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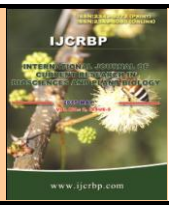

Original Research Article

Application of Exogenous Ascorbic Acid on Tomato (*Solanum lycopersicum* L.) Seeds under NaCl Salinity Stress

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Abstract

This study aimed to explain the role of ascorbic acid (AsA) for reducing the effect of salinity stress on special cultivar of tomato plant. The tomato seeds cv Bonus F1 soaked in AsAat (0.75 mM) for 12 hours in the dark. Planted seeds in trays of cork contains 218 eye for 14 days, transplanted the seedlings plant to plastic containers containing a mixture of sand/peat-moss (1:2).Each pot contained 7 seedling plants were irrigated using different concentrations (1500, 3000, 4500, 6000 ppm) of NaCl. Fresh and dry weights for shoot and root, leaf area decreased significantly with increasing salinity concentrations, after 42 days compared with control, whereas in the present of AsA the growth parameters increased significantly in the presence ofAsA0.75 mM under saline and non-saline stress. The evident recorded a significantly increased the Chl. a, Chl. b, carotenoids, total chlorophyll and total pigment of tomato plant leaves showed a progressive increase significantly with increasing NaCl salinity, also the chloroplast pigments increasing more in the present of AsA (0.75 mM) under saline and non-saline conditions compared with the control. The data provide strong support to the hypothesis that exogenous of ascorbic acid reduces the harmful effects of salinity and increases resistance to salinity in tomato plant.

Keywords

Ascorbic acid
Chlorophyll
Leaf area
Salinity stress
Tomato

Introduction

In arid and semi-arid regions, the soil salinity is one of the major stresses, which can severely limit crop production. The deleterious effects of salinity on plant growth are associated with (1)- low osmotic potential of soil solution (water stress), (2)- nutritional imbalance, (3)- specific ion effect (salt stress), or (4)- a combination of these factors (Ashraf et al., 2001). Saline-sodic irrigation water, coupled with the low annual rainfall and high evaporation and transpiration in the arid and semi-arid regions, have resulted in

accumulation of soluble salts in the soil solution and of cations (especially Na^+) on exchange sites, which can alter the structure and, consequently, affect the soil hydraulic conductivity. While, salinity stress is more important stress which can limit plant productivity (Hasegawa et al., 2000; Sameni and Morshedi, 2000). Therefore, understanding the mechanisms of plant tolerance to salinity stress is important (Ayman, 2003; Bartels and Sunkar, 2005). Worldwide, more than 60 million hectares of irrigated land (representing some 25% of the total irrigated land in the world) have been damaged by salt (Cuartero and Fernandez-Munoz, 1999; Mekhaldi et al., 2008; Aliabadi and Maroufi, 2011; Hasanuzzaman et al., 2013).

Using Ascorbic acid for improvement of the growth, may be due to its antioxidant activity of and 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 conditions (Halmer, 2004; Afzal et al., 2005; Piri et al., 2009). Tomato (*Lycopersicon esculentum*) is one of the most consumed vegetables so fresh as processing industry, and is very common in the Mediterranean diet. Tomato has a high content in carotenoids, phenols, vitamin C and E by which have a high capacity antioxidant (Grover et al., 2001; Khachik et al., 2002), tomato is good source of minerals and vitamins (Colla et al., 2002).

The objective of this study was to explain the role of ascorbic acid (AsA) for reducing the effect of salinity stress on special cultivar of tomato plant on leaf area, fresh and dry weight and chlorophyll content.

Materials and methods

Nutrient solutions and salinity treatments

The base nutrient solution used was similar to that applied by Hoagland and Arnon (1950). The solution was held at pH 6 throughout the experiment.

NaCl salinity concentrations

Molar solutions were prepared of NaCl was added to the Hoagland solutions to give four concentrations of salinity as follows: Control (Hoagland), 1500, 3000, 4500 and 6000 ppm salinity**.**

The soil used

The soil used for cultivated tomato plant was the ratio between the sand and peat-moss (1:2 – v:v), added in each pot (diameter 16 cm and depth of 16 cm), by the same ratio of the soil of the volume**.**

Plant material and growth conditions

Selected of the seeds intact, homogeneous in size and free from wrinkles to plant tomatoes cultivar (Bonus F1). Then soaked the seeds for 12 hours in the dark using the following solutions where seeds were divided into 2 groups as follows: First group (1): seeds soaked in distilled water. The second group (2); seed soaked in a solution of AsA concentration 0.75 mM.

The seedling plant transplanted after germinated (14 days) in trays of cork (39 cm × 67 cm), which containing 218 tray diameter eye (3cm and depth 6.5 cm). The tray eyes containing an equal amount of peat-moss only mixture thoroughly with water so distributed one seed in each eye tray and left the seeds to grow under greenhouse conditions at temperature of 18^oC $\pm 1^oC$ (night) 22^oC $\pm$ 2^oC (day) and relative humidity varied between 60 - 70%. The tomato seeds watering using distilled water until the true leaf appearance then transferred to another pots (diameter 16 cm and depth of 16 cm) which containing the sandy soil washed by diluted hydrochloric acid (1N HCl) and washed thoroughly with distilled water more five times. Used the same pots, and each pot containing the same volume of washing sandy soil and peat moss, (1: 2 - v : v). The sand culture technique and nutrient solution were similar to those adopted by Hewitt (1952); Hoagland and Arnon (1950) respectively.

