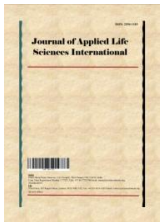

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Influence of Ascorbic Acid, Gibberellic Acid and *Moringa oleifera* Extract for Alleviating Salinity Stress by Enhancing Antioxidant Enzymatic Activity and Some Physiological Studies on Two Tomato Cultivars

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Authors' contributions

This work was carried out in collaboration between both authors. Authors IMAA and HEAE designed the study, wrote the protocol, initiated the experiments, collected the data, performed the statistical analysis, managed the literature review and wrote the final draft of the manuscript. Both authors read and approved the final manuscript.

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ABSTRACT

This study aimed to explain the influence of Ascorbic acid (ASA), Gibberellic Acid (GA3) and *Moringa oleifera* Leaf Extract (MLE) for alleviating salinity stress by enhancing antioxidant enzymatic activity as follow: Super Oxide Dismutase (SOD), Catalase (CAT), Ascorbate Peroxidase (APX), and Glutathione Reductase (GR); nitrogenous components (proline and total amino acids) and some inorganic mineral nutrient elements in two tomato cultivars, cv. Cobra (resistant) and cv. Newton (sensitive) under salinity stress. Germination tomato seeds after soaking in ASA (0.75 mM); GA3 (0.05 mM) and MLE (5%), transplanted to plastic containers containing a mixture of sand/peat-moss (1:2). The tomato seeds for both cultivars watering using distilled water until the true leaf appearance then irrigated with NaCl salinity concentrations (0.0, 50, 100, 150, 200 mM) alternative

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with Hoagland nutrient solution. The experiment was carried out under greenhouse conditions with temperature 18°C±1°C (night) & 22°C±2°C (day) and relative humidity varied between 60 - 70%. Overall, the results indicated that the organic and inorganic components in tomato plants for both cultivars increased significantly in the present of ASA, GA3 and MLE under salinity stress respectively compared with control, there by reduces the harmful effects of salinity and increases resistance to salinity stress more than in the absent of ASA, GA3 and MLE. The data provide strong support to the hypothesis that exogenous application of ASA, GA3 and MLE reduced the harmful effects of NaCl concentrations and increases resistance to salinity in cv. Cobra and cv. Newton respectively. The evident recorded a significantly increased the antioxidant enzymes activity, proline, total amino acids and inorganic macro-mineral nutrient elements (N^{+3}, P^{+3}, K^{+}, Ca^{+2} & Mg^{+2}) and micro-nutrient mineral elements (Mn^{+2}, Fe^{+3} & B^{+2}) but after soaked the seeds in ASA, GA3 and MLE, these components tended to increase more compared with the control. Whereas, the tomato seeds soaked before planting in ASA, GA3 and MLE which leads to remarkably increasing more for all antioxidant enzymatic activity, nitrogenous components and inorganic mineral nutrient elements contents respectively. The relationship between compatible solutes (osmolytes) here are the strategies that plants have developed to tolerate salt stress and produced new strains adapted to salinity stress.

Keywords: Ascorbic acid; gibberellic acid; Moringa oleifera; proline; enzyme; salinity stress; Lycopersicon esculentum; antioxidant enzymes super oxide dismutase; catalase; Ascorbate Peroxidase; glutathione reductase; elements.

ABBREVIATIONS

ASA : Ascorbic acid
GA3 : Gibberellic Acid
MLE : Moringa Leaf Extract
SOD : Super Oxide Dismutase
CAT : Catalase
APX : Ascorbate Peroxidase
GR : Glutathione Reductase
ROS : Reactive oxygen species

1. INTRODUCTION

Salinity is the accumulation of excessive salt contents in the soil which eventually results in the inhibition of crop growth and leads to crop destruction. Millions of hectares of land, throughout the world, are too saline to produce economic crop yields and land becomes more unproductive each year as a result of salt accumulation. Agriculture plays a pioneering role in economic development in most countries, especially in Saudi Arabia [1]. Abiotic stress includes all of the high salinity; drought, extreme temperatures and oxidative stress due to chemical toxicity are key points that affect crop yield by affecting plant growth and productivity. Salinity is one of the important constraints and better understanding of the mechanisms that enable plants to adapt to salinity stress and maintain growth will ultimately help in the selection of stress tolerant cultivars for exploiting saline soils [2]. Salinity is one of the most important abiotic stress factors that limit plant growth, photosynthesis, and productivity [3]. As with other development processes, the various techniques that plants use to counter the adverse effects of abiotic stress require the arrangement of complex hormone signaling pathways to activate the acclimatization of plants to stress conditions [4].

Ascorbic Acid (ASA) is one of the most powerful antioxidants, adding ascorbic acid (vitamin C) to involve in many physiological processes in plants. It has an essential function in the defense against plant antioxidants, in the elongation and cell division as well as in the optimization of photosynthesis [5]. El Sayed et al. [6] have shown that ascorbic acid (ASA) is one of the most powerful antioxidants. Adding ascorbic acid (vitamin C) to the tomato seedling could reduce the synthesis of active oxygen species and thereby increase resistance to salt stress. Ascorbic acid (ASA) is associated with chloroplasts and plays a role in improving the oxidative stress of photosynthesis. Ascorbic acid (ASA) has many of other roles in cell division and protein modification. El Sayed et al. [6-7] reported that exogenous application of ascorbic acid mitigated the dangerous effect of salinity on tomato plants and increased the growth, yield macro and micronutrients of tomato plants and an improvement in fruit quality.

Gibberellic Acid (GA3) are plant hormones that are associated with various plant growth and development processes [8-9]. Maggio et al., [10]

said that gibberellins (GA3) play a central role in heavy metal detoxification and tolerance to salt stress by improving the activity of antioxidant enzymes and preventing lipid peroxidation. Miceli et al. [11] confirmed that gibberellins (GAs) are growth hormones that contribute greatly to a variety of physiological activities. Akhtar et al. [12] found that the use of gibberellin as a leaf sprays increased the accumulation of proline, the potassium content under salt stress.

Moringa oleifera, L. plants has been reported to be a rich source of many minerals such as Ca, P, Na, Mg, K, Fe and others that can be valorized for nutrition balance in plants[13]. *Moringa oleifera* extract is an ability to improve plant development because it is rich in amino acids, phenols and essential elements [14]. *Moringa oleifera* really shows a talent for nature and a "miracle plant" with incalculable advantages that can be used to improve plants that grow under biotic stress. *Moringa oleifera* leaf extract (MLE) has an antimicrobial and antioxidant effect and can be used in the food processing and packaging industries [15]. Moringa's leaves have several macro elements such as Mg& Ca which leads to an increase in the activity of antioxidants [16]. Abd El-Rahman and Mohamed [17] stated that *Moringa oleifera* leaf extraction contains plant growth regulators, antioxidants, certain nutrients, and organic and inorganic chemicals that were used to promote plant growth and development to induce biotic and abiotic stress tolerance, which leads to a higher tolerance economic return.

Due to the importance of Tomato (*Solanum lycopersicum*) plant is widely used as a model crop for fruit development but also for diverse physiological, cellular, biochemical, molecular and genetic studies. It is considered to be the most important vegetable crop in the world. Tomato plant is a rich source of lycopene and vitamins. Lycopene may help to counteract the harmful effects of substances called "free radicals", which are thought to contribute to age-related processes and a number of types of cancer, including, but not limited to, those of prostate, lung, stomach, pancreas, breast, cervix, colorectum, mouth and esophagus [18-19]. Tomato cultivars may differ in their sensitivity to salinity stress; thus, the selection of salt tolerant cultivars may help to improve the performance of tomato plants in saline conditions [20]. Tomato (*Lycopersicon esculentum,* L.) plant are important sources for important nutritious, provide a balanced source of vitamins A, C, and E, which are necessary for maintaining good human health, it's also contains folic acid, pantothenic acid; Biotin, Vitamin K and inhibitors related to Vitamin E [21 -23] .

