

FOLIAR APPLICATION OF ASCORBIC ACID MITIGATES SODIUM CHLORIDE INDUCED STRESS IN EGGPLANT (*SOLANUM MELONGENA* L.)

SAMIN JAN¹, MUHAMMAD HAMAYUN², SHER WALI^{1*}, ASMAT BIBI²,
HUMAIRA GUL² AND FAZLI RAHIM³

¹Department of Botany, Islamia College, Peshawar, Pakistan

²Department of Botany, Abdul Wali Khan University, Mardan, Pakistan

³Department of Botany, Bacha Khan University, Charsadda, Pakistan

*Corresponding author's email: sherwali@icp.edu.pk; Ph: +92-314-9198098

Abstract

The current work was designed to test the effect of sodium chloride on germination, seedling establishment, vegetative growth, yield, chemical contents and ionic composition of eggplant. The consequences of foliar application of ascorbic acid (AA) on mitigation of adverse effects of sodium chloride were also tested. The seeds of *Solanum melongena* were germinated using NaCl (60 mM, 100 mM) and ascorbic acid (100 and 200 mM). High levels of salinity significantly affected the seed germination and seedling fresh and dry weights. Plants grown under salinity stress with foliar application of ascorbic acid showed significant increase in germination percentage and seedlings growth as compare to control plants. Sodium chloride stress showed adverse effects on plant height, root length, number of leaves, leaf area, fresh and dry biomass, total chlorophyll, carbohydrates and proteins as compared to untreated plants. The relative water content, electrolyte leakage were increased and Na⁺ and K⁺ ions balance was disturbed in different plant parts. Ascorbic acid (100 and 200ppm) enhanced all the growth parameters affected adversely by sodium chloride stress.

Key words: Foliar application, Mitigate, Eggplant, Ascorbic acid.

Introduction

Abiotic stressors are most harmful when present in combinations (Mittler, 2002). Abiotic stresses are of many forms. Some abiotic stress factors are most common and easiest to be identified; many others less recognizable are there affecting environment constantly. The basic most stressors include: extreme temperatures, high winds, flood, drought, and other natural disasters including tornados and wildfires. The lesser known stressors occur generally on a small scale and are less noticeable. These stressors include high radiation, poor edaphic conditions, compaction, contamination and quick rehydration of germinating seeds (Palta & Karim, 2006). Salinity becomes a serious concern when soluble salts occur in excessive concentrations of in the soil or water. NaCl is the most common salt causing salinity stress (Ghazi & Al-Karaki, 2006). Soil salinization is one among the major factors limiting crop production especially in arid and semi-arid conditions. More than 60,00,000 hectares irrigated land has been damaged by salinization (Cuartero & Fernandez-Munoz, 1999; Mekhaldi *et al.*, 2008). *Solanum melongena* belongs to family Solonaceae. The leaves and young stems are usually eaten as vegetable when cooked or steamed. The unripe fruits are eaten which are roasted in curries and the ripe fruits are cooked edible. Aubergine is a hardy crop that yields well under stress conditions, including drought. Productivity increased from 12.6 tons per hectare in 1987-88 to 16.5 tons per hectare in 2005-06. *S. melongena* is used in medicines to cure asthma, diabetes, bronchitis, cholera and dysuria. Leaves both fresh and dry and fruit are considered effective in reducing cholesterol level of the blood (Sutarno *et al.*, 1994). Eggplant has solanine which is a glycol-alkaloid poison. It occurs naturally in parts of the plant including leaves, fruits, and tubers. Solanine has fungicidal and pesticidal properties which are the plant's natural defense mechanisms. Solanine has sedative and anticonvulsant properties and has been used as a treatment of bronchial asthma, cough and cold (Singh & Rai, 2005).

Materials and Methods

Germination studies: For the current work seeds of *Solanum melongena* were obtained from the Nursery of Mardan. Seeds were surface sterilized by soaking in 0.1% HgCl₂ for 3 minutes, followed by thoroughly rinsing in distilled water. The sterilized seeds were germinated using different concentrations of NaCl and Ascorbic acid. Different concentrations of sodium chloride, control (non-saline), 60mM NaCl (EC=8.5mS/cm) and 100mM (EC=11.73mS/cm) were selected as stress concentrations. The seeds were divided in three equal parts. Each part was separately soaked for 2 hours in (i) distilled water (control), (ii) 100ppm ascorbic acid and (iii) 200ppm ascorbic acid. From every treatment 10 seeds were selected and placed in separate Petri-plates (15 cm diameter) having filter papers (Whatman No. 1) with each concentration of NaCl. The seeds were germinated in dark at 28 ± 1°C. The number of seeds germinated was recorded 24 and 36 hours after transfer into petriplates. On the 9th day, seedling growth in terms of length, fresh and dry weights was recorded. For dry weight measurement, seedlings were dried at 70°C for 24 hours.

Growth experiment: Sterilized seeds having uniform size were washed with distilled water and sown for studying various growth parameters. The application of the treatments was done in three following sets.

Set I= Different NaCl concentrations without ascorbic acid.

Set II= Different NaCl concentrations with 100ppm ascorbic acid applied foliarly.

Set III = Different NaCl concentrations with 200ppm ascorbic acid applied foliarly.

Four Kg soil was taken in earthen pots of uniform size having basal hole for leaching purpose. Three seeds were sown in each pot and at three leaves stage the pots were irrigated with full strength Hoagland solution. Each Set comprised 4 pots with specific treatments. Each pot was irrigated with 1L of tap water/salt solution twice a week. The plants were harvested at maturity (90 days after sowing) to analyze various morpho-physiological, biochemical and yield attributes.

Electrolyte leakage (EL): Electrolyte leakage (EL) was calculated following the method of Lutts *et al.* (2004) with minor modifications. 0.3g Plant material was washed with 15ml of deionized water and incubated for 2 hours at 25°C in test tubes. Initial electrical conductivity of the solution (L_1) was determined. Samples were then autoclaved at 120°C for 20 minutes and final electrical conductivity (L_2) was measured after equilibrium at 25°C. EL was measured using the following formula:

$$EL (\%) = L_1/L_2 * 100$$

Relative water content (RWC): RWC was determined following Mata & Lamattina (2001). Fresh weight of leaves (FW) was measured and leaves were subjected placed in distilled water for two hours and their turgor weight (TW) was measured. Leaf samples were placed in a pre-heated oven at 80°C for 48 hours in order to obtain dry weight (DW). Relative water content (RWC) was calculated using the following formula:

$$RWC (\%) = (FW - DW) / (TW - DW) * 100$$

Estimation of the mineral of vegetative parts: Leaf, stem and root samples were taken at grand period of growth to analyze Na^+ & K^+ cations. 0.5gm of each sample was taken for ash weight after oven drying. 50ml of deionized water was added to ash and dilutions of this mixture were made for the analysis of minerals. Cations concentrations in the samples were measured by PFP1 Flame Photometer.