Seedling of tomato plant was transferred from cork trays to plastic pots, each pot containing 7 transplanting (seedling plant) then left the seedling for one days and then irrigated using NaCl salinity with different concentrations (1500; 3000; 4500; 6000 ppm) in Hoagland solution (nutrient solution) and using a Hoagland solution as control in the presence or absent the AsA.

Irrigation process four NaCl salinity (1500; 3000; 4500; 6000 ppm) concentration in addition to Hoagland solution (nutrient solution) by using a hand spray control the distribution of salt and avoid the accumulation of salts in one place of pot, irrigated

plants on average once every two days with a fixed amount of each concentration brines by 400 ml.

Growth parameters determination

At 28 days after transplanting, a random sample (3 plants) was taken from each experimental unit to measure The leaf area (cm^2/leaf) assessed using the leaf No. 3 from the lower, by a Portable Area Meter (*Area Meter Model CI, 202*). The shoot and root fresh after weighing, dried at 80°C reweighed, fresh and dry weights (g/plant) of shoot and root every time harvesting and placing samples fresh in oven for drying at a temperature of 80°C for 72 h until proven weight then was weighing in the balance of digital for dry weight.

Pigment analysis

The (leaves was used to determine chlorophyll a, chlorophyll b, carotenoids, total chlorophyll concentration. Total chlorophyll of plants was extracted in 85% (v/v) aqueous acetone and the pigment content of the extract obtained was measured spectrophotometrically at wavelengths E 664; E 645; E 452 nm according to the method of Metzner et al. (1965). The pigment fractions were then calculated as mg/g leaf fresh weight for each treatment.

Statistical analysis

Statistical analysis of the data was fed to the computer and analyzed using IBM SPSS software package version 20.0. Quantitative data were described using mean and standard deviation or standard error of mean for normally distributed data. The distributions of quantitative variables were tested for normality using Kolmogorov-Smirnov test, Shapiro-Wilk test and D'Agstino test, also Histogram and QQ plot were used for vision test. If it reveals normal data distribution, parametric tests was applied. If the data were abnormally distributed, non-parametric tests were used. For normally distributed data, comparison between different groups was analyzed using *F*-test (*ANOVA*). To find the effect between stages, AsA.(mM) and NaCl ppm and their interactions two way *ANOVA* was used. Significance test results are quoted as two-tailed probabilities. Significance of the obtained results was judged at the 5% level (Leslie et al., 1991; Kirkpatrick and Feeney, 2013).

Results

Leaf area (cm^2/leaf)

The results showed that the leaf area affected by all salinity concentrations Fig. 1 and Table 1. Generally, leaf area decreased significantly ($p<0.001$) with increasing NaCl salinity concentrations compared with control. Whereas, in the presence of AsA (0.75 mM), the leaf area increased significantly ($p\leq0.001$) with different salinity treatments at (42 Days). Overall, the analysis of variance (*ANOVA*) between different concentrations of NaCl and presence or absence ofAsA at growth stage (42 Days) indicated that the *F* test was highly significant at $p\leq0.001$. Sumalan and Carmen (2002) found that the leaf area represents an important physiologic index in characterization of intensity to some metabolic process (growing, transpiration, photosynthesis, respiration, etc.).

Fig. 1: Effects of AsA (0.75 mM) on leaf area (cm^2/leaf) of tomato plants grown for 42 days in nutrient solution under salinity stress with different concentrations (0.0, 1500, 3000, 4500 and 6000 ppm NaCl) . Values are means of 3 replicates.

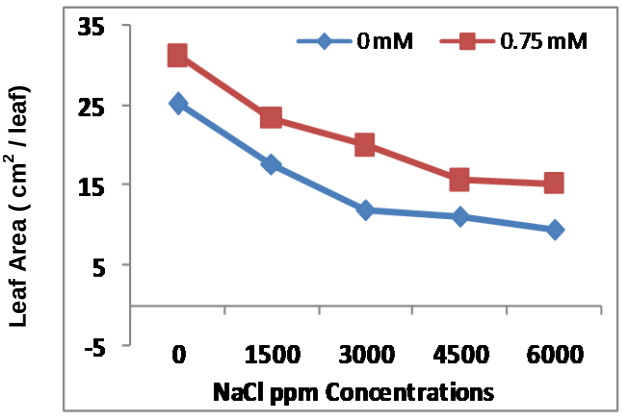


Table 1. Effects of AsA (0.75 mM) on leaf area (Cm^2/Leaf) of tomato plants grown for 42 days under salinity stress with different concentrations.