The present study was conducted to assess the role of ASA, GA3 and *Moringa oleifera* Leaves Extract (MLE) pre-treatment in alleviating the adverse effects of salinity stress by enhancing the antioxidant *enzymes activity as follow; [*Super Oxide Dismutase (SOD), Catalase (CAT), Ascorbate Peroxidase (APX), and Glutathione Reductase (GR)], proline; total amino acids; and inorganic macro & micro Mineral nutrient elements of tomato cultivars cv. Cobra (resistant) and cv. Newton (sensitive) under salinity stress, by soaking the seeds before germination for 12 h in ASA (0.75 mM), GA3 (0.05 mM) and *Moringa oleifera* Moringa leaves extract (MLE - 5%) then cultivated both cultivars under NaCl salinity concentrations (0.0, 50, 100, 150 & 200 mM) to produce new strains adapted to salinity stress and selection of salt tolerant cultivars may help to improve the performance of tomato plants in saline conditions.

2. MATERIALS AND METHODS

2.1 NaCl Salinity Concentrations

Prepared Molar solution (1M) NaCl concentrations, from a molar solution, prepare different concentrations of NaCl (0.0, 50, 100, 150 and 200 mM).

2.2 The Soil Used

The soil used for cultivated tomato plant was the ratio between the peat- moss with agricultural perlite (agrolite) (3:1) then add sand, as a ration (2:1–v: v), in each pot (diameter 16 cm and depth of 16 cm), completed by the same size in each pot using the ratio from the peat moss/soil sand (2:1-v: v).

2.3 Tomato Plant Species and Culture Techniques

The plant used in this study is tomato *(Lycopersicon esculentum*, L.) seeds for both cultivars (cv. Cobra and cv. Newton) which are resistant and sensitive respectively to salinity stress. Both cultivars are characterized by its earliness, high yield ability, uniform ripening and disease tolerance. The tomato seeds obtained from Al-Dakhil Agriculture and Trading Establishment, and were undertaken in the

greenhouse at Al Qassim city, Kingdom of Saudi Arabia.

2.4 Nutrient Solutions

The base nutrient solution used was similar to that applied by Hoagland and Arnon [24] and was composed of: (2.5 x 10^{-7} M KNO_3, 5 x 10^{-4} M KH_2PO_4, 2.5 x 10^{-5} M $Ca(NO_3)_2$, 10^{-3} M $MgSO_4$). A supplementary solution the essential trace element was added to the nutrient solution and this contained 2.3 x 10^{-7} M H_2BO_4, 7x10^{-8} M $MnCl_2$, 7 x 10^{-9} M $ZnSO_4$, $7H_2O$, 2.5 x 10^{-7} $CuSO_4$, $5H_2O$, 6 x 10^{-9} M $(NH_4)MoO_4$ and 1.6 x 10^{-6} M Ferric Citrate. The solution was held at pH 6 throughout the experiment alternative with salinity different concentrations.

2.5 Ascorbic Acid (ASA -0.75 mM)

Ascorbic acid (ASA - 0.75 mM) obtained from *Sigma Chemical Company, UK*, was initially dissolved in a little amount of distilled water and the final volume was reached, using distilled water.

2.6 Gibberellic Acid (GA3 -0.05 mM)

Gibberellic Acid (0.05mM) obtained from *Somatco Laboratory Chemicals Company, Saudi Arabia,* was initially dissolved in a little amount of distilled water and the final volume was reached, using distilled water.

2.7 Moringa Leaves Extract (MLE)

For preparation of moringa leaves extract take one kilogram (1 kg) from moringa leaves (air-dried under shade for two weeks and grounded to reach powder) then mixed with one litre of ethyl alcohol (80% aqueous) using a blender. The extract was purified by filtering twice through (Whatman No. 1) filter paper. After purification the extract was subjected to a rotary evaporator to fully evaporate the alcohol and get the crude extract. The concentrations 5% were prepared by take from the crude extract 5 ml and diluted with 95 ml distilled water for prepare MLE 5% 100 ml [24].

2.8 Germination and Transplanting Tomato (*Lycopersicon esculentum*, L.)

Selected seeds of tomato (*Lycopersicon esculentum*, L.) plant for two cultivars, (cv. Cobra and cv. Newton) intact, homogeneous in size and free from wrinkles. Then soaked the seeds for 12 hours in the dark (1) 1st group, seeds soaked in distilled water (control). (2) 2nd group, seeds soaked in a solution of ascorbic acid (ASA - 0.75 mM). (3) 3rd group, seeds soaked in a solution of gibberellic acid (GA3 - 0.05 mM). (4) 4th group, seeds soaked in Moringa Leaves Extract (MLE - 5%). After germination (15 days) transplanted in pots (diameter 16 cm and depth of 16 cm), each pot containing the same volume of washing sandy soil and peat moss, (1: 2 - v: v), under greenhouse conditions at temperature of 18°C±1°C (night) 22˚C±2˚C (day) and relative humidity varied between 60 - 70% the sand culture technique and nutrient solution were similar to those adopted by Hoagland and Arnon [25].

2.9 Irrigation System

Irrigation system by using different NaCl salinity concentrations (50; 100; 150 and 200 mM), alternative with nutrient Hoagland solution. To avoid the accumulation of salts in pot and improve the growth, using a hand spray for irrigation system. The irrigated system was applied twice a week (once every two days) by 400 ml different NaCl concentrations, alternative the same amount of water.

2.10 Salinity and Plant Growth

Harvest plants have been growth stages started from transplanting the seedling plants the growth stage after 70 days from transplanting (84 days). Determined all growth parameters by using three replicates for each treatment, three plants for each treatment were washed with distilled water, blotted thoroughly and then divided into root and shoot.

2.11 Estimation of Antioxidant Enzymes Activity

For Super Oxide Dismutase (SOD), Catalase (CAT), Ascorbate Peroxidase (APX), and Glutathione Reductase (GR) extraction, leaf samples about (0.5 g) were homogenized in 8ml of 0.1 M phosphate buffer (pH=7.5) on ice bath and each homogenate was centrifuged at 4°C in Beckman refrigerated centrifuge for 15 min at 15000×g. The supernatant was used for enzyme activity assay by Esfandiari et al. [26].

2.12 Superoxide Dismutase (SOD) Activity Enzyme

The plant sample supernatant was used for enzyme activity assay according to Esfandiari et al. [26] within 12h of extraction. Superoxide dismutase (SOD) activity was estimated by recording the decrease in absorbance of superoxide nitro blue tetrazolium complex by the enzyme according to Gupta et al. [27].

2.13 Catalase (CAT) Activity Enzyme

Extraction of soluble proteins by a frozen sample of 0.5 g tomato leaves was homogenized in 8 ml of 50 mM cold phosphate buffer at pH 7.5 modified from Beauchamp and Fridovich [28]. The homogenates were centrifuged at 4000 rpm for 20 min and the supernatant was used as a crude extract for enzymatic assay Catalase (CAT) was measured according to Aebi [29].

