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Alleviation of Adverse Effects of Salinity Stress on Tomato (*Solanum lycopersicum*, L.) Plants by Exogenous Application of Ascorbic Acid

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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 AsA at (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. Total available carbohydrates (reducing sugars, non-reducing (sucrose) and polysaccharides), proteins and proline for both shoot and root of the tomato plant under salinity stress in the present of ascorbic acid (0.75 mM) more than in the absent of AsA compared with the control for growth stage (42 days). 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: Salinity, Ascorbic acid, Tomato, Carbohydrates, Proline, Proteins.

1. INTRODUCTION

Salinity is currently one of the most severe abiotic factors limiting agricultural production and known to adversely affect production of most crops worldwide. In arid and semiarid lands, the plants are subjected through their life cycle to different stresses; some of these plants can tolerate these stresses in different ways depending on plant species and the type of stress **[1-4].** With respect to more than 60 millions hectare from the world regions 25% of regions in all over the world face with salinity problem, doing of project in along with decreasing of salt soil damage is necessary. In saline soil, salt induced water deficit is one of the major constraints for plant growth and depends on the plant genotype and environmental conditions **[5-8].**

Ascorbic acid (AsA) is one of the most powerful antioxidants; the supply of ascorbic acid (vitamin C) to tomato seedling might decrease the synthesis of active oxygen species (AOS) and thereby increase resistance to salt stress **[9-10].** According to the FAO, tomato considered the second most cultivated vegetable in the world, after potato, the annual production of nearly 10^8 ton of fresh tomato in 3.7 × 10^6 ha worldwide, China, the USA and Turkey being the leading producers **[11-13].** 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. **[14-15].** The objective of this study aimed to explain the role of ascorbic acid (AsA) for reducing the effect of salinity stress on special cultivar of tomato plant on carbohydrates, proline, and total soluble proteins.

2. MATERIALS AND METHODS

Nutrient Solutions and Salinity Treatments: The base nutrient solution used was similar to that applied by **Hoagland and Arnon [16].** 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):

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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 [17]; Hoagland and Arnon [16]** 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

Physiological Studies:
Chemical content:
Carbohydrate Analysis: Oven dry plant material (300mg) 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 h., then centrifugation and filtered. The filtrate was used for determination of the direct reducing value (DRV) and total reducing value (TRV), while the residue was dried at 80^oC for determination of polysaccharide **[18-19].**

Direct Reducing Value (DRV): For the determination of DRV (including all free monosaccharide's), was carried out by evaporation 0.1 ml of cleared borate buffer extract was reduced to dryness and then mixed with 1 ml of the modified Nelson solution **[19].** 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 was added **[20]** and the colour mixture was diluted to a definite volume and intensity measured at 700 nm using a colorimeter (Model *GENSYS 10 S VIS Spectrophotometer Thermo Scientific*).

Total Reducing Value (TRV) (Sucrose): For the determination of total reducing value (TRV), a 0.2 ml of the cleared extract filtrate was mixed with distilled 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 37^0C for 30 min. Thereafter, the reducing value was determined as described before for DRV. The difference between the value obtained from this step and that of the DRV is an estimated of sucrose, in terms of glucose.

Estimation of polysaccharides: According to **Naguib [18],** the remaining residue (10 mg) were mixed with 0.2 ml of 1% taka diastase enzyme and 0.1 ml acetate buffer (6 ml acetic acid 0.2 N + 4 ml sodium acetate buffer 0.2 N), completed to 3 ml left over night at 28^oC and then centrifuged. The reducing value of 1 ml of the filtrate was estimated as proceeded above.

Protein Analysis: Three hundred (300 mg) of oven dry plant material extracted using 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 h, then centrifuged and filtered. The filtrate was used for the determination of total soluble proteins, while the residue was dried at 80^oC for the determination of total insoluble proteins **[21].**

Equal volume of a and b were mixed just before use to give Lowry B. 300 mg oven dry sample extracted overnight in borate buffer. 1 ml sample of extract containing 300 mg proteins were added to 5 ml of alkaline copper solution, (50 ml of 2% Na_2CO_3 prepared in 0.1 N NaOH +1 ml of 0.5% $CuSO_4.5H_2O$ prepared in 1% Na-K-tartarate), shacken to ensure complete mixing, then allowed to stand for 10 min. at room temperature. 0.5 ml of diluted Folin phenol reagent (1:1/v:v) was added and reading made after a period of 20 min., the absorbance of the blue colour produced was measured spectrophotometrically at 720 nm. Three samples were measured for each treatment above. Total soluble proteins (TSP) contents were expressed as mg/100 g dry weight.

Estimation of proline: Proline content was determined calorimetrically according to the method of **Bates *et al.* [22].** Acid ninhydrin reagent was prepared by warming 1.25 g ninhydrin in 30 ml glacial acetic acid and 20 ml 6 M phosphoric acid, with agitation until dissolved. At 4 ºC, the reagent remains stable for 24 hr.

Extraction: Dry plant materials (0.1 g) were homogenized in 10 ml of 3 % aqueous sulfo-salicylic acid for 3 h. to extract the proline from the tissue. The extract was centrifuged at 1500 rpm for 10 min.

Procedure: 2 ml of the supernatant was mixed with 2 ml glacial acetic acid and 2 ml fresh acid ninhydrin in a test tube for one hour at 100 ºC. The reaction was terminated in an ice bath, and the mixture was extracted with 4 ml toluene. The extract was vigorously stirred for 20s using a test tube stirrer. Therefore, the chromophore containing toluene (1 ml, upper phase) was aspirated from the aqueous phase. The absorbance was read at wavelength 520 nm using toluene as a blank. The proline concentration was determined using a standard curve of proline and calculated on a dry weight basis as mg proline / 100 g dry weight.