AsA. (mM) / NaCl (ppm)	0.00	0.75	F_1	p
Control	25.20 ± 0.25	31.20 ± 0.37	384.235*	<0.001*
1500	17.70 ± 0.39	23.50 ± 0.35	193.146*	<0.001*
3000	11.87 ± 0.33	20.03 ± 0.45	196.948*	<0.001*
4500	11.11 ± 0.29	15.64 ± 0.40	92.010*	<0.001*
6000	9.44 ± 0.53	15.23 ± 0.49	82.849	<0.001*
F_2	899.756*	752.345*		
p	<0.001*	<0.001*		
Overall Two Way Analysis of Variance (*ANOVA*)	NaCl ppm Conc.		F = 6564.992*	p<0.001*
	AsA(mM) Conc.		F = 1598.693*	p<0.001*
	NaCl ppm Conc. × AsA(mM)		F = 25.411*	p<0.001*

Values expressed are mean ± SEM; F_1: F test (ANOVA) Comparison between different concentrations of AsA in each concentration of NaCl at 42 days; F_2: F test (ANOVA) Comparison between different concentrations of NaCl in each concentration of AsA at 42 days.

Ejaz et al. (2012) they found that the leaf area per plant was significantly reduced under salt stress, while AsA applications markedly improved the inhibitory effects of salt on plants. The leaf area of salt-stressed plants was 90 cm^2, which showed an increasing trend in plants with AsA supply through irrigation treatment. The maximum recorded value was 205 cm^2 at 1.0 mMAsA level. Likewise, in the case of foliar spray, a gradual increase in leaf area was observed with increasing AsA levels. When salt treatments were supplemented with foliar spray of AsA (1.0 mM) the leaf area increased from 96 cm^2 to 173 cm^2 this results agree with data presented here. Also, Jamil et al. (2007), Zhao et al. (2007), and Yilmaz and Kina (2008) found that the exposure to salinity by NaCl reduced leaf area. Numerous studies showed the affection of leaf area negatively by using different concentrations of NaCl (Raul et al., 2003; Netondo et al., 2004; Mathur et al., 2006; Chen et al., 2007; Zhao et al., 2007; Yilmaz and Kina, 2008; Rui et al., 2009). Also, the response of tomato plant increased by NaCl salinity concentrations which a significant decrease in leaf area with application elevated salt treatment, also decrease in plant height and number of fruits per plant was seen as the salt concentration increased (Babu et al., 2012).

Fresh and dry weight (g/plant)

Overall, shoot and root fresh and dry weight of tomato plant decreased significantly (p<0.001) with increasing NaCl salinity concentrations, however, the shoot and root fresh and dry weight at 4500 and 6000 ppm NaCl showed dramatically depression compared with control as shown in Fig. 2 a, b, c and d and Tables 2 a, b, c and d. Whereas, the statistical analysis indicated that the shoot and root fresh and dry weight of tomato plants increased significantly at $p\leq0.001$ in the present of AsA under saline and non- saline conditions. AsA (0.75 mM) tended to increasing shoot and root fresh and dry weight under saline and non-saline conditions at (42 Days) than control.

Overall, the two ways analysis of variance (ANOVA) between different concentrations of NaCl and AsA at 42 days, indicated that the F test highly significant at $p\leq0.001$.The results presented here agree with the results, which indicated that the fresh weight in four tomato cultivars decreased due to salinity stressed, while after application of AsA the promoted significantly vegetative growth of rosemary plant (Youssef and Talaat, 2003; Ali et al., 2011).

The increasing in fresh weight of the shoot system may be due to the ability of the plant to increase the size of its sap vacuoles, which allows for the collection of a lot of water, and this in turn dissolves salt ions that have accumulated and leads to the subsequent increase in fresh weight (Munns, 2002). The studies on interaction between salinity and antioxidants focus on growth, yield and some physiological changes has been done by all of the Abd El-Aziz et al. (2006); Hussein et al. (2007); Athar et al. (2008).

Table (2a). Effects of AsA (0.75 mM) on shoot fresh weight (g/Plant) of tomato plants grown for 42 days under salinity stress with different concentrations.

AsA. (mM) / NaCl (ppm)	0.00	0.75	F_1	p
Control	5.499 ± 0.385	6.804 ± 0.154	32.998*	<0.001*
1500	2.462 ± 0.267	5.298 ± 0.173	81.052*	<0.001*
3000	1.311 ± 0.018	2.208 ± 0.170	18.779*	<0.001*
4500	0.937 ± 0.071	1.450 ± 0.053	9.823*	0.002*
6000	0.790 ± 0.038	1.261 ± 0.080	7.463*	0.005*
F_2	84.596*	340.382*		
p	<0.001*	<0.001*		
Overall Two Way Analysis of Variance (*ANOVA*)	NaCl ppm Conc.		F = 1306.640*	p<0.001*
	AsA(mM) Conc.		F = 220.802*	p<0.001*
	NaCl ppm Conc. × AsA(mM)		F = 13.373*	p <0.001*

Values expressed are mean ± SEM; F_1: F test (ANOVA) Comparison between different concentrations of AsA in each concentration of NaCl at 42 days; F_2: F test (ANOVA) Comparison between different concentrations of NaCl in each concentration of AsA at 42 days.

Table (2 b). Effects of AsA (0.75 mM) on root fresh weight (g/Plant) of tomato plants grown for 42 days under salinity stress with different concentrations.