2.14 Ascorbate Peroxidase (APX) Activity Enzyme

Ascorbate peroxidase (APX) activity was measured according to Yoshimura et al. [30] by monitoring the rate of ascorbate oxidation at 290 nm. The reaction mixture contained 0.1 ml of 25 mM phosphate buffer (pH=7), 0.1 ml of 0.1 mM Na- ethylenediaminetetra acetic acid (EDTA), 0.1 ml of 1 mM H_2O_2, 0.2 of 0.25 mM ASA 0.2 ml of the enzyme sample and complete to 3 ml with water.

2.15 Glutathione Reductase (GR) Activity Enzyme

Glutathione reductase (GR) was assayed by recording to increase the absorbance in the presence of oxidized glutathione and 5, 5-dithiobis-2-nitrbenzoic acid**.** The absorbance at 412 nm recorded at 25°C over a period of 5 min on a spectrophotometer. For enzyme specific activity = R2X100/ R1 according to Sairam [31].

2.16 Estimation of Proline Content

Proline content was determined calorimetrically 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°C, the reagent remains stable for 24 h. 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 µg proline/100 g dry weight according to the method of Bates *et al.* [32].

2.17 Estimation of Total Amino Acid Contents

These were determined by the method described by Ya and Tunekazu. [33]. An aliquot of 0.1 ml plant extract was heated in a test tube with 1.9 ml of ninhydrin citrate buffer-glycerol mixture in a boiling water bath for 12 min and cooled at room temperature. Then the tube was well shaken, and the optical density read at 570 nm. A blank was determined with 0.1 ml of distilled water and a standard curve obtained with 0.005 to 0.2 mM g Glycine.

2.18 Inorganic Mineral Nutrient Elements

Cation contents of the milled samples were estimated following the "wet ashing procedure" of the powdered samples as described by Richards [34]. The acid digests of the oven dried samples were analyzed for potassium, calcium, magnesium and determinations. Potassium (K^+) and calcium (Ca^{+2}) contents were determined photometrically using a corning- 400 flam photometer [35-36]. The levels of magnesium (Mg^{+2}) and manganese (Mn^{+2}) contents were determined using an atomic absorption spectrophotometer. The mixed- acid digestion method was used in preparing the sample solution for determination of element content. Phosphorus (P^{+3}) was estimated by the Molybdenum-blue method while Nitrogen was estimated by the Automatic MicroKjeldahl Allen et al. [36] Automatic MicroKjeldahl consists of:

1. Digestion system.
2. Kjeltec distillation system.

Procedure: Take 250 mg oven dry plant materials together with a tablet of mercuric chloride and 6 ml concentrated H_2SO_4 were placed in tubes in digestion system unit the temperature reached 420ºC. After ½ h. the tubes were removed, cooled, and 25 ml distilled water added. Concentrated NaOH was added to make the solution alkaline and then the mixture was distilled, volatile nitrogenous, materials being trapped in a boric acid solution. The latter was subsequently titrated against 0.1 N HCl, using universal indicator (end point from blue to pink), and the total nitrogen (N^{+3}) calculated from the equation:

$$\text{Total Nitrogen } \% = \frac{(\text{ml.acid} - \text{ml.blank}) \times 0.1 \times 14.007}{\text{Wt.sample (mg)}} \times 100 \quad (1)$$

0.1= Normality of acid; 14.007 = Atomic wt. of nitrogen.

Iron was determined and the procedure were similar to that used in the study by Sharma *et al*. [37], while Boron was determined by the ICP - AES technique, measurements being carried out after extraction from the ashed milled samples at 550ºC in a muffle furnace with HCl. The equipment involved Phillips PV 8490 Plasma Source Unit Linked to a spectraspan III Echelle spectrometer controlled by an Apple II_e microcomputer. The inductively Coupled Plasma Atomic Emission Spectrometer technique (*ICP-AES*) involves a microcomputer controlled inductively coupled plasma emission spectrometer. The computer also stores the corrected intensity values from the samples and then calculates the concentration of analytic with reference to the calibration graph. The computer finds the best – fit line using least squares analysis, it calculates the intensity which should occur for each concentration using the specific time calculated concentration in ppm described by Allen *et al*. [36].

1. Measured standards.
2. Measured Samples.
3. Calibration Procedure

2.19 Statistical Analysis

Statistical analysis of the data was fed to the computer and analyzed using IBM SPSS software package version 20.0. For normally distributed data, comparison between different groups was analyzed using *F*-test (*ANOVA*). To find the effects between stages, Ascorbic acid, Gibberellic Acid (mM), Moringa Leaves Extract (%) and NaCl ppm and their interactions two ways *ANOVA* was used. Significance test results are quoted as two-tailed probabilities. Significance of the obtained results was judged at the 5% level [38-39].

3. RESULTS AND DISCUSSION

3.1 Antioxidant Enzyme Activity

Overall, Data presented in Figs. ($1_{A, B, C \& D}$) and Tables ($1_{A, B, C \& D}$) indicated that the Super Oxide Dismutase (SOD), Catalase (CAT), Ascorbate Peroxidase (APX), Glutathione Reductase (GR) (Units/mg protein) antioxidant enzyme activity contents increased significantly ($P \leq 0.001$) in leaves of tomato plant for both cultivars with increasing NaCl salinity concentrations (50, 100, 150 & 200 mM NaCl) in the presence or absence of ASA (0.75 mM), GA3 (0.05 mM) and MLE (5%) compared with control. The role of ASA on tomato plant for both cultivars have been alleviated the effect of salinity on the antioxidant enzyme activities in leaves more than GA3 and MLE except the APX enzyme decreased with increase salinity concentration (200 mM NaCl) with cv. Newton compared with control. The antioxidant enzyme activates increased significantly ($p \leq 0.001$) more in cv. Cobra than in cv. Newton especially in the present of ASA more than with GA3 and MLE compared with control. Overall the statistical analysis indicated that the two ways analysis of variance (*ANOVA*) between different concentrations of salinity stress ASA, GA3 and MLE in two tomato cultivars indicated that the F test and *LSD* test highly significant at $P \leq 0.001$.

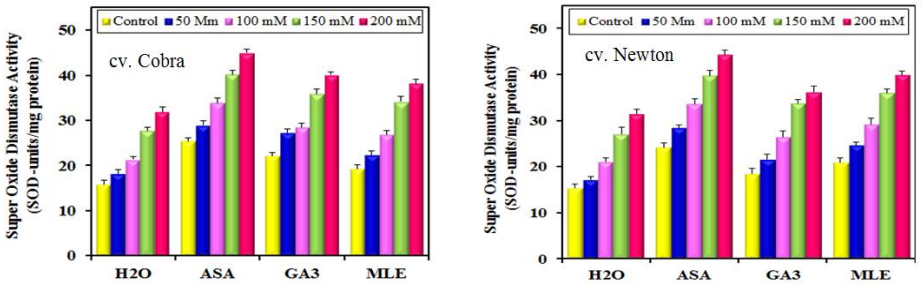

Fig. (1A). Influence of ASA, GA3 and MLE on Super Oxide Dismutase Activity (SOD- units/mg. protein) contents in shoot of tomato (*Lycopersicon esculentum*, L. cv. Cobra & cv. Newton) under salinity stress

Table (1A). Statistical analysis for influence of ASA, GA3 and MLE on super oxide dismutase activity (SOD- units/mg. protein) contents in shoot of tomato (*Lycopersicon esculentum*, L. cv. Cobra & cv. Newton) under salinity stress