Statistical Analysis: Statistical analyses of the data were 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, comparisons between different groups were analyzed using *F*-test (*ANOVA*). To find the effect between stage, 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 **[23-24].**

3. Results and Dissection.

1. Applications of AsA on Direct Reducing Value (Monosaccharide's; mg/100g Dry Weight) in Shoots and Roots of Tomato Plant Response to NaCl Salinity Stress:

The impact of AsA concentration on monosaccharide's contents of tomato shoot and root under NaCl saline stress are shown in **Fig.(1 a & b) and Table (1)**. Monosaccharide's contents increased significantly ($p \leq 0.001$) in both shoot and root of tomato plant compared with control at (42 Days). However, the significantly increased ($p \leq 0.001$) the contents of monosaccharide was more in the present of AsA (0.75 mM) under saline or non-saline stress compared to control at (42 Days). A more pronounced increase in monosaccharide contents were obtain with application of AsA with different concentrations (from 1500 > 3000 > 4500 > 6000 ppm NaCl). Generally, the monosaccharide contents increased in shoot more than the root at different NaCl salinity in the present or absent of AsA (0.75 mM) compared to control. Overall the analysis of variance (*ANOVA*) between different concentrations of NaCl and presence or absence of of AsA at growth stage (42 Days) indicated that the *F* test highly significant at $P \leq 0.001$.

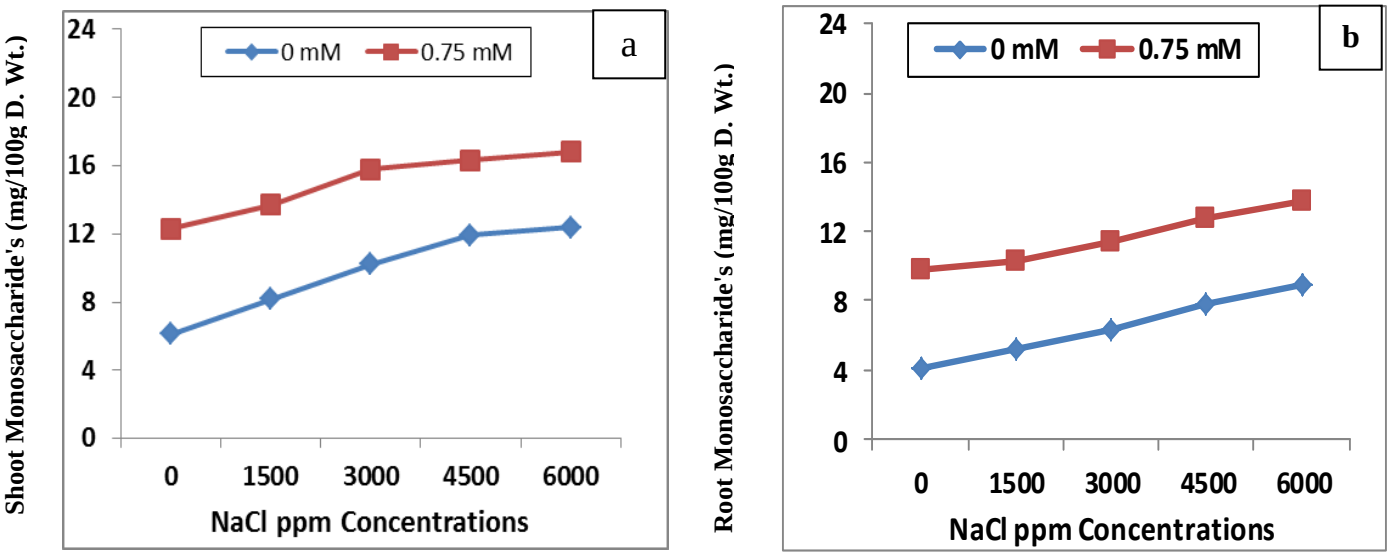

Fig. (1): Exogenous Effect of AsA (0.75 mM) on Shoot (a) and Root (b) Monosaccharide's (mg/100g D. Wt.) Contents of Tomato Plants Grown for 42 Days under Salinity Stress with Different Concentrations (0.0, 1500, 3000, 4500 & 6000 ppm NaCl). The Means of three replicates. Bars indicate – Standard Error (p = 0.05).

Table (1): Exogenous Effect of AsA (0.75 mM) on Shoot (a) and Root (b) Monosaccharide's (mg/100g D. Wt.) Contents of Tomato Plants Grown for 42 Days Under Salinity Stress With Different Concentrations (0.0, 1500, 3000, 4500 & 6000 ppm NaCl). The Means of Three Replicates. Bars Indicate – Standard Error (p = 0.05).

AsA. (mM) / NaCl (ppm)	*Shoot*				*Root*			
	0.00	0.75	F_1	*p*	0.00	0.75	F_1	*p*
Control	6.12 ± 0.38	12.30 ± 0.40	122.247*	<0.001*	4.08 ± 0.40	9.81 ± 0.37	38.686*	<0.001*
1500	8.17 ±0.57	13.71 ± 0.36	44.809*	<0.001*	5.17 ± 0.33	10.32 ± 0.37	42.327*	<0.001*
3000	10.23 ± 0.55	15.81 ± 0.39	57.335*	<0.001*	6.38 ± 0.19	11.37 ± 0.28	76.522*	<0.001*
4500	11.91 ± 0.21	16.31 ± 0.32	43.300*	<0.001*	7.81 ± 0.36	12.71 ± 0.20	98.877*	<0.001*
6000	12.38 ± 0.37	16.80 ± 0.21	42.051*	<0.001*	8.98 ± 0.50	13.80 ± 0.21	40.853*	<0.001*
F_2	36.396*	31.141*			28.426*	31.723*		
p	<0.001*	<0.001*			<0.001*	<0.001*		
Overall The Two Ways Analysis of Variance (*ANOVA*)	NaCl ppm Conc.		F = 198.285*	*p*<0.001*	NaCl ppm Conc.		F = 194.502*	*p*<0.001*
	AsA (mM) Conc.		F = 349.282*	*p*<0.001*	AsA (mM) Conc.		F = 430.225*	*p*<0.001*
	NaCl ppm Conc.xAsA (mM)		F =2.113*	*p*=0.013*	NaCl ppm Conc. x AsA (mM)		F = 1.117	*p*=0.350

2. Applications of AsA on Total Reducing Value (Sucrose; mg/100g Dry Weight) in Shoots and Roots of Tomato Plant Response to NaCl Salinity Stress:

