resistance to salt stress reduces lipid peroxidation. *Journal of Experimental Botany*. Vol. 52: 2207-2211.
127. Shalata, A.; Mittova, V.; Volokita, M.; Guy, M.; Tal, M. (2001). Response of the cultivated tomato and its wild salt-tolerant relative *Lycopersicon pennellii* to salt-dependent oxidative stress: The root antioxidative system. *Physiol. Plant*. Vol.112: 487-494.
128. Shao, H. B.; Chu, L. Y.; Zhao, H. L.; Kang, C. (2008). Primary antioxidant free radical scavenging and redox signaling pathways in higher plant cells. *Int. J. Biol.Sci*. Vol. 4 (1): 8-14.
129. Shi, H. X.; Tadashi, L. (2001). Influence of Different Concentrations of Nutrient Solution and Salt Supplement on Tomato Fruit Quality and Yield. *China Vegetables*. Vol. 4: 9-11.
130. Slatyer, R. O. (1957). The influence of progressive increases in total soil moisture stress on transpiration, growth and internal water relationships of plants. *Aust. J. Biot. Sa*. Vol. 10: 320-336.
131. Smimoff, N.; Conklin, P.; Loewus, F. A. (2001). Biosynthesis of ascorbic acid in plants: a renaissance. *Ann. Rev. Plant Physiol. Plant Mol. Biol*. 52: 437-440.
132. Smirnoff, N. (1996). Botanical Briefing: The function and metabolism of ascorbic acid in plants. *Annals of Botany*. Vol. 78: 661-669.
133. Smirnoff, N.; Wheeler, G. L. (2000). Ascorbic acid in plants: biosynthesis and function. *Critical Reviews in Biochemistry and Molecular Biology*. Vol. 35(4): 291-314.
134. Snedecor, G. W.; Cochran, W. B. (1967). *Statistical Methods*. 6th Ed. Iowa State University Press.
135. Soltani Nezhad, F.; Ehsanpour, A.A.; Hosseini, S.M. (2011). Effect of salt stress on acid phosphatase activity and phosphorus content of *lycopersicon peruvianum* L. under in vitro culture. *Malays. Appl. Biol*. Vol. 40(1): 7-11.
136. Stewart, G. R. and Lee, J. A. (1974). The role of proline accumulation in halophytes. *Planta* 120: PP: 279-289
137. Sumalan, R.; Carmen, D. (2002): *Fiziologie vegetala-lucrari practice*, Editura Marineasa, Timisoara. p.106.
138. Summart, J.; Thanonkeo, P.; Panichajakul, S.; Prathepha, P.; McManus, M. T. (2010). Effect of salt stress on growth, inorganic ion and proline accumulation in Thai aromatic rice, Khao Dawk Mali 105, callus culture. *African Journal of Biotechnology*. Vol. 9 (2): 145-152.
139. Taffouo, V. D.; Wamba, O. F.; Yombi, E.; Nono, G.V.; Akoa, A. (2010a). Growth, yield, water status and ionic distribution response of three bambara groundnut (*Vigna subterranean* (L.) verdc.) landraces grown under saline conditions. *Int. J. Bot*. Vol. 6 (1): 53-58.
140. Taffouo, V. D.; Nouck, A. H.; Dibong, S. D.; Amougou, A. (2010b). Effects of salinity stress on seedling growth, numeral nutrients, and total chlorophyll of some tomato (*Lycopersicum esculentum*, L.) cultivars. *African Journal of Biotechnology*. Vol. 9 (33): 5366-5372.
141. Talaat, N. B. (2003). Physiological studies on the effect of salinity, ascorbic acid and putrescine of sweet pepper plant. Ph. D. thesis, Fac. Of Agric., Cairo, Univ., Egypt.
142. Tejera, N. A.; Campos, R.;Sanjuan, J.; Lluch, C. (2004). Nitrogenase and antioxidant enzyme activities in *Phaseolus vulgaris* nodules formed by *Rhizobium tropici* isogenic strains with varying tolerance to salt stress. *Journal of Plant Physiology*. Vol.161:329-338.
143. Traber, M. G.; Stevens, J. F. (2011). Vitamins C and E: Beneficial effects from a mechanistic perspective. *Free Radical Biology Medicine*. Vol. 51(5): 1000-1013.
144. Weatherly, P. E. (1950). Studies in the water relation of cotton plants. I- The field measurement of water deficits in leaves. *New Phytol*. Vol. 49:81-97.
145. Weatherly, P. E.; Barr, C. (1962). Examination of the relative turgidity technique for estimating water deficits in leaves. *Aust. J. Biol. Sci*. Vol.15(3):413-418.
146. Xiong, L.; Zhu, J. K. (2002). Molecular and genetic aspects of plant responses to osmotic stress. *Plant, Cell and Env*. Vol. 25:131-139.
147. Ya, P. L.; Tunekazu, T. (1966). An improved colorimetric determination of amino acids with the use of ninhydrin. *Anal. Biochem.*, 14 : 71 - 77.
148. Yang, S. L.; Lan, S.S.; Gong, M. (2009): Hydrogen peroxide-induced proline and metabolic pathway of its accumulation in maize seedlings. *J. Plant Physiol*. Vol.166: 1694- 1699.
149. Zhu, J.K. (2000). Genetic analysis of plant salt tolerance using *Arabidopsis*. *Plant Physiol*. Vol. 124: 941-948.
150. Zhu, J.K. (2001). Plant salt tolerance. *Trends in Plant Science*. Vol. 6: 66-71.
151. Zidan, M. A. (1991). Alleviation of salinity stress on growth and related parameters in wheat sprayed with thiamine, nicotinic acid, or pyridoxine. *Arab Gulf J. Scient. Res*. 9: 103-117.