NaCl Conc. (mM)	Super Oxide Dismutase (SOD) Activities (Units/mg protein)							
	cv. Cobra				cv. Newton			
Statistical Analysis (*ANOVA*)	H_2O	ASA	GA3	MLE	H_2O	ASA	GA3	MLE
F	61.853*	70.755*	78.104*	70.248*	46.945*	67.653*	40.183*	65.233*
p	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*
LSD	2.677	3.004	2.560	2.972	3.123	3.152	3.785	3.059

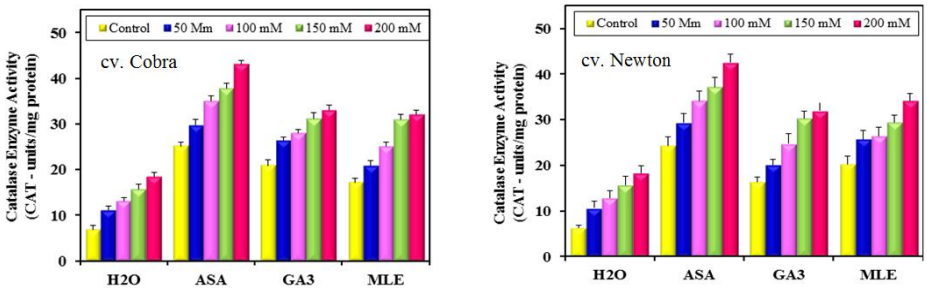

Fig. (1B). Influence of ASA, GA3 and MLE on catalase enzyme activity (CAT- units/mg. protein) contents in shoot of tomato (*Lycopersicon esculentum*, L. cv. Cobra & cv. Newton) under salinity stress

Table (1B). Statistical analysis for influence of ASA, GA3 and MLE on catalase enzyme activity (CAT- units/mg. protein) contents in shoot of tomato (*Lycopersicon esculentum*, L. cv. Cobra & cv. Newton) under salinity stress

NaCl Conc. (mM)	Ascorbate Peroxidase (APX) Activities (Units/mg protein)							
	cv. Cobra				cv. Newton			
Statistical Analysis (*ANOVA*)	H_2O	ASA	GA3	MLE	H_2O	ASA	GA3	MLE
F	19.090*	14.222*	41.515*	34.263*	9.804*	10.009*	10.820*	19.212*
p	<0.001*	<0.001*	<0.001*	<0.001*	<0.002*	<0.002*	<0.001*	<0.001*
LSD	2.964	5.441	2.920	3.394	5.117	6.724	5.667	5.911

Radical protective mechanisms are enzymatic antioxidant system that includes the superoxide dismutase found in various cell compartments; Catalyzes enzymes are a conversion from two O_2^- radicals to H_2O_2 and O_2 by converting it to be water [40-41]. Moreover, there remain some important enzyme systems that play the important role in ROS scavenging by working around ascorbate-glutathione cycle such as glutathione reductase [42]. Plants that are exposed to high salinity condition can be stressed with reactive oxygen species (ROS) such as superoxide (O_2^-), hydrogen peroxide (H_2O_2) and hydroxyl radicals (OH^-). These existed ROSs have much ability to harm plant tissues due to their highly reactive properties [43]. Naturally, some plants can develop several protective mechanisms that can effectively eliminate or reduce the ROSs at different stress induced deterioration levels and the ability has been known to be varied in species and varieties [44].

Khalid and Aftab [45] reported that the Antioxidant enzymes were superoxide dismutase (SOD) activities was recorded after 30 d. from grown *Solanum tuberosum,* L. under salt stress were increased with GA3 under NaCl stress, therefore, the exogenous application of GA3 played a positive role and significantly affected of the biochemical parameters. Superoxide dismutase (SOD) activities tended to be increased at low, dropped at medium and turned back again at high salt concentration, this phenomenon can be simply explained by enzyme optimum that the salt concentrations

within activity decrease range may be out of enzyme's optimal margins [46].

Glutathione Reductase (GR) found in chloroplasts as well as in mitochondria and cytoplasm, GR catalyzes the rate limiting last step of ascorbate-glutathione pathway. Salt stress caused an increase in GR activity and the elevated levels of GR activity perhaps could increase the ratio of $NADP^+$/ NADPH [47]. Baisak et al. [48 showed that Increase in the glutathione reductase activity in plants resulted in the accumulation of glutathione levels and ultimately confers the tolerance of plants. Ascorbate Peroxidase (APX) in Plant are widely distributed in all higher plants and one of its main functions is connected with its role as a part of the defense enzyme complex in the cells, ensuring the detoxification of the activated oxygen forms. This function is very important in the formation of the

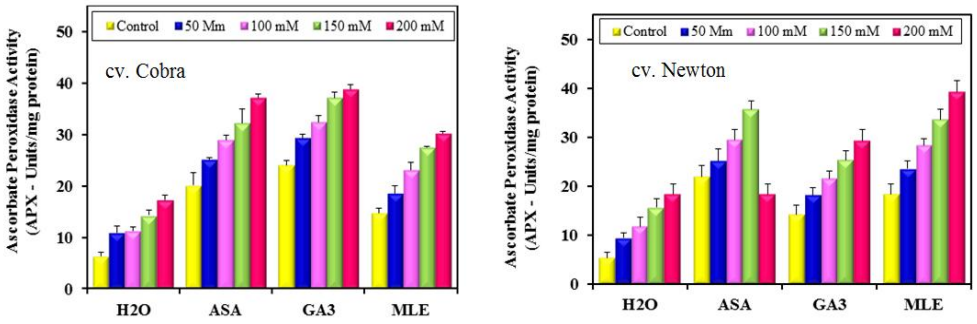

Fig. (1C). Influence of ASA, GA3 and MLE on Ascorbate Peroxidase Activities (APX - units/mg. protein) Contents in Shoot of Tomato (*Lycopersicon esculentum*, L. cv. Cobra & cv. Newton) Under Salinity Stress

Table (1C) Statistical Analysis for Influence of ASA, GA3 and MLE on Ascorbate Peroxidase Activities (APX - units/mg. protein) Contents in Shoot of Tomato (*Lycopersicon esculentum*, L. cv. Cobra & cv. Newton) Under Salinity Stress

NaCl Conc. (mM)	Catalase (CAT) Activities (Units/mg protein)							
	cv. Cobra				cv. Newton			
Statistical Analysis (*ANOVA*)	H_2O	ASA	GA3	MLE	H_2O	ASA	GA3	MLE
F	23.413*	50.086*	21.909*	44.629*	9.437*	12.376*	15.649*	8.068*
p	<0.001*	<0.001*	<0.001*	<0.001*	<0.002*	<0.001*	<0.001*	<0.004*
LSD	2.914	3.114	3.174	3.041	4.722	6.314	5.265	5.655

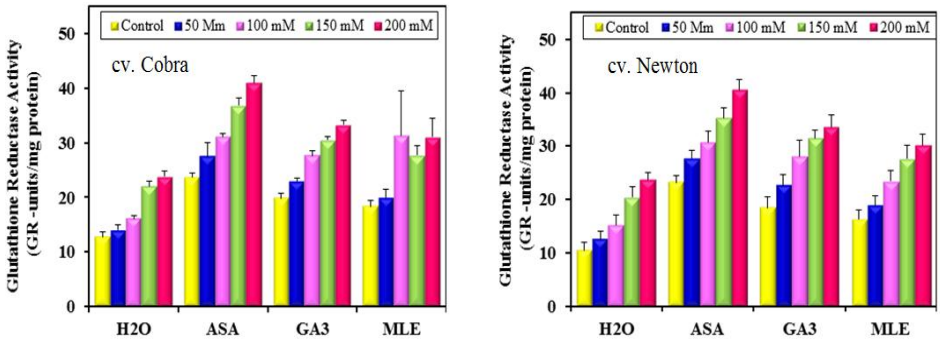

Fig. (1D). Influence of ASA, GA3 and MLE on glutathione reductase activities (GR - units/mg. protein) contents in shoot of tomato (*Lycopersicon esculentum*, L. cv. Cobra & cv. Newton) under salinity stress