AsA. (mM) / NaCl (ppm)	0.00	0.75	F_1	p
Control	0.603 ± 0.014	1.255 ± 0.032	226.913*	<0.001*
1500	0.335 ± 0.006	1.043 ± 0.034	295.754	<0.001*
3000	0.146 ± 0.003	0.326 ± 0.020	51.023*	<0.001*
4500	0.098 ± 0.001	0.187 ± 0.003	43.385*	<0.001*
6000	0.060 ± 0.003	0.121 ± 0.003	42.403*	<0.001*
F_2	1025.756*	522.544*		
p	<0.001*	<0.001*		
Overall Two Way Analysis of Variance (*ANOVA*)	NaCl ppm Conc.		F = 2474.655*	p<0.001*
	AsA(mM) Conc.		F = 677.074*	p<0.001*
	NaCl ppm Conc. × AsA(mM)		F = 69.815*	p <0.001*

Values expressed are mean ± SEM; F_1: F test (ANOVA) Comparison between different concentrations of AsA in each concentration of NaCl at 42 days; F_2: F test (ANOVA) Comparison between different concentrations of NaCl in each concentration of AsA at 42 days.

Table (2 c). The Effects of AsA (0.75 mM) on shoot dry weight (g/Plant) of tomato plants grown for 42 days in nutrient solution under salinity stress with different concentrations.

AsA. (mM) / NaCl (ppm)	0.00	0.75	F_1	p
Control	0.605 ± 0.014	0.670 ± 0.028	25.008*	<0.001*
1500	0.529 ± 0.016	0.497 ± 0.023	76.313*	<0.001*
3000	0.127 ± 0.001	0.188 ± 0.014	13.239*	<0.001*
4500	0.083 ± 0.007	0.119 ± 0.003	6.003*	0.010*
6000	0.036 ± 0.009	0.091 ± 0.007	4.087*	0.032*
F_2	452.098*	211.943*		
p	<0.001*	<0.001*		
Overall Two Way Analysis of Variance (*ANOVA*)	NaCl ppm Conc.		F = 1646.842*	p<0.001*
	AsA(mM) Conc.		F = 152.259*	p<0.001*
	NaCl ppm Conc. × AsA(mM)		F = 11.940*	p <0.001*

Values expressed are mean ± SEM; F_1: F test (ANOVA) Comparison between different concentrations of AsA in each concentration of NaCl at 42 days; F_2: F test (ANOVA) Comparison between different concentrations of NaCl in each concentration of AsA at 42 days.

Fig. 2 a, b, c and d: Effects of AsA (0.75 mM) on fresh and dry weight (g/Plant) of tomato plants (shoots and roots) grown for 42 days under salinity stress with different concentrations (0.0, 1500, 3000, 4500 and 6000 ppm NaCl). Values are means of 3 replicates.