Chlorophyll content: Chlorophyll content was estimated following the protocol of Maclachlam & Zalick (1963). 0.1g of fresh leaves was macerated with a little sand washed with acid and 3 ml. of 80% acetone. Centrifugation of the mixture was done at 1000rpm for 5 minutes at 15-30°C. The debris was thrice washed with 1 ml. of 80% acetone. The supernatant was pooled and with acetone the volume was made upto 7 ml. Optical density of the solutions was recorded at 480, 510, 663nm and 645nm, on spectrophotometer. Chlorophyll a, b and Carotenoid contents were calculated using the following formula:

$$\text{Chlorophyll 'a' (mg/g fresh weight)} = \frac{12.3D_{663} - 0.86D_{645}}{d \times 1000 \times w} \times V$$

$$\text{Carotenoid (mg/g fresh weight)} = \frac{7.6D_{480} - 1.49D_{510}}{d \times 1000 \times w} \times V$$

$$\text{Chlorophyll 'b' (mg/g fresh weight)} = \frac{12.3D_{663} - 0.86D_{645}}{d \times 1000 \times w} \times V$$

Total carbohydrate content: Total carbohydrate content was calculated through modified anthrone reagent (Fales, 1951). Ethyl alcohol and sulphuric acid were incorporated into the reagent as the colored product stabilized. Anthrone (about 400mg) was dissolved in 200 ml of mixed solution of 95% ethyl alcohol and 85% sulfuric acid by heating. The solution was mixed and cooled now. Modified anthrone reagent, 10 ml, was added to 1 ml of extract solution. The color developed in boiling water bath following Seifter *et al.* (1950). The absorption was reported at 620 nm.

Protein extraction: Protein content was determined using bovine serum albumin as standard (Bradford, 1976; Bollag & Edelstein, 1991). 100 mg leaf samples were homogenized with 3ml extraction buffer (50mM Tris-HCl (pH: 7.5), 2mM EDTA, 1mM 2-Mercaptoethanol and 1mM DTT). Samples were centrifuged at 14000rpm for 25 minutes at 4°C. The supernatants isolated were used for protein assay.

Estimation: 0.5 ml of supernatant was taken and volume was raised to 1.0 ml with phosphate buffer. Bradford reagent (50mL) was added and absorbance at 595nm was recorded against reagent blank. Bovine serum albumin was used as standard and a standard calibration curve was drawn. The standard curve revealed concentrations of proteins in samples.

Experimental design and statistical analysis: Completely randomized Design (CRD) was used with 3 NaCl and 3 ascorbic acid levels in three replicates. Collected data was analyzed statistically by using SPSS for the analysis of variance (ANOVA) and the means compared by Duncan's multiple range test ($p < 0.05$).

Results and Discussion

Germination and seedling stage: High levels of salinity significantly ($p < 0.05$) inhibit the seed germination (Table 1). Inhibition of germination and early seedling growth stages were sensitive to salinity. Soluble salts at high salinity levels significantly suppressed growth in different crop plants (Siddiqui *et al.*, 2008). Seed germination percentage exhibited increase at low level of salinity (60 mM). Growth stimulating effect at low salinity concentrations on plant may have resulted from the enhancing effect of the low concentrations of chloride ions on various physiological processes such as photosynthesis, osmoregulators and enzymatic activities. Chloride acts as a cofactor of a Mn containing O_2 evolving enzyme which is sensitive to NH_2OH (Kelley & Izawa, 1978). High level of salinity exhibited decrease in germination percentage. Duan *et al.* (2007) reported reduced seed germination at high salinity level in *Suaeda salsa*. Seed germination can be affected by Salinity stress by the reduction of water uptake leading to water stress (osmotic effect) caused due to ionic toxicity and/or imbalance, Na^+ and Cl^- ions accumulation and inhibition of the uptake of essential nutrients such as K^+ causing nutritional imbalance or accumulation of all these results (Taamalli *et al.*, 2004).

Table 1. Effect of different concentrations of Ascorbic Acid and sodium chloride on germination, water content and electrolyte leakage of *Solanum melongena*.

| Treatment | | Control | 100ppm | 200ppm |
|------------------------|-------------|---------------------|---------------|---------------|
| | | | Ascorbic acid | Ascorbic acid |
| Germination percentage | Control | | | |
| | Mean | 66.667 | 70.000 | 76.667 |
| | SE | ± 6.666 | ± 10.000 | ± 3.333 |
| | 60 mM NaCl | | | |
| | Mean | 73.333 | 70.000 | 63.333 |
| | SE | ± 3.333 | ± 10.000 | ± 6.666 |
| | 100 mM NaCl | | | |
| | Mean | 63.333 | 83.333 | 46.667 |
| | SE | 3.333 | 3.333 | 12.019 |
| Relative water content | Control | | | |
| | Mean | 52.226a | 41.006a | 65.146a |
| | SE | ± 23.726 | ± 4.620 | ± 4.264 |
| | 60 mM NaCl | | | |
| | Mean | 51.376a | 52.333a | 78.723a |
| | SE | ± 13.502 | ± 6.633 | ± 17.773 |
| | | (-1.627) | (+27.621) | (+20.84) |
| | 100 mM NaCl | | | |
| | Mean | 79.483a | 56.29a | 78.576a |
| | SE | ± 18.348 | ± 9.336 | ± 17.091 |
| | | (+52.189) | (+37.27) | (+20.615) |
| | | LSD _{0.05} | 65.716 | 24.674 |
| Electrolyte leakage | Control | | | |
| | Mean | 27.062a | 59.183a | 41.107a |
| | SE | ± 14.918 | ± 17.711 | ± 4.807 |
| | 60 mM NaCl | | | |
| | Mean | 46.076a | 47.243a | 26.863b |
| | SE | ± 1.458 | ± 1.102 | ± 2.957 |
| | | (+70.262) | (-20.174) | (-34.651) |
| | 100 mM NaCl | | | |
| | Mean | 35.707a | 101.873a | 29.126b |
| | SE | ± 6.83 | ± 46.849 | ± 1.803 |
| | | (+31.945) | (+72.131) | (-29.145) |
| | | LSD _{0.05} | 32.91 | 100.09 |

At seedling stage root and shoot fresh and dry weights increased at low level of salinity and decreased at high levels of salinity (Fig. 1). Similar results were reported for fresh and dry weights due to salinity by Varma (1981) and Savvas & Lenz (2000) on eggplant. A similar effect was also observed in *Zea mays* seedlings as stated by Al-Moaikal (2006). However, increase in fresh and dry weights of eggplant at low salinity levels may be due to more growth at those levels by the uptake of more salts (Basalah 1991).