Table (1D). Statistical analysis for influence of ASA, GA3 and MLE on glutathione reductase activities (GR - units/mg. protein) contents in shoot of tomato (*Lycopersicon esculentum*, L. cv. Cobra & cv. Newton) under salinity stress

NaCl Conc. (mM)	Glutathione Reductase (GR) Activities (Units/mg protein)							
	cv. Cobra				cv. Newton			
Statistical Analysis (*ANOVA*)	H_2O	ASA	GA3	MLE	H_2O	ASA	GA3	MLE
F	33.852*	23.065*	56.585*	2.194	10.829*	14.167*	7.748*	8.337*
p	<0.001*	<0.001*	<0.001*	<0.143	<0.001*	<0.001*	<0.004*	<0.003*
LSD	2.692	4.585	2.291	13.064	5.280	5.680	7.091	6.380

metabolic response of plants to different stress factors; APXs protect cells against harmful concentration of hydro peroxides [49]. It has been reported that elevated antioxidant levels could be associated with salt tolerance of plants [50]. Plant Ascorbate Peroxidase has attracted industrial attention due to their usefulness in multiple applications including clinical diagnosis and laboratory experiments [51].

The changes in CAT activity depend on the species, the development and metabolic state of the plant, as well as on the duration and intensity of the stress [52]. Catalase (CAT) which is involved in the degradation of hydrogen peroxide into water and oxygen is the most effective antioxidant enzymes in preventing oxidative damage, CAT activity profiles against different NaCl concentrations exhibited the same trend as what SOD was and the assumptions of changed patterns can be explained in the same way. Increased CAT activities under salinity stress in *Cassia angustifolia* L. [53], maize [54], *Sesamum indicum* [55] and wheat [56] were similar to our finding and depend on salt tolerance potential of plant's varieties. Studies have suggested that peroxidases played a role in auxin metabolism, lignification, suberization, cross-linking of cell wall components, self-defense against pathogens and senescence [57-58].

3.2 Nitrogenous Organic Components

3.2.1 Proline contents (µg/100g Dry Weight)

Overall, the proline contents in shoot and root of tomato plant for both cultivars increased highly significant at ($p \leq 0.001$), with increasing NaCl salinity concentrations in the presence or absence of ASA, GA3 and MLE, also, proline contents increased significantly ($p \leq 0.001$) in shoot more than roots for both cultivars compared with control as shown in Fig. (2) & Table (2). The results shown that the impact of ASA on the proline contents in shoot tended to increased highly significantly ($p \leq 0.001$) for cv. Cobra under NaCl salinity compared with GA3 and MLE. While, the role of ASA has been alleviated the effect of salinity on tomato plant for both cultivars by increasing proline contents in shoot and root more than with GA3 and MLE compared with control. So, the all of these results it has been found that the proline contents in shoot and root increased significantly ($p \leq 0.001$) more in cv. Cobra than in cv. Newton especially in the present of ASA, GA3 the MLE respectively compared with control. Overall the statistical analysis indicated that the two ways analysis of variance (*ANOVA*) between different concentrations of salinity stress, ASA, GA3 and MLE in two tomato cultivars indicated that the F test and *LSD* test highly significant at $P \leq 0.001$. Proline is an important component which helps reduce the deleterious effects of stressors and accelerate recovery processes during the period after exposure to stress on wheat plants [59]. Proline has been reported to reduce NaCl-induced stress by increasing Rubisco's oxygenase and carboxylase activities [60]. Therefore, the accumulation of proline content to protect plants from free radical damage by deleting the oxygen single [61]. Proline accumulation is one of the many adaptations of plants to salinity stress [62-63]. It has also been widely advocated that proline accumulation can be used as a selective parameter for salt stress tolerance [64].

Kaur and Asthir [65] they said that the proline is a cyclic, low molecular weight amino acid among the major compatible solutes and is known to enable osmotic adjustments in plants under stressful environments. Increasing salt levels reduced significantly enzyme activity and antioxidant activity as well, photosynthesis, has a significant impact on the quantity and quality of tomato yield by influenced enzyme activity, increased ROC release and the formation of antioxidants in tolerant genotype, also, the morpho-physiological properties were significantly reduced [66-68].

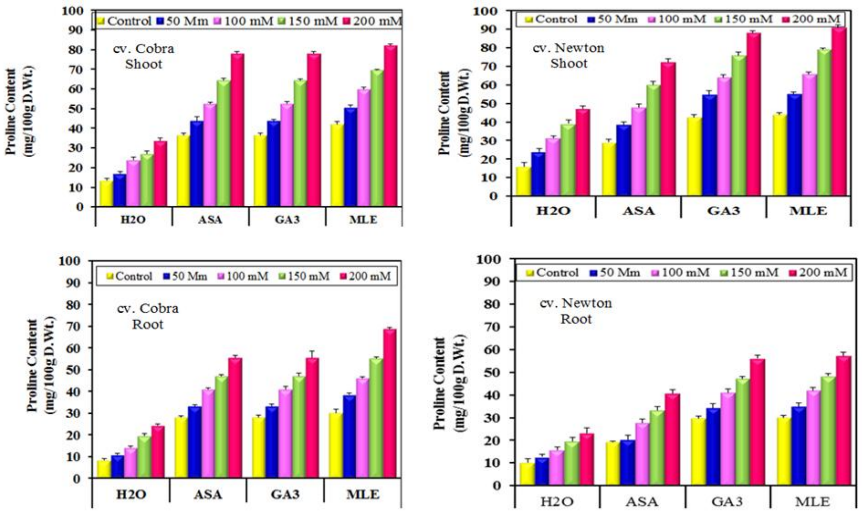

Fig. (2). Influence of ASA, GA3 and MLE on proline (mg/100g D. Wt.) contents in shoot and root of tomato (*Lycopersicon esculentum*, L. cv. Cobra & cv. Newton) under salinity stress

Table (2). Statistical analysis for influence of ASA, GA3 and MLE on Proline (mg/100g D. Wt.) contents in shoot and root of tomato (*Lycopersicon esculentum*, L. cv. Cobra & cv. Newton) under salinity stress

NaCl Conc. (mM)	Proline (mg/100g D. Wt.)							
Statistical	cv. Cobra				cv. Newton			
Analysis (*ANOVA*)	H_2O	ASA	GA3	MLE	H_2O	ASA	GA3	MLE
Shoot								
F	43.069*	157.551*	260.696*	238.510*	48.624*	111.802*	129.898*	311.963*
p	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*
LSD	3.852	4.163	3.236	3.196	5.546	5.124	4.920	3.327
Root								
F	94.212*	133.604*	46.403*	260.199*	9.529*	38.247*	47.614*	74.061*
p	<0.001*	<0.001*	<0.001*	<0.001*	0.002*	<0.001*	<0.001*	<0.001*
LSD	2.125	2.982	5.060	2.947	5.241	4.605	4.775	3.930

3.2.2 Total amino acids contents (mg/100g Dry Weight)

Total amino acid contents (mg/100g. D. Wt.*)* in shoot and root tomato plant for both cultivars increased progressively with increasing NaCl salinity, also total amino acids Contents increased in the present of ASA, MLE &GA3 respectively compared with control as shown in Fig. (3) & Table (3). Overall, total amino acid contents of tomato plant shoot and root increased significantly ($p \leq 0.001$) with increasing NaCl salinity concentrations. Overall the statistical analysis indicated that the two ways analysis of variance (*ANOVA*) between different concentrations of salinity stress, ASA, GA3 and MLE in two tomato cultivars indicated that the *F test* and *LSD test* highly significant at $P \leq 0.001$.