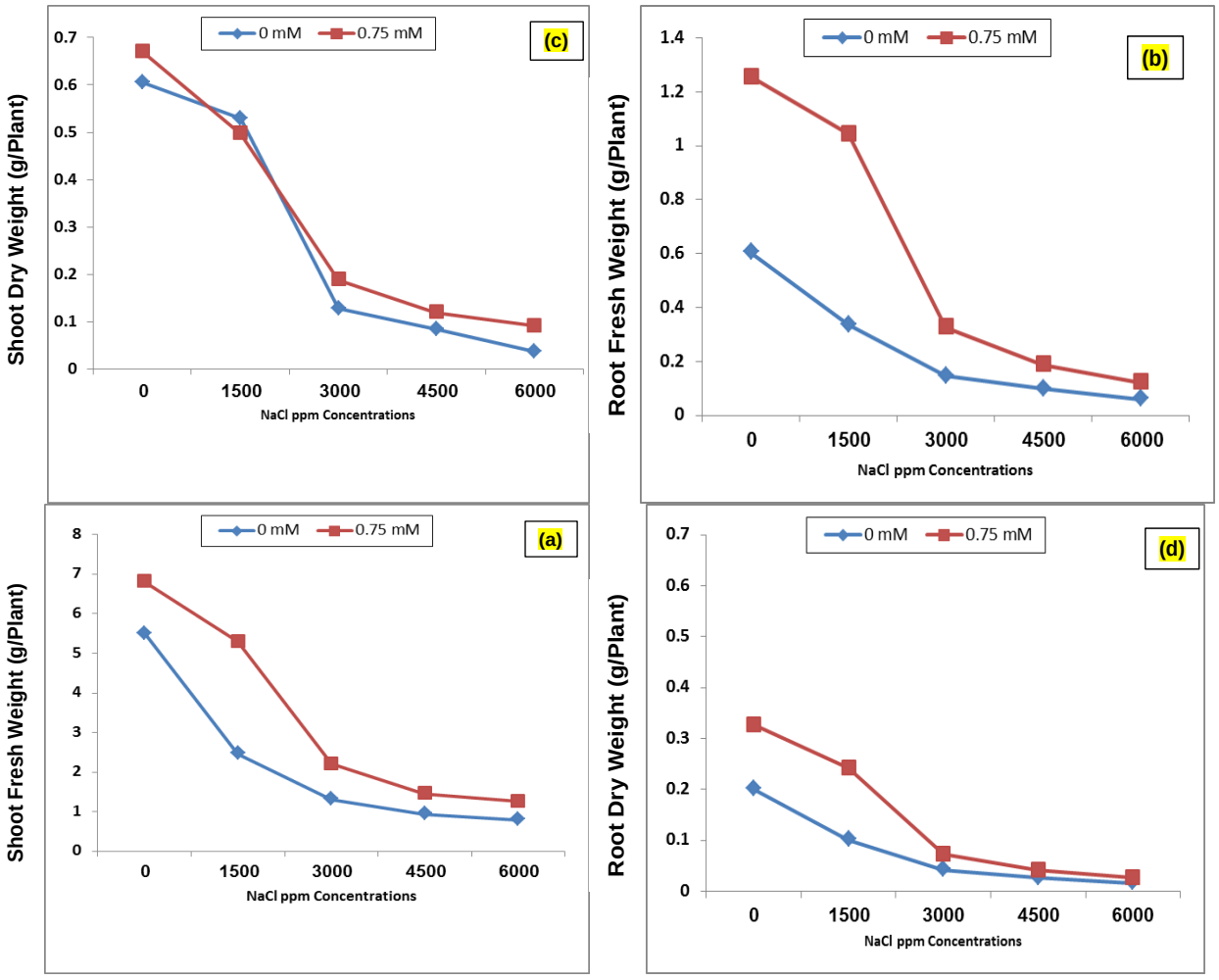


Table (2 d): The Effects of AsA (0.75 mM) on root dry weight (g/Plant) of tomato plants grown for 42 days in nutrient solution under salinity stress with different concentrations.

AsA. (mM) / NaCl (ppm)	0.00	0.75	F_1	p
Control	0.200 ± 0.006	0.326 ± 0.003	84.027*	<0.001*
1500	0.101 ± 0.004	0.241 ± 0.009	86.678*	<0.001*
3000	0.042 ± 0.003	0.072 ± 0.002	37.732*	<0.001*
4500	0.026 ± 0.001	0.041 ± 0.001	23.829*	<0.001*
6000	0.016 ± 0.001	0.026 ± 0.001	15.873*	<0.001*
F_2	84.027*	958.841*		
p	<0.001*	<0.001*		
Overall Two Way Analysis of Variance (*ANOVA*)	NaCl ppm Conc.		F = 1669.101*	P<0.001*
	AsA(mM) Conc.		F = 225.177*	p<0.001*
	NaCl ppm Conc. × AsA(mM)		F = 32.869*	p<0.001*

Values expressed are mean ± SEM; F_1: F test (ANOVA) Comparison between different concentrations of AsA in each concentration of NaCl at 42 days; F_2: F test (ANOVA) Comparison between different concentrations of NaCl in each concentration of AsA at 42 days.

The results presented here agree with the results by Khan et al. (2010) found that the application of AsA as seed soaking increased the shoot fresh and dry weight under saline and non-saline conditions. AsA (50 mg L^{-1}) applied as seed soaking increased the root dry weight under saline conditions. Contrary to what we have reached applied AsA (100 mg L^{-1}) as soaking seed increased both of the shoot fresh and dry weight under saline and non-saline conditions as compared to the other concentration of the AsA, these results disagree with this studies results.

The results presented here agree with results by Hajer et al. (2006) who found that the stem, leaves and root dry weights of cultivated three varieties of tomato plant was decreased with salinity increased. Also, the effect of NaCl stress on the growth of tomato plants is reflected in lower dry weights. The reduction of the plant organs dry weight due to increased salinity may be a result of a combination of osmotic and specific ion effects of Cl^- and Na^+ (Al-Rwahy, 1989; Afzal et al., 2005; Turan et al., 2007a; Hajiboland et al., 2010; Taffouo et al., 2010). Many studies have shown that the fresh and dry weights of the shoot system are affected, either negatively or positively, by changes in salinity concentration, type of salt present or type of plant species (Bayuelo-Jiménez et al., 2002; Jamil et al., 2005; Niaz et al., 2005; Saqib et al., 2006; Turan et al., 2007b; Saffan, 2008; Rui et al., 2009; El-Bastawisy, 2010; Memon et al., 2010; Taffouo et al., 2009; 2010;). The significant decrease in dry weight of shoot is due to salinity in all the tomato cultivars (Ali et al., 2011).

Afzal et al. (2006) observed that the wheat (*Triticum aestivum*) seedling raised from primed seeds with 50 ppm ascorbic acid had significantly higher lengths and fresh and dry weight of shoot than other treated or non-primed seeds under non-saline and saline conditions, agree with the resulted presented here, agree with results Khafagy et al. (2009) they found that the effect of NaCl salinity levels up to 6000 ppm (11.88 dsm^{-1}) on sweet pepper decreased all of the plant height, root length and shoot fresh as well as dry weights. This effect increased consistently and rapidly with increasing salinity level, as compared to non-stressed plants. While, AsA increased plant height, root length, fresh and dry weights of shoot. Pre-soaking with AsA (100 ppm) proved to be more effective in this respect. AsA counteracted the harmful effects of salinity on plant height and root length at all salinity levels used. Sminoff (1996) and Tarraf et al. (1999) mentioned that the AsA has been implicated in regulation of cell division. Hussein et al. (2011) found that the salt stress in the wheat plants (3000 to 6000 ppm) decreased plants height, number and surface of leaves, dry weight of both stem and leaves, compared to the unstressed plants this resulted agree with this resulted presented. But spraying AsA improvement the parameters of growth and yield and decreased the salt effect.