The application of AsA at (0.75 mM) by soaking the seeds of tomato plant before cultivated enhanced significantly ($p \leq 0.001$), the total reducing value (TRV - sucrose) at all NaCl saline concentrations compared with control. However, the interaction between salinity (1500, 3000, 4500 & 6000 ppm) and AsA concentration resulted an improved the sucrose contents in shoot and root of tomato plants at (42 Days) as shown in **Fig.(2 a& b) and Table (2).** Generally, the TRV (sucrose) contents increased significantly ($p \leq 0.001$) in shoot more than the root at different NaCl salinity in the present or absent of AsA at growth stage (42Days). Overall the analysis of variance (*ANOVA*) between different concentrations of NaCl and AsA at (42Days) indicated that the *F* test highly significant at $P \leq 0.001$. The data presented by **Bartels and Sunkar [25]** they reported that the different of physiological studies was offered which under salinity condition of non-structural carbohydrate such as sucrose and hexanes accumulated with the rate of this accumulation and concentration increasing in different plant species can be different. Increasing of starch hydrolyze which it needs to hydrolytic enzymes activity resulted an increasing in total available carbohydrates concentration. Also, these results have been confirmed by the mention of the emergence of increased of sugar content under condition of salinity stress compared with control plants on tomato plant **[26]** and on barley plant **[27].**

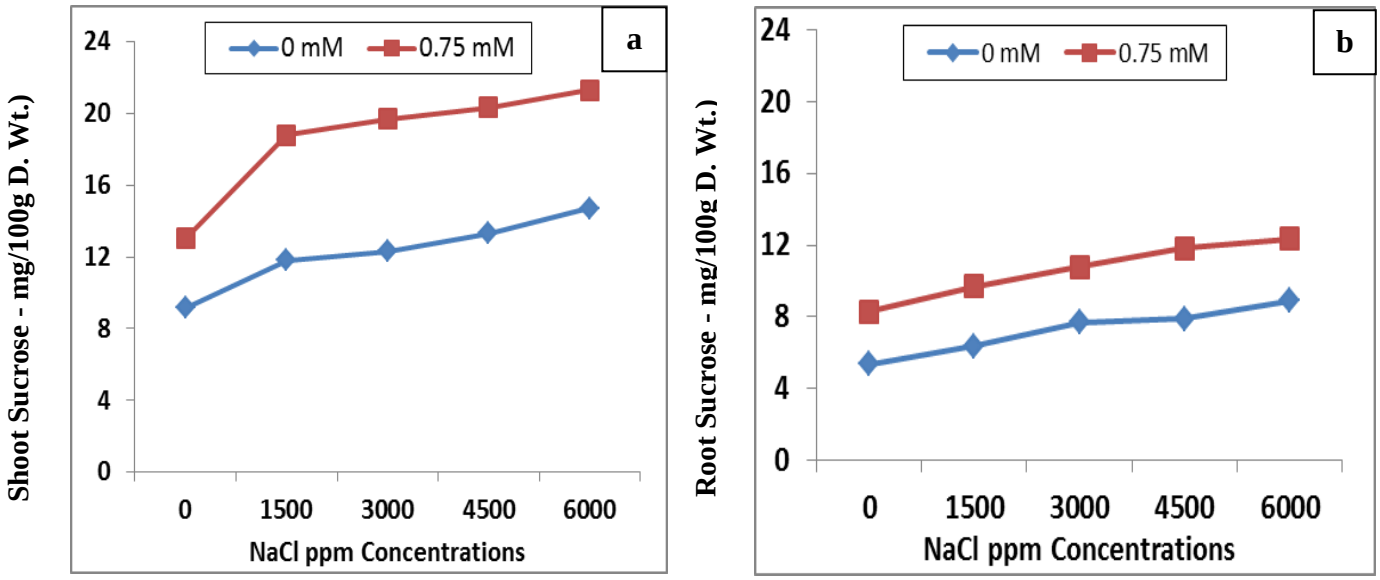

Fig. (2): Exogenous Effect of AsA (0.75 mM) on Shoot (a) and Root (b) Total Reducing Sugar (Sucrose - mg/100g D. Wt.) Contents of Tomato Plants Grown for 42 Days under Salinity Stress with Different Concentrations (0.0, 1500, 3000, 4500 & 6000 ppm NaCl). The Means of three replicates. Bars indicate – Standard Error (p = 0.05).

Table (2)): Exogenous Effect of AsA (0.75 mM) on Shoot (a) and Root (b) Total Reducing Sugar (Sucrose - mg/100g D. Wt.) Contents of Tomato Plants Grown for 42 Days Under Salinity Stress With Different Concentrations (0.0, 1500, 3000, 4500 & 6000 ppm NaCl). The Means of Three Replicates. Bars Indicate – Standard Error (p = 0.05).

AsA. (mM) / NaCl (ppm)	*Shoot*				*Root*			
	0.00	**0.75**	F_1	*p*	**0.00**	**0.75**	F_1	*p*
Control	9.17 ± 0.38	13.01 ±0.37	51.013*	<0.001*	5.37 ± 0.29	8.31 ± 0.37	147.706*	<0.001*
1500	11.81 ± 0.36	18.80 ± 0.33	75.673*	<0.001*	6.38 ± 0.23	9.70 ± 0.29	17.361*	<0.001*
3000	12.30 ± 0.46	19.70 ± 0.37	62.161*	<0.001*	7.70 ± 0.18	10.79 ± 0.22	15.364*	<0.001*
4500	13.31 ± 0.48	20.31 ± 0.52	69.201*	<0.001*	7.90 ± 0.27	11.83 ± 0.46	19.772*	<0.001*
6000	14.71 ± 0.38	21.31 ± 0.13	129.827*	<0.001*	8.89 ± 0.21	12.34 ± 0.37	28.291*	<0.001*
F_2	24.431*	79.446*			33.188*	21.574*		
p	<0.001*	<0.001*			<0.001*	<0.001*		
Overall The Two Ways Analysis of Variance (*ANOVA*)	NaCl ppm Conc.		F = 543.764*	*p*<0.001*	NaCl ppm Conc.		F = 177.745*	*p*<0.001*
	AsA(mM) Conc.		F = 534.368*	*p*<0.001*	AsA(mM) Conc.		F = 198.182*	*p*<0.001*
	NaCl ppm Conc.x AsA (mM)		F = 2.297*	*p*=0.007*	NaCl ppm Conc. x AsA (mM)		F = 0.618	*p*=0.863