The accumulation of osmolytes, especially which of proline, is a common phenomenon in plants. Besides its role as an osmolyte, proline contributes to scavenging reactive oxygen species (ROS), stabilizing subcellular structures, modulating cell redox homeostasis, supplying energy and functioning as a signal [69].

Although proline accumulation is a common response to salt stress in tomato, the extent of its accumulation varies between tolerant and sensitive genotypes. Indeed, our findings revealed that proline accumulation increases. To withstand salt stress, plants accumulate compatible solutes such as proline, which decreases the cytoplasmic osmotic potential, facilitating water absorption, and (ROS) molecules [70-71].

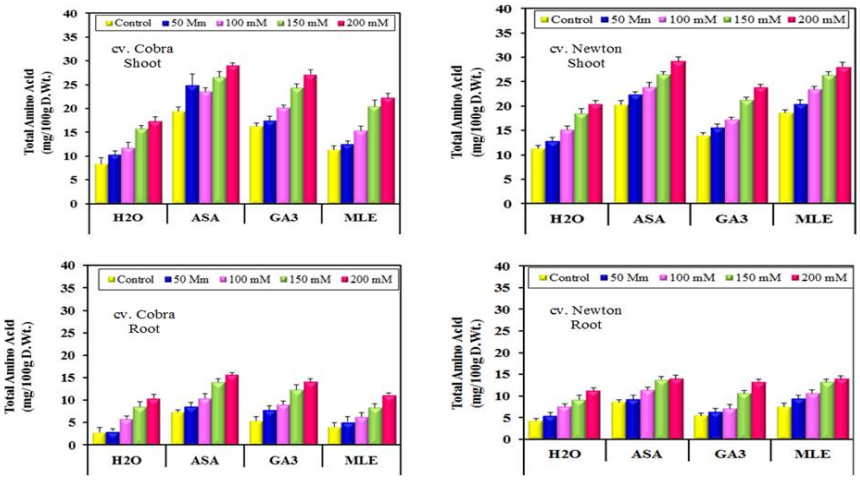

Fig. (3). Influence of ASA, GA3 and MLE on total amino acid (mg/100g D. Wt.) contents in shoot and root of tomato (*Lycopersicon esculentum*, L. cv. Cobra & cv. Newton) under salinity stress

Table (3). Statistical analysis for influence of ASA, GA3 and MLE on total amino acid (mg/100g D. Wt.) contents in shoot and root of tomato (*Lycopersicon esculentum*, L. cv. Cobra & cv. Newton) under salinity stress

NaCl Conc. (mM)	Total Amino Acid (mg/100g D. Wt.)							
	cv. Cobra				cv. Newton			
Statistical Analysis (*ANOVA*)	H_2O	ASA	GA3	MLE	H_2O	ASA	GA3	MLE
Shoot								
F	15.763*	7.314*	35.885*	26.895*	29.696*	22.178*	51.008*	30.782*
p	<0.001*	<0.005	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*
LSD	2.973	4.197	2.424	2.935	2.220	2.331	1.790	2.254
Root								
F	18.271*	20.147*	20.652*	12.760*	16.976*	14.485*	24.351*	14.150*
p	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*
LSD	2.469	2.490	2.424	2.497	2.162	2.107	2.107	2.266

In addition, Hildebrandt et al. [72] they found that amino acids in plants have various outstanding functions which use during protein biosynthesis, they are also building blocks for various other biosynthetic pathways and play a central role in signaling processes and in the response to plant stress. In general, the pool sizes of the 20 amino acids differ greatly and change dynamically depending on the developmental and physiological state of the plant cell. Kahlaoui *et al*. [73] indicated that a reduction in the synthetase activities of proline oxidase was found in both tomato varieties when irrigated with salt water, but this was found to be applied exogenously at the lower proline concentration. Salinity stress caused significant gradual increases in free amino acids with increases in salinity levels. These results agree with the results observed by Rady et al. [74] on wheat plant, Sadak et al. [75] on sunflower plant and Sadak &Abd Elhamid [76] on flax plant where, they concluded that salinity stress was capable of acting as activators of free amino acids accumulation.

The accumulation of amino acids in flax plant exposed to stress may be attributed to the disturbance in amino acid metabolism. Furthermore ASA, GA3 and MLE significantly enhanced the stimulatory role of salt stress on production of total free amino acids. Proline protects plants from salinity stress, mainly by maintaining osmotic adaptation, ROS trapping and modulating antioxidant metabolites and important enzymatic components of the antioxidant defense system. Phytohormones regulate pro-production and stress tolerance. It is therefore expected that there is a close

relationship between phytohormones and pro-metabolism. Elucidating these relationships could improve understanding of the regulatory issues involved in phytohormone-mediated pro-metabolism. The unavailability of nutrients for plants under salt and drought stress has many inevitable consequences for plants [77].

3.3 Inorganic Minerals Nutrient Element Components (mg/100g Dry Weight)

3.3.1. Macro-minerals nutrient elements (mg/100g Dry Weight)

Nitrogen, Potassium, Phosphorous, Magnesium and Calcium Contents (mg/100g Dry Weight): Overall, the shoot N^{+3}, P^{+3}, K^{+}, Mg^{+2} & Ca^{+2}contents increased highly significant at ($p \leq 0.001$) with increasing NaCl salinity concentrations (gradually 50 then 100, 150 and 200 mM) of tomato shoot and root for both cultivars in the presence or absence of ASA, GA3 and MLE compared with control. The results indicated that the shoot and root macro-nutrient elements (N^{+3}, P^{+3}, K^{+}, Mg^{+2} & Ca^{+2}) contents increased highly significant at ($p \leq 0.001$) especially with 200 mM NaCl concentration compared with control as shown in Figs. (4, 5, 6, 7 & 8) and Tables (4, 5, 6, 7 & 8). The results shown that the influences of ASA was more effective increasing significantly ($p \leq 0.001$) of all macro-nutrient elements (N^{+3}, P^{+3}, K^{+}, Mg^{+2} & Ca^{+2}) contents in tomato shoot and root for both cultivars under NaCl salinity concentrations than GA3 and MLE. While, the macro-nutrient elements (N^{+3}, P^{+3}, K^{+}, Mg^{+2} & Ca^{+2}) contents increased significantly in shoot more than in root for both cultivars compared with control. So, the role of ASA on tomato plant for both cultivars have been alleviated the effect of salinity by increasing the shoot macro-nutrient elements (N^{+3}, P^{+3}, K^{+}, Mg^{+2} & Ca^{+2}) contents more than GA3 and MLE compared with control. Consequently, the all of these results it has been found that the contents of macro-nutrient elements (N^{+3}, P^{+3}, K^{+}, Mg^{+2} & Ca^{+2}) contents increased significantly ($p \leq 0.001$) in tomato shoot and root more in cv. Cobra than in cv. Newton, especially in the present of ASA more than GA3 and MLE respectively compared with control. So, macro-mineral nutrient elements increased significantly more in the presence of ASA, GA3 & MLE than in the absence respectively compared with control. Overall, the statistical analysis indicated that the two ways analysis of variance (*ANOVA*) between different concentrations of salinity stress, ASA, GA3 and MLE in two tomato cultivars indicated that the *F test* and *LSD test* highly significant at $P \leq 0.001$.