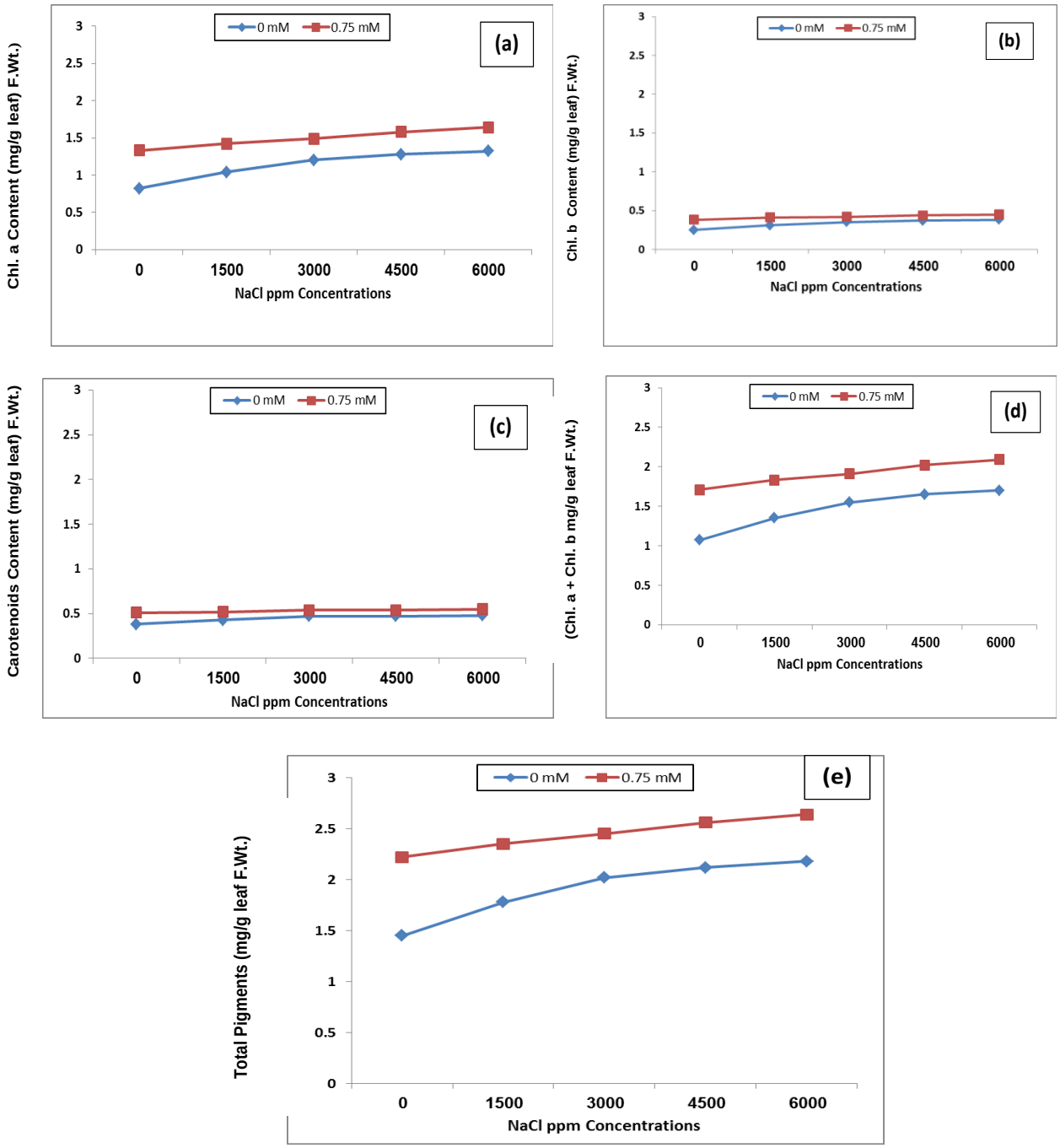
Application of AsA on chloroplast pigment contents response to salinity stress

The chlorophyll a, chlorophyll b, total chlorophyll, carotenoids, and total pigment contents of tomato plant leaves increased progressively with increasing NaCl salinity at 42 days, also the chloroplast pigments increased in the present of AsA. The data presented by Zhu (2001); Munns (2002); Tejeraet al. (2004); Bartels and Sunkar (2005) they found that the NaCl salt stress 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. Chlorophylls (a and 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. Changes of pigment contents under salt stress are used as parameter for selection of tolerant and sensitive cultivars in crop plants (Eryilmaz, 2007).

Chlorophyll a, b and carotenoids content (mg/g leaf fresh weight)

Overall, the chlorophyll a, chlorophyll b, total chlorophyll, carotenoids, and total pigment contents of tomato plant leaves increased significantly ($p \leq 0.001$) with increasing NaCl salinity concentrations and age of tomato plant (42 days) as shown in Figs. 3 a, b, c, d and e) and Tables (3 a, b, c, d and e). The NaCl salinity induced to increased the content of chlorophyll a, chlorophyll b, total chlorophyll, carotenoids, and total pigment contents in leaves of tomato plant significantly ($p \leq 0.001$) whereas in the present of AsA increased more compared with control plants. Overall the two ways analysis of variance (*ANOVA*) between different concentrations of NaCl and AsA at growth stage indicated that the *F* test highly significant at $p \leq 0.001$.