Plants grown under salinity stress as well as ascorbic acid showed significant ($p < 0.05$) increase in germination percentage and seedlings growth as compare to control plants. Ascorbic acid and salicylic acid application results in a reduction in the harmful effects of salinity stress (Afzal *et al.*, 2006). Addition of ascorbic acid (2.0 mM) improved the germination and growth of the seedlings of *Arabidopsis* in 100 mM NaCl and so reversed the deleterious effects by NaCl stress suggestive of the fact that under NaCl stress changes in the redox state took place and ultimately proved

to be helpful to avoid the formation of reactive oxygen species (ROS) (Borsani *et al.*, 2001).

Growth parameters: Some major abiotic stresses have existed long before agricultural practices were started; salinity is one of these stresses. It has obvious effects on the reduction of agricultural production globally, resulting in increased food problems, especially in developing countries (Munns, 2007; Ashraf *et al.*, 2008). Plant height showed significant reduction when irrigated with different levels of saline water in comparison to control (Fig. 1). Plant height decrease is more pronounced at high salt concentration.) The decrease in plant height at salinity stress may possibly be due to several reasons including reduced photosynthesis with ultimate limited supply of carbohydrate, reduced growth of shoots and roots due to reduction of turgor in expanding tissues caused by lowered water potential (Alam *et al.*, 2004). Imbalance in mineral supply caused by excess or deficiency might have directly affected on growth. This imbalance is induced by changes in concentrations of specific ions in the growth medium (Zhu, 2002).

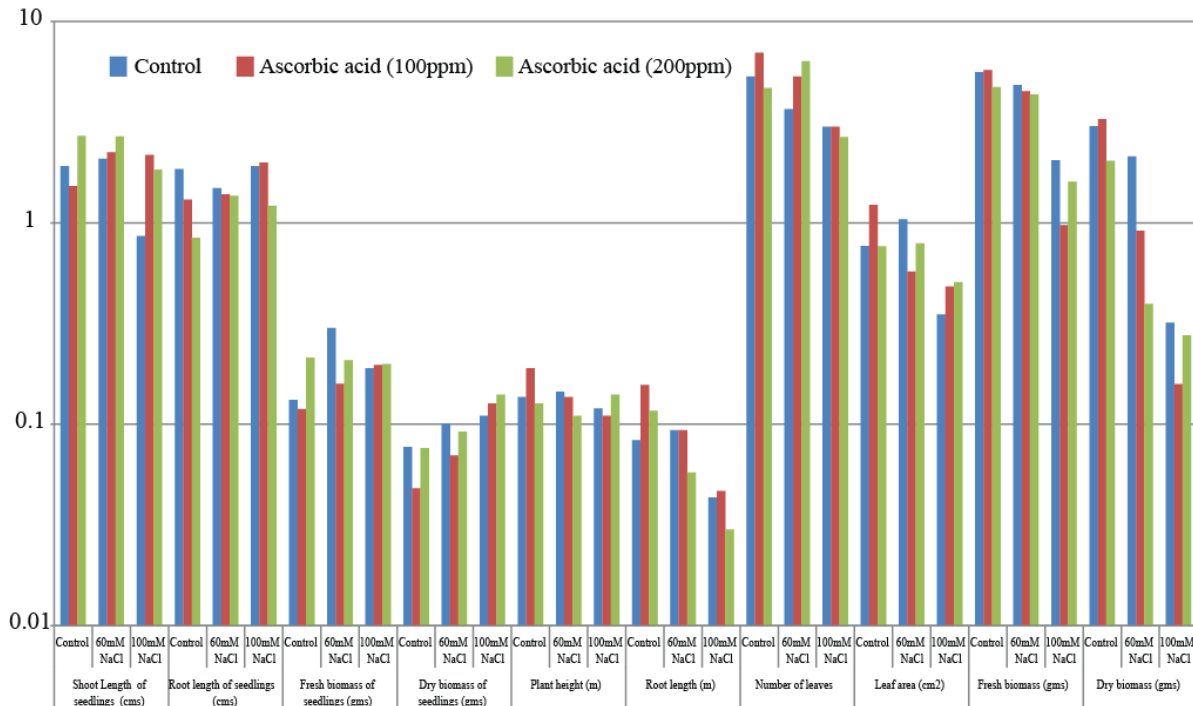


Fig. 1. Effect of different concentrations of sodium chloride and ascorbic acid on various growth parameters of *Solanum melongena*.

Root length showed significant ($p < 0.001$) reduction under different salinity levels (Fig. 1). Game *et al.* (2007) reported reduction in root length as a result of salinity. Significant ($p < 0.05$) reduction in both the number of leaves and average leaf size resulted in reducing total leaf area (Fig. 1). Total leaf area was reduced due to salinity (Gebauer *et al.*, 2004; Ramoliya & Pandey, 2002).

Plants treated with ascorbic acid (100ppm and 200ppm) showed increase in growth parameters (plant height, root length, number of leaves, leaf area) both in saline and non-saline environments as compared to control plants. Ascorbic acid has beneficial effect on root length which can be attributed to the involvement of ascorbic acid in root elongation regulation, cell expansion and cell vacuolation (Smirnov, 1996).

High salt concentrations have adverse effects on fresh and dry biomass of *Solanum melongena* under this study (Fig. 1). Exposure to salinity conditions causes most of the crop plants to suffer and show growth decline which was due to water stress, ion imbalance and toxicities, or all of these (Kurt *et al.*, 1986). The decrease in plant growth due to salt stress may be due to physiological drought caused by low water potential of soil solution and osmotic variations in plants due to increased cellular ionic concentration, resulting to deform the macromolecules by disrupting water associated to them (Schwarz, 1985). The application of ascorbic acid (100 and 200ppm) showed enhancement of growth parameters. The effect of ascorbic acid on plant growth may be due to the substantial role of ascorbic acid in many metabolic and physiological processes and counteracting the adverse effects of salinity (Shadadad *et al.*, 1999). Foliar application of ascorbic acid on lemongrass had positive effect on growth parameters (Tarraf *et al.*, 1999). Beneficial effects of the exogenous application of ascorbic acid in mitigating

partially the adverse effects of salt stress on growth (Ahmed-Hamad & Monsaly, 1998).