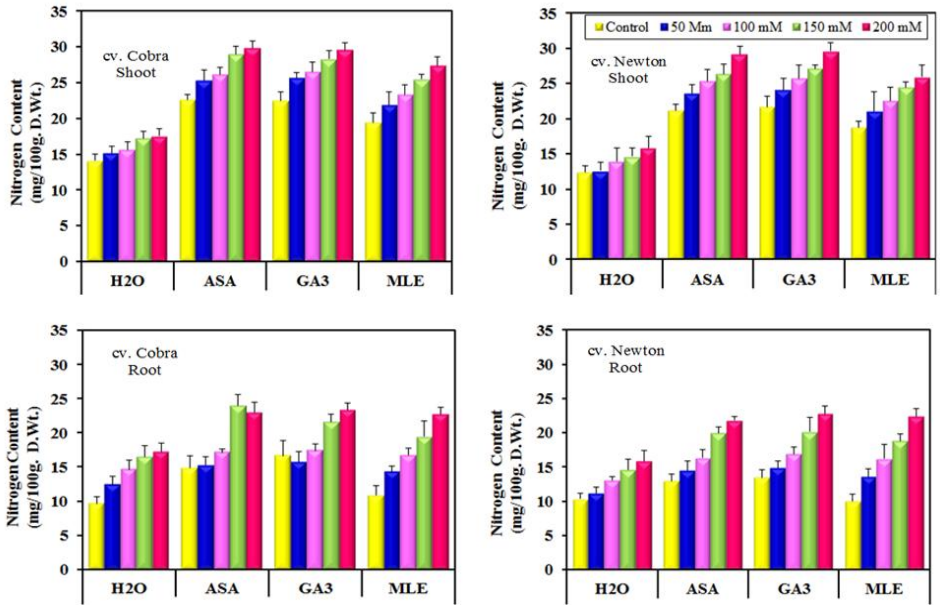

Fig. (4). Influence of ASA, GA3 and MLE on Nitrogen (N^{+3}) (mg/100g D. Wt.) contents in shoot and root of tomato (*Lycopersicon esculentum*, L. cv. Cobra & cv. Newton) under salinity stress

Table (4). Statistical analysis for influence of ASA, GA3 and MLE on Nitrogen (N^{+3}) (mg/100g D. Wt.) contents in shoot and root of tomato (*Lycopersicon esculentum*, L. cv. Cobra & cv. Newton) under salinity stress

NaCl Conc. (mM)	Nitrogen Nutrient Elements (mg/100g D. Wt.)							
	cv. Cobra				cv. Newton			
Statistical Analysis (*ANOVA*)	H_2O	ASA	GA3	MLE	H_2O	ASA	GA3	MLE
Shoot (N^{+3})								
F	2.078	7.551*	6.236*	5.434*	1.047	5.724*	4.424*	2.631
p	<0.159	<0.005*	<0.009*	<0.014*	<0.430	<0.012*	<0.026*	<0.098
LSD	3.115	3.371	3.469	4.199	4.383	3.970	4.512	5.486
Root (N^{+3})								
F	5.806*	9.634*	5.610*	10.484*	4.241*	11.954*	8.270*	13.191*
p	<0.011*	<0.002*	<0.012*	<0.001*	<0.029*	<0.001*	<0.003*	<0.001*
LSD	4.112	4.444	4.497	4.474	3.530	3.380	4.178	4.111

Our results finding agree with Farahat et al. [78] reported that the all of nitrogen, phosphorus and potassium contents in both shoots and roots increased gradually with increasing the levels of ascorbic acid. So, ascorbic acid (ASA) protects metabolic processes against H_2O_2 and other toxic derivatives of oxygen affected many enzyme activities, minimize the damage caused by oxidative processes through synergistic function with other antioxidants and stabilize membranes [79].

Nitrogen proved beneficial in ameliorating the salinity triggered oxidative damage to significant extent. Antioxidant components, both enzymatic and non-enzymatic, increased due to N supplementation conferring its active involvement in their expression levels [80]. Also, with increasing salinity levels, the phosphorus content of shoots & roots decreased in all NaCl concentrations. In contrast, by increasing of salt concentration in the culture medium, phosphorus content decreased significantly in roots compared to untreated plants [81].

The general lack of recognition of the limiting role of calcium Ca^{+2} is due in part to the fact that some important plant functions are controlled by changes in very small physiologically active pools of Ca^{+2} within the cytoplasm. Furthermore, the low mobility of (Ca^{+2}) makes the rates of its uptake and distribution limiting processes for many key plant functions [82]. Potassium (K^+) has been considered to play a role in osmotic stress and salt toxicity remediation, and some studies show inhibition of K^+ influx by NaCl in the cytosol [83].

Table (5). Statistical analysis for influence of ASA, GA3 and MLE on phosphorous (P^{+3}) (mg/100g D. Wt.) contents in shoot and root of tomato (*Lycopersicon esculentum*, L. cv. Cobra & cv. Newton) under salinity stress

NaCl Conc. (mM)	Phosphorous Nutrient Elements (mg/100g D. Wt.)							
	cv. Cobra				cv. Newton			
Statistical Analysis (*ANOVA*)	H_2O	ASA	GA3	MLE	H_2O	ASA	GA3	MLE
Shoot (P^{+3})								
F	1.376	19.737*	28.180*	13.405*	13.967*	21.408*	32.511*	26.113*
p	<0.310	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*
LSD	4.133	3.813	3.642	4.365	3.420	3.598	3.309	3.629
Root (P^{+3})								
F	0.950	4.528*	4.656*	2.866	1.025	3.300	4.599*	2.336
p	<0.475	<0.024*	<0.022*	<0.081	<0.440	<0.057	<0.023*	<0.126
LSD	3.410	3.270	3.534	4.051	2.790	4.246	3.474	3.825

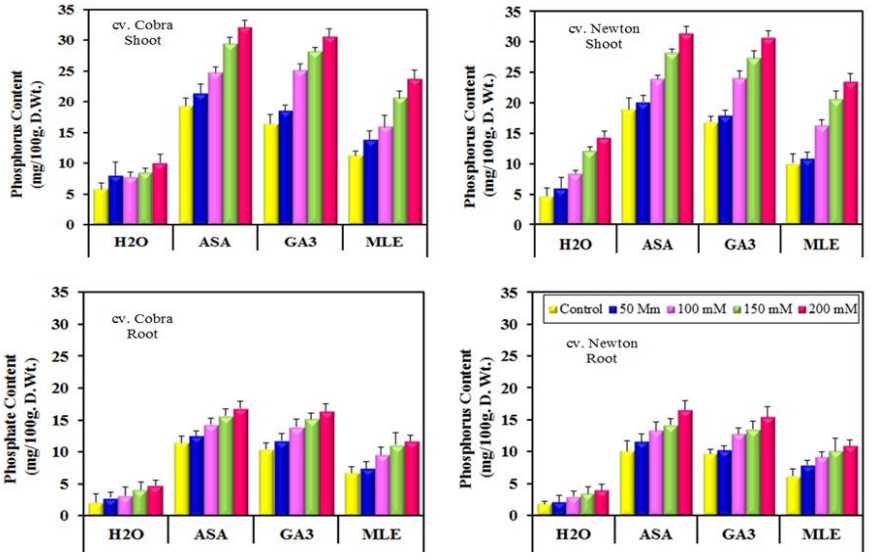

Fig. (5). Influence of ASA, GA3 and MLE on Phosphorous (P^{+3}) (mg/100g D. Wt.) contents in shoot and root of tomato (*Lycopersicon esculentum*, L. cv. Cobra & cv. Newton) under salinity stress