Fig. 3 a, b, c, d and e: The Effects of AsA (0.75 mM) on (a) chlorophyll a, (b) chlorophyll b, (c) carotenoids, d) chlorophyll contents and (e)total pigments(mg/g f. wt. leaf) of tomato plants grown for 42 days under salinity stress with different concentrations (0.0, 1500, 3000, 4500 and 6000 ppm NaCl). Values are means of 3 replicates.



The results of this study agrees with the data presented by Ashraf et al. (2003) they found that the marked increase in the chlorophyll content with impact of salt stress on the *Hibiscus esculentus* plants. The results presented here disagree with the results obtained by Hajeret al. (2006) they found that in tomato, the salinity stress decreased chlorophyll b more than chlorophyll a content, and both of them with increasing salinity. Also, Stoeva and Kaymakanova (2008) observed that the chlorophyll a, chlorophyll b

and carotenoids decreased with increasing salinity (100 mMNaCl) on beans plant. Also, Di Martino et al. (2003) found that the exposed of spinach (*Spinacia oleraceae*) plant to salinity stress, decreased both of chlorophyll a and chlorophyll b content.The results of this study agrees with the results obtained by Farahat et al. (2013) who 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 and b contents and total carbohydrates % of shoots and roots were reduced as external salinity in irrigation water increased.

Table 3 a: The Effects of AsA (0.75 mM) on chlorophyll a (mg/g F. Wt. Leaf) of tomato plants grown for 42 days under salinity stress with different concentrations.

AsA. (mM) / NaCl (ppm)	0.00	0.75	F_1	p
Control	0.82 ± 0.07	1.33 ± 0.01	52.109*	<0.001*
1500	1.04 ± 0.06	1.42 ± 0.04	56.516*	<0.001*
3000	1.20 ± 0.04	1.49 ± 0.01	77.694*	<0.001*
4500	1.28 ± 0.04	1.58 ± 0.02	39.904*	<0.001*
6000	1.32 ± 0.05	1.64 ± 0.04	27.396*	<0.001*
F_2	45.613*	76.857*		
p	<0.001*	<0.001*		
Overall Two Way Analysis of Variance (*ANOVA*)	NaCl ppm Conc.		F = 605.237*	p<0.001*
	AsA(mM) Conc.		F = 307.232*	p<0.001*
	NaCl ppm Conc. × AsA(mM)		F = 3.374*	p<0.001*

Values expressed are mean ± SEM; F_1: F test (ANOVA) Comparison between different concentrations of AsA in each concentration of NaCl at 42 days; F_2: F test (ANOVA) Comparison between different concentrations of NaCl in each concentration of AsA at 42 days.

Table 3 b: The Effects of AsA (0.75 mM) on chlorophyll b (mg/g F. Wt. Leaf) of tomato plants grown for 42 days under salinity stress with different concentrations.

AsA. (mM) / NaCl (ppm)	0.00	0.75	F_1	p
Control	0.25 ± 0.02	0.38 ± 0.04	11.540*	0.001*
1500	0.31 ± 0.04	0.41 ± 0.04	6.000 *	0.010*
3000	0.35 ± 0.02	0.42 ± 0.02	6.378*	0.008*
4500	0.37 ± 0.02	0.44 ± 0.03	3.995*	0.034*
6000	0.38 ± 0.02	0.45 ± 0.04	2.488	0.111
F_2	17.127*	2.018		
p	<0.001*	0.168		
Overall Two Way Analysis of Variance (*ANOVA*)	NaCl ppm Conc.		F = 37.309*	p<0.001*
	AsA(mM) Conc.		F = 16.752*	p<0.001*
	NaCl ppm Conc. × AsA(mM)		F = 0.235	p = 0.999

Values expressed are mean ± SEM; F_1: F test (ANOVA) Comparison between different concentrations of AsA in each concentration of NaCl at 42 days; F_2: F test (ANOVA) Comparison between different concentrations of NaCl in each concentration of AsA at 42 days.

Table 3 c: The Effects of AsA (0.75 mM) on carotenoids (mg/g F. Wt. Leaf) of tomato plants grown for 42 days under salinity stress with different concentrations.

AsA. (mM) / NaCl (ppm)	0.00	0.75	F_1	*p*
Control	0.38 ± 0.02	0.51 ± 0.03	18.709*	<0.001*
1500	0.43 ± 0.01	0.52 ± 0.01	49.115*	<0.001*
3000	0.47 ± 0.01	0.54 ± 0.01	5.964*	0.010*
4500	0.47 ± 0.01	0.54 ± 0.01	15.940*	<0.001*
6000	0.48 ± 0.04	0.55 ± 0.05	3.478	0.051
F_2	12.686*	1.181		
p	0.001*	0.376		
Overall Two Way Analysis of Variance (*ANOVA*)	NaCl ppm Conc.		F = 79.508*	p<0.001*
	AsA(mM) Conc.		F = 61.005*	p<0.001*
	NaCl ppm Conc. × AsA(mM)		F = 0.557	p = 0.908

Values expressed are mean ± SEM; F_1: *F* test (ANOVA) Comparison between different concentrations of AsA in each concentration of NaCl at 42 days; F_2: *F* test (ANOVA) Comparison between different concentrations of NaCl in each concentration of AsA at 42 days.