Biochemical analysis: Plants treated with different NaCl concentrations exhibited reduction in chlorophyll-a, chlorophyll-b, total chlorophyll and chlorophyll a/b ratio (Table 2). This decrease in chlorophyll contents may have caused by reduced biosynthesis or increase in the degradation of chlorophyll molecules under salinity stress. The breakdown of ultra-structure of chloroplasts may have resulted by the Na^+ toxicity directly or salt induced oxidative damage (Santos, 1998). In addition to this the high salts levels caused the chloroplast structure, number and size to be disturbed affecting total chlorophyll content (El-Banna & Attia, 1999). The chloroplasts are disrupted due to oxidative stresses causing decrease in chlorophyll content and photosynthetic reactions (Rahman *et al.*, 2000). Chlorophyll concentrations are reduced probably by the inhibitory effect of the ions accumulated (Ali, 2004). A decrease in chlorophyll concentration in salinized plants could be attributed to increased activity of the chlorophyll-degrading enzyme chlorophyllase (Reddy & Vora 1986). Plants treated with ascorbic acid (100ppm) exhibited significant ($p < 0.05$) increase in chlorophyll-a, b, total chlorophyll and chlorophyll a/b ratio in saline environment (60mM NaCl) and increased in chlorophyll a, b and total chlorophyll contents in control while plants treated with 200ppm ascorbic acid showed increased in chlorophyll-a, total chlorophyll and chlorophyll a/b ratio in saline environment (60mM NaCl) compared to control. Application of Glycinebetaine (GB) and ascorbic acid at two different levels reduced the ill effects of salt stress on chlorophyll a & b concentrations due the lesser activity of chlorophyllase, an enzyme degrading chlorophylls (Mishra & Sharma, 1994). GB and ascorbic acid decreased

the toxic ions especially sodium concentration, while increased in some ions such as magnesium which needed for chlorophyll synthesis (Shaddad, *et al.*, 1999). The leaf photosynthetic efficiency is increased by high K⁺ concentration possibly by the increase in number of chloroplasts/ cell, number of cells per leaf and hence leaf area (Possingham, 1980).

Foliar application of ascorbic acid in the absence of salinity stress led to increased photosynthesis pigments especially at the higher concentrations (200ppm) (Table 2). This increase appears to be more noticeable on chlorophyll a compared to chlorophyll b and carotenoids. This may be due to modulation of the membrane fluidity in similar manner to cholesterol. Ascorbic acid also play role in membrane permeability to ions & molecules (Foyer & Noctor, 2000), stabilizing membrane structure (Blokhuin *et al.*, 2002) and involvement both in the electron transport of Photosystems and oxidized system of chloroplasts.

Plants treated with different concentrations of NaCl exhibited significant (p<0.05) reduction in carbohydrates (Table 2). The effect of carbohydrates accumulation in photosynthesis is significant in the source leaves but not in young sink leaves (Araya *et al.*, 2006). Plants treated with 100 and 200ppm ascorbic acid showed significant (p<0.05) promotion in carbohydrates in saline media. Similar results are reported on rosemary plant (Youssef & Talaat, 2003).

Plants treated with different concentrations of NaCl exhibited significant (p<0.05) reduction in protein contents, more pronounced at increased salinity level (Table 2). Reduction in protein content under salinity stress may be due to disturbance in nitrogen metabolism or inhibition of nitrate absorption (El-Zeiny *et al.*, 2007). The reduction in protein content of various plant tissues under saline conditions may be due to the inhibition of transamination process, the increase of proteolysis or the decrease of protein synthesis (Baraka, 2008).

One of the early symptoms of salinity stress in plant tissue is the decrease in relative water content (RWC). Plants treated with salinity 60mM NaCl exhibited reduction in relative water content (Table 1). This reduction of RWC in stressed plants may be associated with a decrease in plant vigor and was observed in many plant species (Halder & Burrage 2003; Lopez *et al.*, 2002). Reduction in leaf turgor potential directly affects many important morphological and physiological processes (Jones & Turner, 1978). They reported that although RWC was decreased, leaf osmolality increased the slow development of water deficits resulted not only in osmotic adjustment, but also a decrease in leaf tissue elasticity. Similar trends could be seen in the results of other authors (Alberico & Cramer, 1993; Shalaby *et al.*, 1993).

Table 2. Effect of different concentrations of Ascorbic Acid and sodium chloride on chlorophyll a, b, total chlorophyll, carotenoids, total sugars and proteins of *Solanum melongena*.