3. Applications of AsA on Polysaccharides (mg/100g Dry Weight) in Shoots and Roots of Tomato Plant Response to NaCl Salinity Stress:

Overall, NaCl salinity concentrations increased significantly ($p \leq 0.001$) in shoots and roots polysaccharides content compared to control. Whereas, increasing polysaccharides content in shoot and root of tomato plants in the present of AsA with concentration (0.75 mM) was more increased than in the absent of AsA as shown in **Fig.(3 a & b) and Table (3).** The polysaccharides content increased significantly ($p \leq 0.001$) more at high NaCl salinity especially with both NaCl concentrations (4500 & 6000 ppm) with applied AsA concentration (0.75 mM) compared with control. Generally, the polysaccharides content increased in shoot more than the root at different NaCl salinity concentrations in the present or in the absent of AsA concentration at (42 days). Overall the analysis of variance (*ANOVA*) between different concentrations of NaCl in and AsA at 42 days indicated that the *F* test highly significant at $P \leq 0.001$.

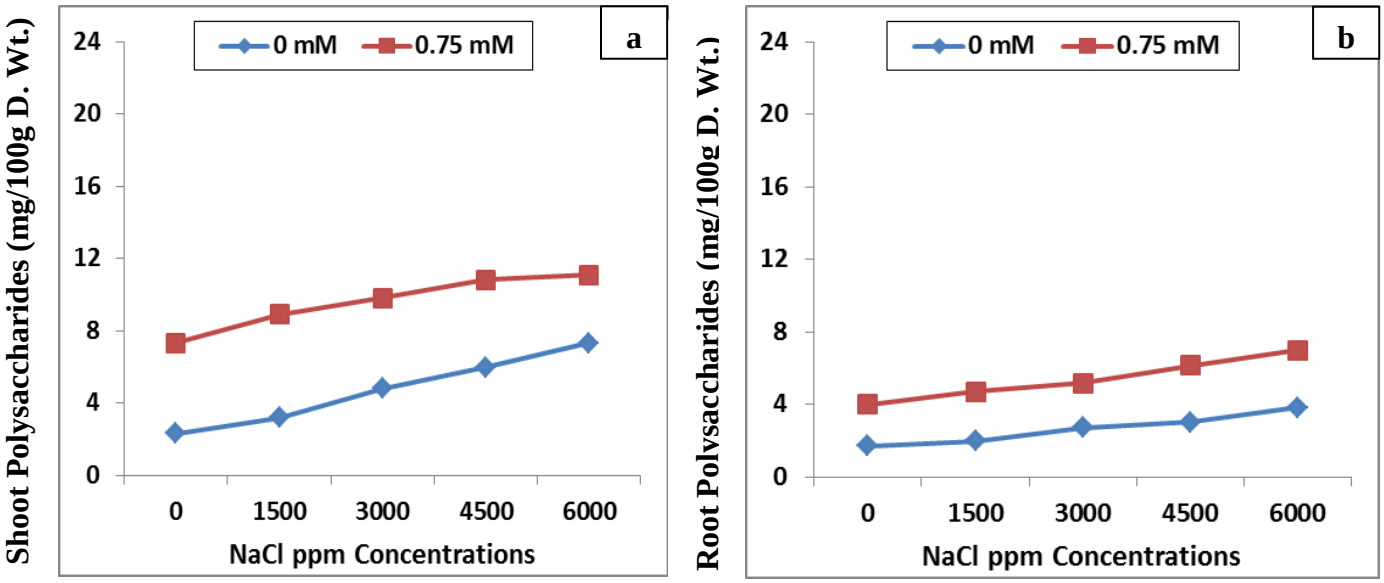

Fig. (3): Exogenous Effect of AsA (0.75 mM) on Shoot (a) and Root (b) Polysaccharides (mg/100g D. Wt.) Contents of Tomato Plants Grown for 42 Days under Salinity Stress with Different Concentrations (0.0, 1500, 3000, 4500 & 6000 ppm NaCl). The Means of three replicates. Bars indicate – Standard Error (p = 0.05).

Table (3): Exogenous Effect of AsA (0.75 mM) on Shoot (a) and Root (b) Polysaccharose (mg/100g D. Wt.) Contents of Tomato Plants Grown for 42 Days Under Salinity Stress With Different Concentrations (0.0, 1500, 3000, 4500 & 6000 ppm NaCl). The Means of Tthree Replicates. Bars Indicate – Standard Error (p = 0.05).

AsA. (mM) / NaCl (ppm)	*Shoot*				*Root*			
	0.00	0.75	F_1	*p*	0.00	0.75	F_1	*p*
Control	2.31 ± 0.03	7.31 ± 0.05	2636.657*	<0.001*	1.70 ± 0.05	3.98 ± 0.02	1182.234*	<0.001*
1500	3.17 ± 0.04	8.91 ± 0.04	2713.277*	<0.001*	1.98 ± 0.05	4.70 ± 0.03	1171.915*	<0.001*
3000	4.81 ± 0.06	9.81 ± 0.05	1760.934*	<0.001*	2.70 ± 0.04	5.17 ± 0.05	1407.064*	<0.001*
4500	5.98 ± 0.03	10.82 ± 0.05	2054.353*	<0.001*	3.01 ± 0.05	6.13 ± 0.03	1233.498*	<0.001*
6000	7.32 ± 0.04	11.09 ± 0.05	1503.526*	<0.001*	3.81 ± 0.04	6.98 ± 0.06	1110.569*	<0.001*
F_2	2398.593*	1047.796*			327.384*	767.621*		
p	<0.001*	<0.001*			<0.001*	<0.001*		
Overall The Two Ways Analysis of Variance (*ANOVA*)	NaCl ppm Conc.		F = 16326.533*	*p*<0.001*	NaCl ppm Conc.		F = 2323.208*	*p*<0.001*
	AsA(mM) Conc.		F = 22188.783*	*p*<0.001*	AsA(mM) Conc.		F = 4282.622*	*p*<0.001*
	NaCl ppm Conc. x AsA (mM)		F = 217.784*	*p*<0.001*	NaCl ppm Conc. x AsA (mM)		F = 18.621*	*p*<0.001*