Table 3 d: The Effects of AsA (0.75 mM) on chlorophyll contents (mg/g F. Wt. Leaf) of tomato plants grown for 42 days under salinity stress with different concentrations.

AsA. (mM) / NaCl (ppm)	0.00	0.75	F_1	*p*
Control	1.07±0.057	1.71±0.031	60.040*	<0.001*
1500	1.35±0.026	1.83±0.030	111.467*	<0.001*
3000	1.55±0.051	1.91±0.020	57.392*	<0.001*
4500	1.65±0.00	2.02±0.025	60.603*	<0.001*
6000	1.70±0.055	2.09±0.068	20.525*	<0.001*
F_2	104.408*	45.379*		
p	<0.001*	<0.001*		
Overall Two Way Analysis of Variance (*ANOVA*)	NaCl ppm Conc.		301.27	<0.001*
	AsA(mM) Conc.		131.39	<0.001*
	NaCl ppm Conc. × AsA(mM)		2.19	<0.001*

Values expressed are mean ± SEM; F_1: *F* test (ANOVA) Comparison between different concentrations of AsA in each concentration of NaCl at 42 days; F_2: *F* test (ANOVA) Comparison between different concentrations of NaCl in each concentration of AsA at 42 days.

Table 3 e: The Effects of AsA (0.75 mM) on total pigments (mg/g F. Wt. Leaf) of tomato plants grown for 42 days under salinity stress with different.

AsA. (mM) / NaCl (ppm)	0.00	0.75	F_1	*p*
Control	1.45±0.035	2.22±0.023	293.755*	<0.001*
1500	1.78±0.035	2.35±0.027	267.642*	<0.001*
3000	2.02±0.022	2.45±0.010	211.249*	<0.001*
4500	2.12±0.014	2.56±0.019	182.696*	<0.001*
6000	2.18±0.036	2.64±0.040	89.866*	<0.001*
F_2	315.691*	125.324*		
p	<0.001*	<0.001*		
Overall Two Way Analysis of Variance (*ANOVA*)	NaCl ppm Conc.		361.27	<0.001*
	AsA(mM) Conc.		191.39	<0.001*
	NaCl ppm Conc. × AsA(mM)		3.79	<0.001*

Values expressed are mean ± SEM; F_1: *F* test (ANOVA) Comparison between different concentrations of AsA in each concentration of NaCl at 42 days; F_2: *F* test (ANOVA) Comparison between different concentrations of NaCl in each concentration of AsA at 42 days.

Verma and Mishra (2005); Ziaf et al. (2009) found that the low level salt (50 mM/L NaCl) increased carotenoids level in leaves of both *Brassica juncea* and hot pepper cultivars than the control respectively under salinity stress this results agrees with the results obtained here. Whereas, the carotenoids content in tomato leaves plant increased with salinity stress, but, its disagree with Tort and Turkyilmaz (2004) they observed that the NaCl salinity stress (0.0, 120, 180, and 240 mM) reduced leaf content of carotenes barley seedlings (*Hordeum vulgare* L.).

Also, the results obtained by Mustard and Renault (2006) registered a reduction in carotene content in seedlings of dogwood (*Cornus sericea* L.) under salinity stress. These results reinforce the results obtained by Shah (2007), Beltagi (2008), and Ekmekçi and Karaman (2012), the carotenoids content increasing at low and moderate NaCl levels as compared with control. Increasing the chlorophyll content under salinity stress were observed with these results similar to the results with Abd El Samad (1993 a, b), Misra et al. (1997), and Kusvuran et al. (2008) they observed that the high increased total chlorophyll content under salt stress in different cultivars and this may be due to an increase in the number of chloroplasts in the stressed leaves.

The results presented here disagree with the results obtained by Soltani Nezhad et al. (2011) reported that the salt stress leads to a significant decrease in chlorophyll content was observed at 120 and 150 mM salt in the leaf of tomato (*Lycopersicon peruvianum*, L.) plant. Also, the results presented here disagree with the results obtained by Lee et al. (2004) on *Paspalum vaginatum*, L. and Tort and Turkyilmaz (2004) on barley (*Hordeum vulgare* L.), Siler et al. (2007) on *Centaurium erythraea*, L. and Al-Sobhi et al. (2006) on plants *Calotropis procera* (Ait.) all reported that chlorophyll a; b and total chlorophyll decreased with increasing salt concentrations. Generally, chlorophyll contents were reduced markedly at high salinity concentration treatments especially with aged plants. It may be due to the reason that the total chlorophyll and the proportion of its components depend on the biological process and development stages of the plant and also on the type and concentration of the salt. So, salinity reduced chlorophyll a; chlorophyll b; carotenoids and total chlorophyll significantly with increasing concentrations on wheat (Hassaneinet al., 2009) and on *Vicia faba* L. (Abdul Qados, 2011).

Conclusion

Generally, this study concluded that the ascorbic acid (75 mM) resulted an increased fresh and dry weights for shoot and root, leaf area in tomato plant and mitigate the impact of salinity inhibitory to the plant metabolism. Whereas, the ascorbic acid (75 mM) tended to improvement the growth parameters and increased the amount of chloroplast pigments.

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