| Treatment | Chlorophyll-a | Chlorophyll-b | Total Chlorophyll | a/b Ratio | Carotenoids | Total sugar | Total protein | |
|-----------------------|---------------|---------------|-------------------|------------|---------------|----------------|----------------|-----------|
| | (mg/gm fr.wt) | (mg/gm fr.wt) | (mg/gm fr.wt) | | (mg/gm fr.wt) | (mg/gm dry.wt) | (mg/gm dry.wt) | |
| Without ascorbic acid | Control | | | | | | | |
| | Mean | 9.341a | 0.856a | 10.197a | 12.778a | 0.164a | 2.999a | 0.658a |
| | SE | ± 7.268 | ± 0.140 | ±7.197 | ±10.533 | ±0.016 | ±0.143 | ±0.295 |
| | 60 mM NaCl | | | | | | | |
| | Mean | 1.043a | 1.217ab | 2.262a | 0.791a | 0.215a | 1.514ab | 0.438ab |
| | SE | ± 0.978 | ± 0.101 | ±1.037 | ±0.728 | ±0.003 | ±0.844 | ±0.104 |
| | | (-88.823) | (+42.159) | (-77.822) | (-93.805) | (+30.932) | (-49.506) | (-90.048) |
| | 100 mM NaCl | | | | | | | |
| | Mean | 8.670a | 1.511b | 10.182a | 5.437 | 0.242a | 1.221b | 0.098b |
| | SE | ± 5.407 | ± 0.149 | ±5.489 | ±3.145a | ±0.061 | ±0.007 | ±0.021 |
| | (-7.174) | (+76.436) | (-0.152) | (-57.449) | (+47.47) | (-59.273) | (-97.762) | |
| LSD _{0.05} | 18.201 | 0.458 | 18.425 | 21.99 | 0.085 | 1.71 | 1.71 | |
| 100 ppm ascorbic acid | Control | | | | | | | |
| | Mean | 1.268a | 0.578a | 1.846a | 2.196a | 0.12a | 2.511a | 0.356a |
| | SE | ± 0.220 | ± 0.103 | ±0.324 | ±0.045 | ±0.022 | ±0.214 | ±0.127 |
| | 60 mM NaCl | | | | | | | |
| | Mean | 1.956a | 0.878ab | 2.835a | 2.234a | 0.183a | 2.066a | 0.19a |
| | SE | ± 0.206 | ± 0.106 | ±0.312 | ±0.034 | ±0.024 | ±0.144 | ±0.050 |
| | | (+54.300) | (+52.023) | (+53.587) | (+1.716) | (+53.026) | (-17.732) | (-94.741) |
| | 100 mM NaCl | | | | | | | |
| | Mean | 8.48a | 0.928b | 9.408a | 9.361a | 0.164a | 2.399a | 0.232a |
| | SE | ± 6.292 | ± 0.029 | ±6.278 | ±7.041 | ±0.062 | ±0.025 | ±0.066 |
| | (-568.646) | (+60.550) | (+409.549) | (+326.202) | (+37.111) | (-4.455) | (-93.571) | |
| LSD _{0.05} | 12.587 | 0.302 | 12.575 | 14.067 | 0.141 | 0.519 | 0.305 | |
| 200 ppm ascorbic acid | Control | | | | | | | |
| | Mean | 1.67a | 0.741a | 2.411a | 2.252a | 0.151a | 4.025a | 0.993a |
| | SE | ± 0.373 | 0.164 | 0.538 | 0.01 | 0 | ±0.637 | ±0.223 |
| | 60 mM NaCl | | | | | | | |
| | Mean | 7.445a | 0.872b | 8.318a | 9.269a | 0.224ab | 1.804ab | 0.503a |
| | SE | ± 5.374 | ± 0.070 | ±5.33 | ±7.019 | ±0.018 | ±0.509 | ±0.244 |
| | | (+345.728) | (+17.781) | (+244.94) | (+311.578) | (+48.83) | (-55.157) | (-70.722) |
| | 100 mM NaCl | | | | | | | |
| | Mean | 3.132a | 1.31b | 4.442a | 2.4a | 0.243b | 2.592b | 1.378a |
| | SE | ± 0.123 | ± 0.088 | ±0.212 | ±0.073 | ±0.019 | ±0.015 | ±0.968 |
| | (+87.525) | (+76.775) | (+84.221) | (+6.596) | (+61.207) | (-35.598) | (-19.837) | |
| LSD _{0.05} | 10.766 | 0.399 | 10.711 | 14.025 | 0 | 1.631 | 2.045 | |

Means followed by different letters in the same column differ significantly at 95% probability level according to New Duncan's Multiple Range Test. Figures in parentheses indicate % promotion (+) and reduction (-) over control

The extent of membrane damage by salinity was assessed by an indirect measurement of solute leakage. NaCl stress induced significant increase in electrolyte leakage compared to the control (Table 1). This phenomenon is associated to chain reactions initialized by free radicals (Ghoulam *et al.*, 2002). Lipid peroxidation due to the accumulation of the ROS is the principal cause of membrane damage (Sairam *et al.*, 2005). Maintaining integrity of the cellular membranes under salt stress is considered an integral part of the salinity tolerance mechanism (Stevens *et al.*, 2006). Plants treated with 100ppm ascorbic showed significant ($p < 0.05$) decrease in electrolyte leakage while plants treated with 200ppm ascorbic acid increased in this parameter as compared to control.

Ionic composition: Plants treated with different NaCl concentration exhibited significant ($p < 0.05$) increase in Na^+ concentration in root, stem and leaves, whereas K^+ concentrations and K^+/Na^+ ratio of stem and roots was

decreased (Table 3). NaCl enhances the Na^+ and Cl^- contents and consequently affects the uptake of other minerals elements (Greenway & Munns, 1980). The increase in Na^+ concentrations in plants with salinity may be the result of the ability of plants to use Na^+ to maintain an adequate osmotic adjustment and potential gradient between plant tissues and external solution. Osmotic adjustment is essential for a plant to survive in saline environment (Flowers, 2004). The ions most frequently concerned with plants toxicity are Na^+ and Cl^- , as these are highly soluble in water, readily absorbed and transported to shoots in the transpiration stream and inhibits uptake & transport of K^+ (Hu & Schmidhalter, 1998). High accretion of Na^+ ions in plant roots is probably due to the ability to avoid toxicity of Na^+ , reducing the transport of sodium ions to shoot where these ions may cause ion charge imbalance resulting in specific toxicity and or a regulatory mechanisms in roots that prevent translocation of mineral ions e.g. Na^+ from root to shoot; hence preventing high mobility of Na^+ in phloem (Adams, 1994).

Table 3. Effect of different concentration of Ascorbic Acid and sodium chloride on ionic composition in different plant parts of *Solanum melongena*.