4. Applications of AsA on Total Available Carbohydrates (mg/100g Dry Weight) in Shoots and Roots of Tomato Plant Response to NaCl Salinity Stress:

The responses of total available carbohydrates (TAC) in shoot and root of tomato plant increased significantly ($p \leq 0.001$) under NaCl salinity stress. The direct relation obtained between NaCl salinity and AsA concentration was increased significantly ($p \leq 0.001$) the TAC contents in shoots and roots of tomato plant at growth stage (42 days). It is clear that the TAC contents increased significantly ($p \leq 0.001$) in shoot and root of tomato plants especially in the present of AsA with concentration (0. 75 mM) compared with the control as shown in **Fig.(4 a & b) and Table (4).** Generally, the TAC contents increased in shoot more than the root with different concentrations of NaCl salinity in the present or absent of AsA at (42 days). Overall the analysis of variance (*ANOVA*) between different concentrations of NaCl and AsA at (42 days) indicated that the *F* test highly significant at $P \leq 0.001$. The results of this study agree with

results obtained by **Abd El-Aziz *et al.* [28]** they found that in *Syngonium podophyllum* L. total carbohydrate content significantly increased in plants treated with AsA (100 ppm) compared with control plants.

The results presented here agree with the findings results by **Shahba *et al.* [29]** they found that in tomato plant using NaCl (0, 25, 50, 75 and 100 mM) concentration was increased the soluble sugar in leaf and root tissues. This may be attributed in the accumulation of sugar at stress conditions; a protective mechanism enters the cell via sodium entry. So, some more of this kind of carbohydrate in cell area increases their membrane tolerance and selectivity versus ion entry like sodium and chloride **[30]** in *Calotropis procera* Ait. Also, the results presented here agree with the results obtained by **Al-Sobhi *et al.* [31]** they found that the total soluble and insoluble carbohydrates content in the shoot and root tended to increase with increasing salinity stress in the solution culture and also with plant age which considered playing an important role in the osmotic adjustment.

The results of this study agree with the results obtained by **Fercha *et al.* [32]** they applied the ascorbic acid (AsA) may enhance the salt tolerance in durum wheat. Two weeks old seedling, were subjected to salt stress by adding 25ml of NaCl (150 mm), and treated with the addition of ascorbic acid (0.7 mM) for two weeks after salt stress, the presence of NaCl in the medium induced accumulation of water soluble carbohydrates in leaves.

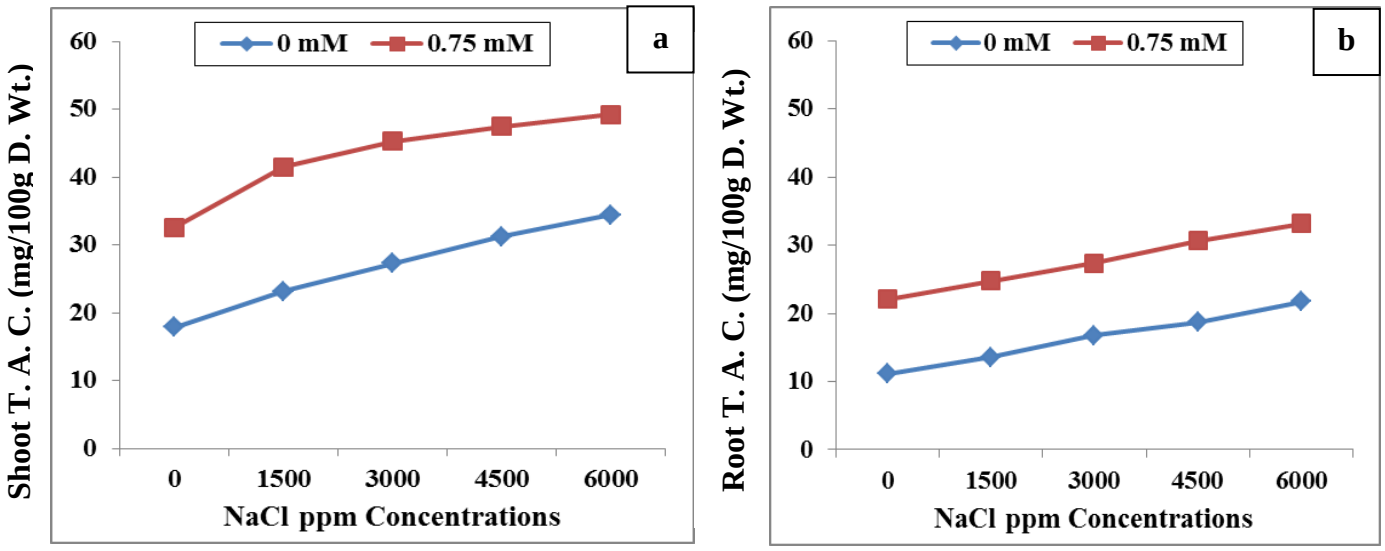

Fig. (4): Exogenous Effect of AsA (0.75 mM) on Shoot (a) and Root (b) Available Carbohydrates (T.A.C. mg/100g D. Wt.) Contents of Tomato Plants Grown for 42 Days under Salinity Stress with Different Concentrations (0.0, 1500, 3000, 4500 & 6000 ppm NaCl). The Means of three replicates. Bars indicate – Standard Error (p = 0.05).

Table (4): Exogenous Effect of AsA (0.75 mM) on Shoot (a) and Root (b) Total Available Carbohydrates (mg/100g D. Wt.) Contents of Tomato Plants Grown for 42 Days Under Salinity Stress With Different Concentrations (0.0, 1500, 3000, 4500 & 6000 ppm NaCl). The Means of Three Replicates. Bars Indicate – Standard Error (p = 0.05).

AsA. (mM) / NaCl (ppm)	*Shoot*				*Root*			
	0.00	0.75	F_1	*p*	0.00	0.75	F_1	*p*
Control	17.87 ± 0.27	32.62 ± 0.29	745.501*	<0.001*	11.15 ± 0.52	22.10 ± .46	75.310*	<0.001*
1500	23.15 ± 0.39	41.51 ± 0.36	925.415*	<0.001*	13.53 ± 0.56	24.72 ± 1.04	72.638*	<0.001*
3000	27.32 ± 0.32	45.32 ± 0.18	1248.894*	<0.001*	16.78 ± 0.27	27.33 ± 0.57	97.704*	<0.001*
4500	31.20 ± 0.53	47.44 ± 0.35	482.245*	<0.001*	18.72 ± 0.50	30.67 ± 0.57	125.552*	<0.001*
6000	34.41 ± 0.70	49.21 ± 0.20	388.295*	<0.001*	21.69 ± 0.51	33.13 ± 0.33	216.568*	<0.001*
F_2	196.506*	537.059*			74.560*	47.976*		
p	<0.001*	<0.001*			<0.001*	<0.001*		
Overall The Two Ways Analysis of Variance (*ANOVA*)	NaCl ppm Conc.		F = 2990.694*	*p*<0.001*	NaCl ppm Conc.		F = 612.855*	*p*<0.001*
	AsA(mM) Conc.		F = 3972.312*	*p*<0.001*	AsA(mM) Conc.		F = 1042.345*	*p*<0.001*
	NaCl ppm Conc. x AsA (mM)		F = 17.993*	*p*<0.001*	NaCl ppm Conc. x AsA (mM)		F = 1.185	*p*=0.293

5. Applications of AsA on Total Soluble Proteins (mg/100g Dry Weight) in Shoots and Roots of Tomato Plant Response to NaCl Salinity Stress:

The total soluble proteins (TSP) content in shoot and root of tomato plant showed a progressive increased significantly ($p \leq 0.001$) with increasing NaCl concentrations in the present or absent of AsA with concentration (0. 75 mM) at growth stage (42 days) as shown in **Fig. (5 a & b) and Table (5).** Whereas, the statistical analysis indicated that the TSP content increased significantly ($p \leq 0.001$), the TSP accumulated in shoot more than in root of tomato plant in the presence or absent of AsA (0.75Mm) at growth stage (42 days). Overall the analysis of variance (*ANOVA*) between

different concentrations of NaCl and AsA at (42days) indicated that the *F* test highly significant at $P \leq 0.001$.

Data presented by **Mahajan and Tuteja [3]** they explained that the major effect of salinity stress was the loss of intracellular water, the plants tended to accumulate many metabolites as "compatible solutes" which prevent the water loss from the cell and protect the cellular proteins.

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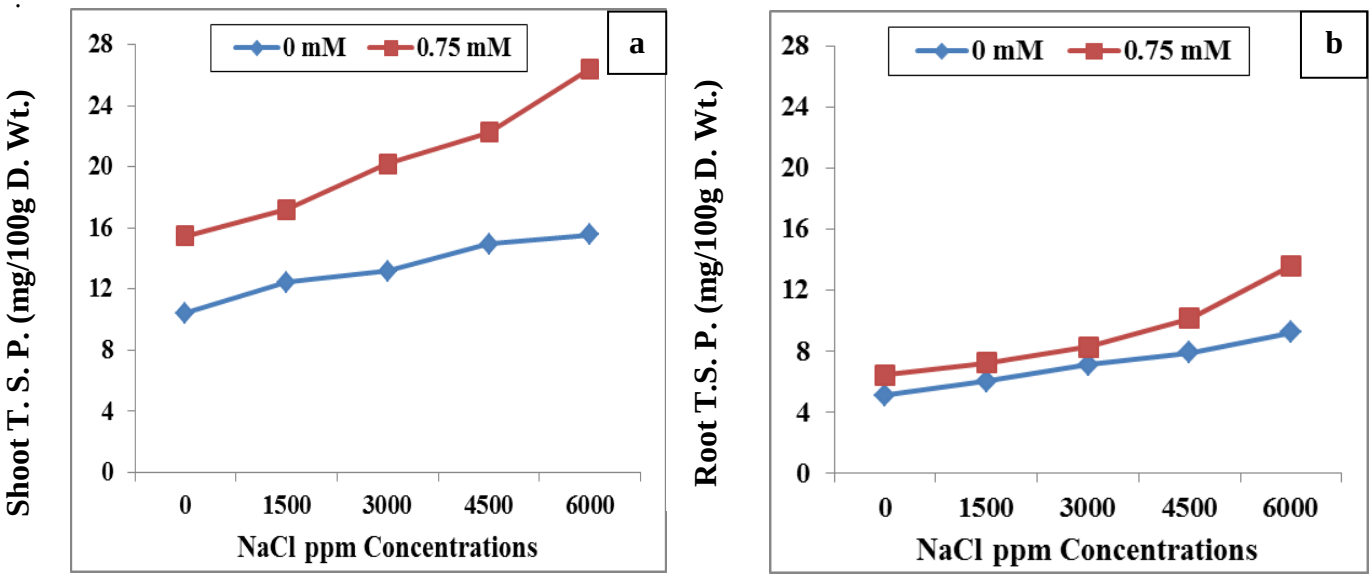

Fig. (5): Exogenous Effect of AsA (0.75 mM) on Shoot (a) and Root (b) Total Soluble Proteins (T.S.P. mg/100g D. Wt.) Contents of Tomato Plants Grown for 42 Days under Salinity Stress with Different Concentrations (0.0, 1500, 3000, 4500 & 6000 ppm NaCl). The Means of three replicates. Bars indicate–Standard Error (p = 0.05).

Table (5): Exogenous Effect of AsA (0.75 mM) on Shoot (a) and Root (b) Total Soluble Proteins (mg/100g D. Wt.) Contents of Tomato Plants Grown for 42 Days Under Salinity Stress With Different Concentrations (0.0, 1500, 3000, 4500 & 6000 ppm NaCl). The Means of Three Replicates. Bars Indicate – Standard Error (p = 0.05).