| Treatment | STEM | | | ROOT | | | LEAVES | | |
|-----------------------|---------------|--------------|--------------------------|---------------|--------------|--------------------------|---------------|--------------|--------------------------|
| | Na^+ | K^+ | K^+/Na^+ | Na^+ | K^+ | K^+/Na^+ | Na^+ | K^+ | K^+/Na^+ |
| Control | | | | | | | | | |
| Mean | 254.226a | 200.85a | 0.815a | 371.45a | 221.26a | 0.735a | 137.923a | 294.32a | 2.141a |
| SE | ± 31.839 | ± 6.63 | ± 0.104 | ± 119.772 | ± 50.167 | ± 0.303 | ± 7.973 | ± 25.816 | ± 0.202 |
| 60 mM NaCl | | | | | | | | | |
| Mean | 296.93a | 221.78a | 0.763ab | 420.823ab | 123.5a | 0.293a | 154.253b | 345.54a | 2.276a |
| SE | ± 41.952 | ± 52.165 | ± 0.202 | ± 41.279 | ± 27.367 | ± 0.053 | ± 13.713 | ± 18.562 | ± 0.25 |
| | (+16.797) | (+10.42) | (-6.481) | (+13.292) | (-44.183) | (-60.062) | (+11.839) | (+17.402) | (+6.299) |
| 100 mM NaCl | | | | | | | | | |
| Mean | 371.91a | 111.28a | 0.31b | 633.803b | 176.93a | 0.28a | 314.41b | 324.48a | 1.035b |
| SE | ± 36.663 | ± 11.607 | ± 0.057 | ± 8.062 | ± 45.118 | ± 0.074 | ± 30.919 | ± 37.185 | ± 0.083 |
| | (+46.29) | (-44.595) | (-61.947) | (+70.629) | (-20.035) | (-61.816) | (+127.959) | (+10.247) | (-51.646) |
| LSD _{0.05} | 128.208 | 107.588 | 0.469 | 253.619 | 145.469 | 0.633 | 69.43 | 97.749 | 0.663 |
| Control | | | | | | | | | |
| Mean | 117.913a | 169.39a | 1.478a | 175.26a | 97.89a | 0.59a | 171.12a | 204.36a | 1.19a |
| SE | ± 14.024 | ± 20.02 | ± 0.234 | ± 27.812 | ± 4.348 | ± 0.109 | ± 12.827 | ± 25.131 | ± 0.085 |
| 60 mM NaCl | | | | | | | | | |
| Mean | 284.51a | 59.67b | 0.211b | 435.696b | 118.69b | 0.271b | 288.19a | 147.81a | 0.515b |
| SE | ± 24.363 | ± 2.251 | ± 0.0139 | ± 19.548 | ± 10.133 | ± 0.015 | ± 16.463 | ± 16.812 | ± 0.066 |
| | (+141.287) | (-64.773) | (-85.658) | (+148.6) | (+21.248) | (-54.004) | (+68.413) | (-27.671) | (-56.662) |
| 100 ppm Ascorbic Acid | | | | | | | | | |
| Mean | 346.84b | 67.6b | 0.204b | 679.726c | 223.21b | 0.331b | 326.6b | 137.8a | 0.432b |
| SE | ± 24.363 | ± 2.251 | ± 0.013 | ± 19.548 | ± 10.133 | ± 0.015 | ± 16.463 | ± 16.812 | ± 0.066 |
| | (+194.148) | (-60.092) | (-86.134) | (+287.839) | (+128.021) | (-43.845) | (+90.86) | (-32.569) | (-63.625) |
| LSD _{0.05} | 103.076 | 43.489 | 0.474 | 154.719 | 59.874 | 0.234 | 104.751 | 71.04 | 0.256 |
| Control | | | | | | | | | |
| Mean | 253.23a | 126.1a | 0.512a | 226.55a | 217.62a | 0.962a | 203.243a | 134.55a | 0.661a |
| SE | ± 18.506 | ± 24.159 | ± 0.125 | ± 11.96 | ± 21.827 | ± 0.093 | ± 3.373 | ± 10.335 | ± 0.043 |
| 60 mM NaCl | | | | | | | | | |
| Mean | 266.493a | 141.18a | 0.541a | 505.233a | 223.47a | 0.484b | 309.81b | 118.69a | 0.390b |
| SE | ± 16.713 | ± 18.33 | ± 0.096 | ± 101.754 | ± 9.121 | ± 0.109 | ± 20.01 | ± 20.421 | ± 0.082 |
| | (+5.237) | (+11.958) | (+5.782) | (+123.011) | (+2.688) | (-49.692) | (+52.433) | (-11.787) | (-40.955) |
| 100 mM NaCl | | | | | | | | | |
| Mean | 219.88a | 86.19a | 0.416a | 509.91b | 259.74a | 0.509b | 483.23c | 114.53a | 0.235b |
| SE | ± 16.713 | ± 18.33 | ± 0.096 | ± 101.754 | ± 9.121 | ± 0.109 | ± 20.01 | ± 20.421 | ± 0.082 |
| | (-13.169) | (-31.649) | (-18.692) | (+125.076) | (+19.354) | (-47.057) | (+137.759) | (-14.879) | (-64.307) |
| LSD _{0.05} | 85.992 | 63.362 | 0.36 | 204.694 | 109.247 | 0.3 | 61.699 | 52.228 | 0.188 |

Means followed by different letters in the same column differ significantly at 95% probability level according to New Duncan's Multiple Range Test. Figures in parentheses indicate % promotion (+) and reduction (-) over control.

Plants treated with ascorbic acid (100ppm) exhibited significant ($p < 0.05$) increase in potassium ions concentration in root in both low (60mM) as well as high salinity stress (100mM). Exogenous supply of ascorbic acid results in the protection of sweet pepper plants against salinity stress; it is probably caused indirectly as a result of its effect on K^+ uptake (Tipirdamaz & Cakirlar, 1990; Shawky, 2003).

It is concluded that NaCl had adverse effects on germination and plant growth and these adverse effects of salt stress were significantly improved by exogenous application of AA. The AA application mostly led to a substantial increase in values of almost all the studied growth and biochemical parameters. This study justifies further work on *Solanum melongena* plants under a broader range of field conditions to further evaluate the possibility of using AA for improving the growth of *Solanum melongena*.