AsA. (mM) / NaCl (ppm)	*Shoot*				*Root*			
	0.00	0.75	F_1	*p*	0.00	0.75	F_1	*p*
Control	10.43 ± 0.11	15.48 ± 0.04	561.980*	<0.001*	5.15 ± 0.06	6.48 ± 0.10	50.674*	<0.001*
1500	12.43 ± 0.10	17.19 ± 0.10	413.884*	<0.001*	6.06 ± 0.07	7.27 ± 0.06	989.735*	<0.001*
3000	13.18 ± 0.07	20.17 ± 0.10	989.735*	<0.001*	7.14 ± 0.05	8.29 ± 0.07	2143.590*	<0.001*
4500	14.95 ± 0.05	22.24 ± 0.06	1836.742*	<0.001*	7.92 ± 0.04	10.18 ± 0.10	1836.742*	<0.001*
6000	15.530 ± 0.103	26.393 ± 0.101	2143.590*	<0.001*	9.25 ± 0.035	13.643 ± 0.044	769.218*	<0.001*
F_2	541.333*	2490.014*			952.471*	1303.709*		
p	<0.001*	<0.001*			<0.001*	<0.001*		
Overall The Two Ways Analysis of Variance (*ANOVA*	NaCl ppm Conc.		F =14481.983*	*p*<0.001*	NaCl ppm Conc.		F = 6565.888*	*p*<0.001*
	AsA(mM) Conc.		F = 9542.033*	*p*<0.001*	AsA(mM) Conc.		F = 1231.047*	*p*<0.001*
	NaCl ppm Conc. x AsA (mM)		F = 71.745*	*p*<0.001*	NaCl ppm Conc. x AsA (mM)		F = 27.558*	*p*<0.001*

Results of a recent study by **Kapoor and Srivastava [33]** on *Vigna mungo*, L. supports our findings in this study, they observed an increase in protein content when increasing salt concentration.

Results of this study coincide with what the **Sibole *et al.* [34]** they reported that the treatment of clover plant (*Medicago citrna*, L.) for 30 days with NaCl salinity concentrations (0.0; 1; 50; 100; 200 mM) increased soluble protein content in the seedlings, compared with control plants. Similarly, **Ashraf and Harris [35]** they found that in salt tolerant cultivars of barley, sunflower finger millet, and rice increased the content of soluble proteins. Also**, Tort and Turkyilmaz [36]** recorded that a big increase in protein content when treating barley (*Hordeum vulgare* L.) plant with 120 mM of sodium chloride.

Protein content can also be affected negatively or positively by salt stress. The salinity stress interferes with nitrogen consumption and absorption, the salt stress condition could have effect on different stages of nitrogen metabolism, such as absorption, ionic reduction and protein synthesis, so NaCl stress severely reduced leaf protein contents in *Phaseolus vulgaris* plants **[37-38].** Doganlar et. al. **[39]** they treated the different varieties (cultivars) of tomato (*Lycopersicon esculentum* Mill.), at seedlings with NaCl (25; 50; 100; 125; 150; 200 mM) concentrations for 96 h with 24 h interval. They found that the pigment and total soluble protein contents of all tomato cultivars were significantly decreased by salt stress depending on time intervals and salt concentrations

6. Applications of AsA on Proline Content (mg/100g Dry Weight) in Shoots and Roots of Tomato Plant Response to NaCl Salinity Stress:

The proline content increased significantly ($P \leq 0.001$) in both shoot and root of tomato plant with increasing NaCl salinity in the present or absent AsA with concentration (0. 75 mM) at growth stage (42 days) as shown in **Fig.(6 a & b) and Table (6).** The level of proline being higher in shoot than root of tomato plant at growth stage (42days). Generally, AsA increased significantly ($p \leq 0.001$) the proline content in shoot and root of tomato plant growing under saline and non-saline conditions at growth stage (42days). Overall the analysis of variance (*ANOVA*) between different concentrations of NaCl and AsA at 42 days indicated that the *F* test highly significant at $P \leq 0.001$

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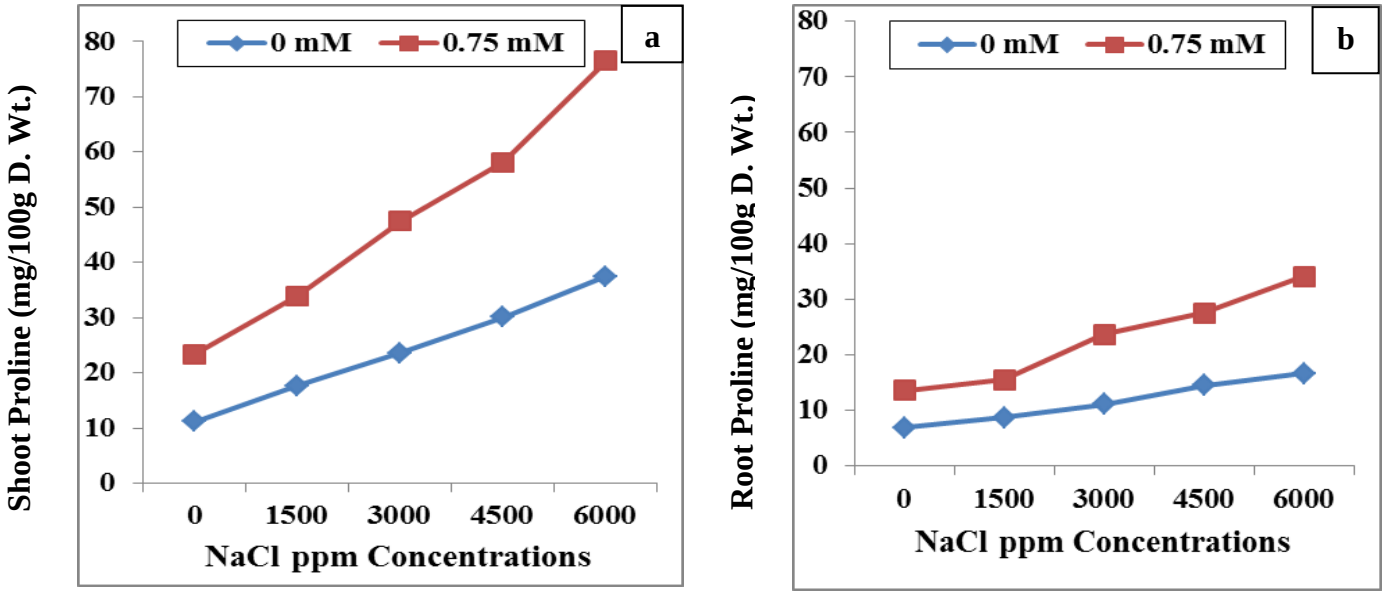