References

- Adams, P. 1994. Nutrition of greenhouse vegetables in NFT and hydroponic systems. *Acta Horti.*, 361: 245-257.
- Afzal, I., S.M.A. Basra, A. Hameed and M. Farooq. 2006. Physiological enhancements for alleviation of salt stress in wheat. *Pak. J. Bot.*, 38: 1649-1659.
- Ahmed-Hamad, A.M. and H.M. Monsaly. 1998. Seed soaking pre sowing in vitamins versus the adverse effects of NaCl salinity on photosynthesis and some related activities of maize and sunflower plants. In: *Proceedings of the XIth International Photosynthesis Conference, Budapest, Hungary*, August, pp. 17-22.
- Alam, M.Z., T.R. Stuchbury, E.L. Naylor and M.A. Rashid. 2004. Effect of salinity on growth of some modern rice cultivar. *J. Agron.*, 3(1): 1-10.
- Alberico, G.J. and G.R. Cramer. 1993. Is the salt tolerance of maize related to sodium exclusion? 1. Preliminary screening of seven cultivars. *J. Plant Nutr.*, 16(11): 2289-2303.
- Ali, Y., Z. Aslam, M.Y. Ashraf and G.R. Tahir. 2004. Effect of salinity on chlorophyll concentration, leaf area, yield and yield components of rice genotypes grown under saline environment. *Int. J. Environ. Sci. & Technol.*, 1(3): 221-225.
- Al-Moaikil, R.M.S. 2006. *Physiological effects of gibberellic acid and polyamine(s) on germination and seedling growth of Zea mays stressed with sodium chloride salinity*. Ph.D. Thesis, Botany Dept., Science College for girls, Dammam.
- Araya, T., K. Noguchi and I. Terashima. 2006. Effects of carbohydrates accumulation on photosynthesis different between source and sink leaves *Phaseolus vulgaris*. *Plant Cell Physiol.*, 47-644.
- Ashraf, M., H.R. Athar, P.J.C. Harris and T.R. Kwon. 2008. Some prospective strategies for improving crop salt tolerance. *Adv. Agron.*, 97: 45-110.
- Baraka, D.M. 2008. Osmotic adjustment of wheat grain germination to hyperosmotic saline by nicotine hormone. *Res. J. Agric. and Biol. Sci.*, 4: 824-831.
- Basalah, M.O. 1991. Effect of salinity on seed germination and growth of squash (*Cucurbita pepo* L.) seedlings. *Arab Gulf J. Res.*, 9(2): 87-97.
- Bollag, D.M. and S.J. Edelman. 1991. Immunoblotting. In *Protein Methods*. Wiley-Liss Inc., New York, NY, pp. 181-211.
- Blokhina, O., E. Virolainen and V. Fagersted. 2002. Antioxidants, oxidative damage and oxygen deprivation stress: A review. *Ann. Bot.*, 179-194.
- Borsani, O., V. Valpuesta and M.A. Botella. 2001. Evidence for a role of salicylic acid in the oxidative damage generated by NaCl and osmotic stress in Arabidopsis seedlings. *Plant Physiol.*, 126: 1024-1030.
- Bradford, M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analyt. Biochem.*, 72: 248-254.
- Cuartero, J. and R. Fernandez-Munoz. 1999. Tomato and salinity. *Sci. Hort.*, 78: 83-125.
- Duan, D., W. Li, H. Ouyang and P. An. 2007. Seed germination and seedling growth of *Suaeda salsa* under salt stress. *Ann. Bot. Fennici.*, 44: 161-169.
- El-Banna, Y. and T. Attia. 1999. Root tip meristematic cell and leaf chloroplast structure in three barley (*H. vulgare*, L.) genotypes exposed to salinity stress. *Cytologia*, 64: 69-76.
- El-Zeiny, H.A., L.B. Abou, M.S. Gaballah and S. Khalil. 2007. Antitranspirant application to sesame plant for salinity stress Augmentation. *Res. J. Agric & Biologic. Sci.*, 3: 950-959.
- Fales, F.W. 1951. The assimilation and degradation of carbohydrates of yeast cells. *J. Biological. Chem.*, 193: 113-116.
- Flowers, T.J. 2004. Improving crop salt tolerance. *J. Exp. Bot.*, 55: 307-319.
- Foyer, C.H. and G. Noctor. 2000. Oxygen processing in photosynthesis: regulation and signaling. *New Phytol.*, 146: 359-388.
- Game, P.B.S., S. Inanga, K. Tanaka and R. Nakazawa. 2007. Physiological response of common bean (*Phaseolus vulgaris* L.) seedlings to salinity stress. *Afri. J. Biotec.*, 6(2): 79-80.
- Gebauer, J., K. El-Siddig, A.A. Salih and G. Ebert. 2004. *Tamarindus indica* L. seedlings are moderately salt tolerant when exposed to NaCl-induced salinity. *Scientia Hort.*, 103: 1-8.
- Ghazi, N. and G.N. Al-Karaki. 2006. Nursery inoculation of tomato with arbuscular mycorrhizal fungi and subsequent performance under irrigation with saline water. *Sci Hort.*, 109: 1-7.
- Ghoulam, C., A. Foursy K. Fares. 2002. Effects of salt stress on growth, inorganic ions and proline accumulation in relation to osmotic adjustment in five sugar beet cultivars. *Env. & Exp. Bot.*, 47: 39-50.
- Greenway, H. and K. Munns. 1980. Mechanisms of salt tolerance in non halophytes. *Ann. Rev. Plant Physiol.*, 31: 149-190.
- Halder, K. P. and S.W. Burrage. 2003. Drought stress effects on water relations of rice grown in nutrient film technique. *Pak. J. Biol. Sci.*, 6: 441-446.
- Hu, Y. and U. Schmidhalter. 1998. Spatial distributions and net deposition rates of mineral elements in the elongating wheat (*Triticum aestivum* L.) leaf under saline conditions. *Planta*, 204: 212-219.
- Jones, M.M. and N.C. Turner. 1978. Osmotic adjustment in leaves of *Sorghum* in response to water deficits. *Plant Physiol.*, 61: 122-126.
- Kelley, P.M. and S. Izawa. 1978. The role of chloride ion in photosystem II. I. Effects of chloride ion on photosystem II electron transport and on hydroxylamine inhibition. *Bioch. Biophys Acta.*, 502: 198-210.
- Kurt, E., A. Jensen and E. Epstein. 1986. Resistance of fully imbibed tomato seeds to very high salinities, *Plant Cell Environ.*, 9: 667-676.
- Lopez, A.D., O.B. Ahmad, M. Guillot, B.D. Ferguson, J.A. Salomon, C.J.L. Murray and K.H. Hill. 2002. World mortality in 2000: Life tables for 191 countries. Geneva WHO crop improvement (Eds.): Staples, R. and G.H. Toenniessen, John Wiley, New York, pp. 171-187.