Fig. (6): Exogenous Effect of AsA (0.75 mM) on Shoot (a) and Root (b) Proline (mg/100g D. Wt.) Contents of Tomato Plants Grown for 42 Days Under Salinity Stress With Different Concentrations (0.0, 1500, 3000, 4500 & 6000 ppm NaCl). The Means of Tthree Replicates. Bars Indicate – Standard Error (p = 0.05).

Table (6): Effects of AsA (0.75 mM) on Shoot and Root Proline (mg/100g D. Wt.) Contents of tomato plants grown for 42 days under salinity stress with different concentrations (0.0, 1500, 3000, 4500 & 6000 ppm NaCl). The Means of Three Replicates. Bars Indicate – Standard Error ($p = 0.05$).

AsA. (mM) / NaCl (ppm)	*Shoot*				*Root*			
	0.00	**0.75**	F_1	p	**0.00**	**0.75**	F_1	p
Control	11.18 ± 0.03	23.41 ± 0.35	180.049*	<0.001*	6.91 ± 0.46	13.61 ± 0.34	73.271*	<0.001*
1500	17.69 ± 0.08	33.97 ± 0.20	2424.170*	<0.001*	8.73 ± 0.09	15.47 ± 0.20	29.920*	<0.001*
3000	23.59 ± 1.10	47.42 ± 0.05	234.115*	<0.001*	11.02 ± 0.64	23.69 ± 0.40	141.955*	<0.001*
4500	30.11 ± 0.05	58.14 ± 0.09	576.934*	<0.001*	14.49 ± 0.20	27.54 ± 0.36	109.340*	<0.001*
6000	37.40 ± 0.751	76.52 ± 0.53	713.581*	<0.001*	16.570 ± 0.981	34.030 ± 0.144	143.719*	<0.001*
F_2	296.501*	4757.470*			48.897*	763.042*		
p	<0.001*	<0.001*			<0.001*	<0.001*		
Overall The Two Ways Analysis of Variance (*ANOVA*	**NaCl ppm Conc.**		F = 1595.031*	p<0.001*	**NaCl ppm Conc.**		F = 1126.685*	p<0.001*
	AsA(mM) Conc.		F = 561.102*	p<0.001*	**AsA(mM) Conc.**		F = 672.381*	p<0.001*
	NaCl ppm Conc. x AsA (mM)		F = 1.519	p=0.108	**NaCl ppm Conc. x AsA (mM)**		F = 1126.685*	p<0.001*

Amino acid (proline) is known to occur widely in higher plants and normally accumulates in large quantities in response to environmental stresses **[40]. Rontein *et al.* [41]** they reported that the proline is a dominant organic molecule that acts as a mediator of osmotic adjustment under salinity stress, a stabilizer of sub-cellular structures, a sink for energy and even a stress-related signal. It is also involved in cell osmoregulation, protection of proteins during dehydration and can act as an enzymatic regulator during stress conditions.

The results presented here agree with the results obtained by **Fercha *et al.* [32]** they found that the effect of AsA on the contents of proline and soluble sugars can suggest that AsA probably improves growth of stressed plants, further to its antioxidant action, by intensification of their potential for osmotic adjustment and activities of growth (cell division and expansion). Proline may also serve as an organic nitrogen reservoir ready to be used after stress relief to sustain both amino acid and protein synthesis **[42-43].**

The results of the study confirmed the findings of the **Chookhampaeng [44]** where he noted that the salinity treatments caused the increased proline content in pepper plant. 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. Also, The results obtained by **Babu *et al.* [45]** they found that the proline accumulation increased with increase in salt stress, this was explained by in tomato cultivar PKM 1 were subjected to 25, 50, 100, 150 and 200 mM NaCl stress. The salt tolerance of tomato cultivars is dependent on the ability to synthesize proline under salinity stress conditions **[46].** Also, the similar results obtained by **Shahba *et al.* [29]** they found that the proline level increased in leaf and root of tomato plant using NaCl concentration (0, 25, 50, 75 and 100 mM).

A large number of plant species accumulate proline in response to salinity stress and that accumulation may play a role in defense against salinity stress. However, data do not always indicate a positive correlation between osmolytes accumulation and an ability to adapt to stress. Salinity increased markedly the proline content in different salt sensitive and tolerant species/cultivars with greater proline accumulation in salt tolerant ones, which is supposed to correlate with the adaptation to salinity **[35, 47-48].**

Increasing of proline level in cotton under salinity stress was recognized due to that proline are consistent smolite, macromolecules conserver and remover of active oxygen producing in during of environmental stress **[49].** Also, increasing of proline content in *Paulownia imperialis* **[50]** and wheat **[51]** was synchronized with increasing of salinity levels.

According to **Eraslan *et al.* [52]** Both NaCl and fertilizer induced salinity significantly increased proline concentration of tomato and pepper plants. Understanding the biosynthesis, degradation, transport and role of proline during stress and signaling events that regulate stress induced accumulation is vital in developing plants for stress tolerance. An increased proline level is a common response of plants to stress of treatments **[53-56].**

5. Conclusion:

Generally, this study concluded that the ascorbic acid (75 mM) resulted an increased the total available carbohydrates (reducing sugars, non-reducing sugars [sucrose] and polysaccharides), proteins and proline for both shoot and root of the tomato plant and mitigate the impact of salinity inhibitory to the plant metabolism. Whereas, the ascorbic acid (AsA 75 mM) tended to reduce the harmful effects of salinity and increases resistance to salinity in tomato plant.

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