- Lutts, S., M. Almansouri and J.M. Kinet. 2004. Salinity and water stress have contrasting effects on the relationship between growth and cell viability during and after stress exposure in durum wheat callus. *Plant Sci.*, 167: 9-18.
- Machlachlam, S. and S. Zalik. 1963. Plastids structure, chlorophyll concentration and free amino acid composition of chlorophyll mutant of Barley. *Can. J. Bot.*, 41: 1053-1062.
- Mata, C.G. and L. Lamattina. 2001. Nitric oxide induces stomatal closure and enhances the adaptive plant responses against drought stress. *Plant Physiol.*, 126: 1196-1204.
- Mekhaldi, A., M. Benkhalifa and M. Belkhdja. 2008. The effects of salinity on gas exchange on different developmental stages of Mung Bean (*Vigna radiata* L. Wilczek). *Int. J. Bot.*, 4(3): 269-275.
- Mishra, S.N. and I. Sharma. 1994. Putrescine as a growth inducer and as a source nitrogen for mustard seedling under sodium chloride salinity. *Indian Journal of Experimental Physiology*, 32: 916-918.
- Mittler, R. 2002. Oxidative stress, antioxidants and stress tolerance. *Trends Plant Sci.*, 7(9): 405-410.
- Munns, R. 2007. Utilizing genetic resources to enhance productivity of salt-prone land. CAB Rev. Perspectives in Agric. Vet. Sci. Nutr. Nat. Res., 2: No. 009.
- Palta, J.P. and F. Karim. 2006. Methohasds for enhancing plant health, protecting plants from biotic and abiotic stress related injuries and enhancing the recovery of plants injured as a result of such stresses. *United States Patent*, 7101828.
- Possingham, J. V. 1980. Plastid replication and development in higher plants. *Ann. Rev. Plant Physiol.*, 31: 113-129.
- Rahman, M.S., T. Matsumuro, H. Miyake and Y. Takeoka. 2000. Salinity-induced ultra-structural alternations in leaf cells of rice (*Oryza sativa* L.). *Plant Prod. Sci.*, 3: 422-429.
- Ramoliya, P.J. and A.N. Pandey. 2002. Effect of increasing salt concentration on emergence, growth and survival of seedlings of *Salvadora oleoides* (Salvadoraceae). *J. Arid Environ.* 51: 121-132.
- Reddy, M.P. and A.B. Vora. 1986. Changes in pigment composition. Hill reaction activity and saccharides metabolism in bajra (*Penisetum typhoides* S & H) leaves under NaCl salinity. *Photosynthetica.*, 20: 50-55.
- Sairam, R.K., G.C. Srivastava, S. Agarwal and R.C. Meena. 2005. Differences in antioxidant activity in response to salinity stress in tolerant and susceptible wheat genotypes. *Biologia Plantrum*, 49: 85-91.
- Santos, C. 1998. *Estudo de alguns mecanismos de regulacao osmotica eda expressao de glutamine sintetase em cellulula de Helianthus annuus L. Snjeitus a stress salino: Seleccao de celulas tolerantes a NaCl* - Ph. D thesis Uni. Aveiro, Portugal.
- Savvas, D. and F. Lenz. 2000. Effects of NaCl or nutrient-induced salinity on growth, yield, and composition of eggplants grown in rockwool. *Sci. Hort.*, 84: 37-47.
- Schwarz, M. 1985. The use of saline water in hydroponics. *Soilless Cult.*, 1: 25-34.
- Seifter, S., S. Dayton, B. Noric and B. Muntwyler. 1950. The estimation of glycogen with the anthrone reagent. *Arch Biochem.*, 25: 191-200.
- Shaddad, M.A., A.F. Radi, A.M. Abdel-Rahman and M.M. Azooz. 1999. Response of seeds of *lupinus termis* and *Vicia faba* to the interactive effect of salinity and ascorbic acid or pyridoxine. *Plant & Soil*, 122: 177-183.
- Shalaby, E.E., E. Epstein and C.O. Qualset. 1993. Variation in salt tolerance among some wheat and *Triticale* genotypes. *J. Agronomy and Crop Sci.*, 171: 298-304. L.) cultivated in Turkey. *Tr. J. Biol.*, 14: 125-148 (In Turkish).
- Shawky, N.B.T. 2003. *Physiological studies on the effect of salinity, ascorbic acid and putrescine on sweet pepper plant*. Ph.D. Thesis, Dept. of Agric. Bot., Faculty of Agric. Univ. of Cairo.
- Siddiqui, M.H., M.N. Khan, F. Mohammad and M.A. Khan. 2008. Role of nitrogen and gibberellic acid (GA3) in the regulation of enzyme activities and in osmoprotectant accumulation in *Brassica juncea* L. under salt stress. *J. Agro. Crop Sci.*, 194: 214-224.
- Singh, J. and M. Rai. 2005. Eggplant (*Solanum melongena* L.): nutritional, medicinal and antioxidant properties, pp. 10-15.
- Shawky, N.B.E.T. 2003. *Physiological studies on the effect of salinity, ascorbic acid and putrescine on sweet pepper plant*. Ph.D. Thesis, Dept. of Agri. Bot., Faculty of Agri. Univ. of Cairo.
- Smirnoff, N. 1996. The function and metabolism of ascorbic acid in plants. *Ann. Bot.*, 78: 661-669.
- Sutarno, H., S. Danimihardja and G.J.H. Grubben. 1993. *Solanum melongena* L. in: Plant resources of south Asia. No. 8. Vegetables. Siemonsma, J.S. & K. Piluek. Pudoc Scientific Publisger, Wageningen, The Nether Lands, 255-258.
- Stevens, J., T. Senaratna and K. Sivasithamparam. 2006. Salicylic acid induces salinity tolerance in tomato (*Lycopersicon esculentum* cv. Roma): associated changes in gas exchange, water relations and membrane stabilisation. *Plant Growth Reg.*, 49: 77-83.
- Taamalli, W., L. Abz, N.B. Youssef, D.D.B. Miled and M. Zarrouk. 2004. Lipid breakdown in sunflower (*Helianthus annuus* L.) seeds during post germinative growth under salt-stress. *Rivista Italian delle Sostanze Grasse*, 81: 90-97.
- Taraf, S.A., K.M. Gamal-El-Din and L.K. Balbaa. 1999. The response of vegetative growth and essential oil of lemongrass (*Cymbopogon citrates* Hort) to foliar application of ascorbic acid, nicotinamid and some micronutrients. *Arab Univ. of Agric. Sci.*, 7: 247-259.
- Tipirdamaz, R. and H. Cakirlar. 1990. Effects of water and salinity stresses on relative water content, proline and betaine composition in two varieties of wheat plants (*Triticum aestivum*. cultivated in Turkey. *Doga Biyol Serisi*, 14: 124, 1990.
- Varma, S.K. 1981. Specific ion effect on the early growth in wheat (*Triticum acstivum* L.) *Indian J. Pl. Physiol.*, 24: 291-294.
- Youssef, A.A. and I.M. Talaat. 2003. Phisiological response of rosemary plants to some vitamins. *Egypt Pharm J.*, 1: 81-93.
- Zhu, J.K. 2002. Salt and drought stress signal transduction in plants. *Ann. J. Plant Biol.*, 14: 267-273.

(Received for publication 15 April 2015)