Babu et al. [84] found that the Potassium content was found in leaves and tomato fruits to be decreasing with increase in salt stress. Labrada et al. [68] reported that increasing salt levels significantly reduced tomato plant growth as well as tomato quality as enzyme activity and antioxidant activity. An increasing salt content increases the Na^+ plant content and the Na^+/K^+ ratio and decreases the K^+ plant content. Sivakumar & Ponnusami [85] realized the increased uptake and accumulations of some nutritive elements as N, P, K, & Ca, and as well as Mg in roots and shoots of several plants by using Moringa leaf extract (MLE) is supposed to accelerate the nutrient uptake and translocation by increasing the root membranes permeability for electrolytes, preventing nutrients fixation and increasing its mobility in soil.

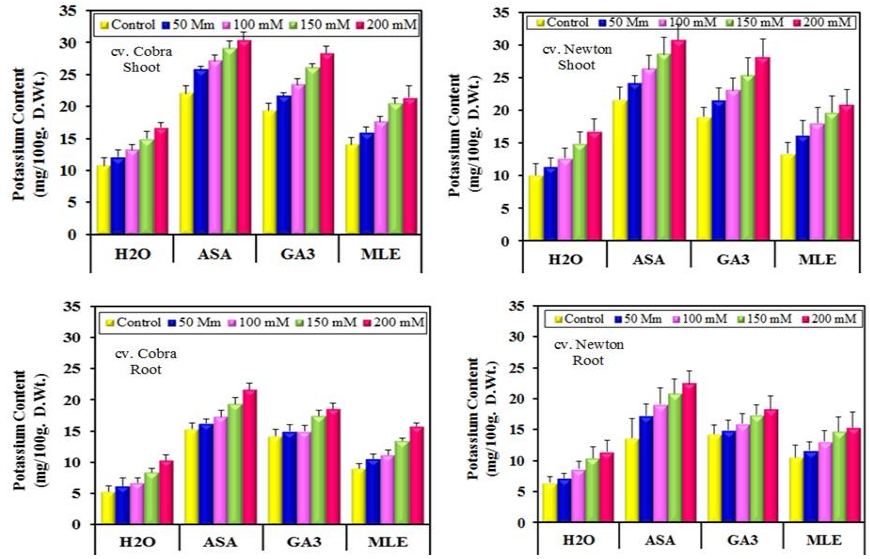

Fig. (6). Influence of ASA, GA3 and MLE on Potassium (K^{+1}) (mg/100g D. Wt.) contents in shoot and root of tomato (*Lycopersicon esculentum*, L. cv. Cobra & cv. Newton) under salinity stress

Table (6). Statistical analysis for influence of ASA, GA3 and MLE on potassium (K^{+1}) (mg/100g D. Wt.) contents in shoot and root of tomato (*Lycopersicon esculentum*, L. cv. Cobra & cv. Newton) under salinity stress

NaCl Conc. (mM)	Potassium Nutrient Elements (mg/100g D. Wt.)							
	cv. Cobra				cv. Newton			
Statistical Analysis (*ANOVA*)	H_2O	ASA	GA3	MLE	H_2O	ASA	GA3	MLE
Shoot (K^+)								
F	5.228*	10.478*	15.397*	7.154*	2.535	2.991	2.613	1.765
p	<0.016*	<0.001*	<0.001*	<0.005*	<0.106	<0.073	<0.099	<0.212
LSD	3.258	3.178	2.852	3.631	5.321	6.544	6.848	6.961
Root (K^+)								
F	4.911*	7.732*	3.279	16.292*	2.292	2.002	0.945	1.015
p	<0.019*	<0.004*	<0.058	<0.001*	<0.131	<0.170	<0.477	<0.445
LSD	2.799	2.854	3.219	2.033	4.325	7.620	5.384	6.273

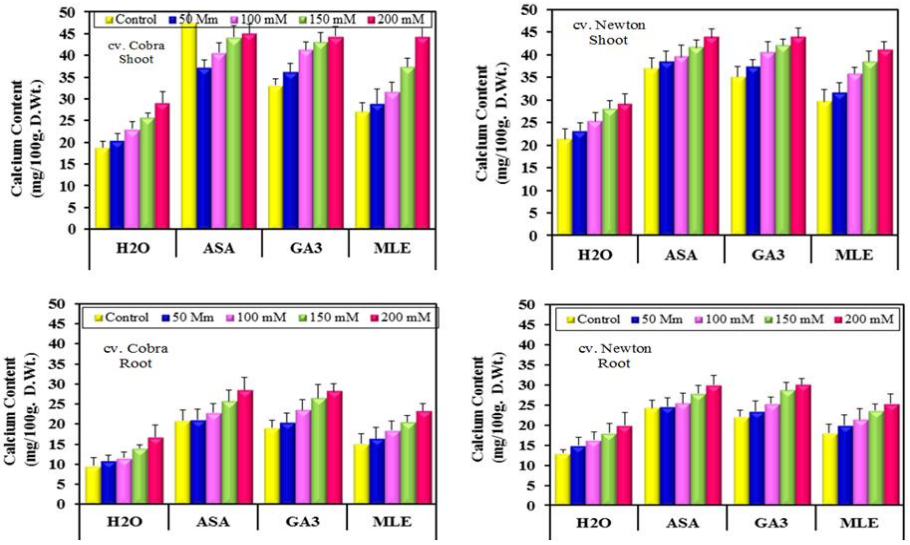

Fig. (7). Influence of ASA, GA3 and MLE on calcium (Ca^{+2}) (mg/100g D. Wt.) contents in shoot and root of tomato (*Lycopersicon esculentum*, L. cv. Cobra & cv. Newton) under salinity stress

Table (7). Statistical analysis for influence of ASA, GA3 and MLE on Calcium (Ca^{+2}) (mg/100g D. Wt.) contents in shoot and root of tomato (*Lycopersicon esculentum*, L. cv. Cobra & cv. Newton) under salinity stress

NaCl Conc. (mM)	Calcium Nutrient Elements (mg/100g D. Wt.)							
	cv. Cobra				cv. Newton			
Statistical Analysis (*ANOVA*)	H_2O	ASA	GA3	MLE	H_2O	ASA	GA3	MLE
Shoot (Ca^{+2})								
F	5.816*	2.861	6.482*	8.696*	2.721	1.685	3.218	5.009*
p	<0.011*	<0.081	<0.008*	<0.003*	<0.091	<0.229	<0.061	<0.018*
LSD	5.354	7.444	5.922	7.487	6.196	6.576	6.277	6.636
Root (Ca^{+2})								
F	2.307	1.493	2.398	2.379	1.574	1.253	3.398	1.564
p	<0.129	<0.276	<0.119	<0.121	<0.255	<0.350	<0.053	<0.258
LSD	5.849	8.339	7.862	6.722	6.796	6.774	5.866	7.278

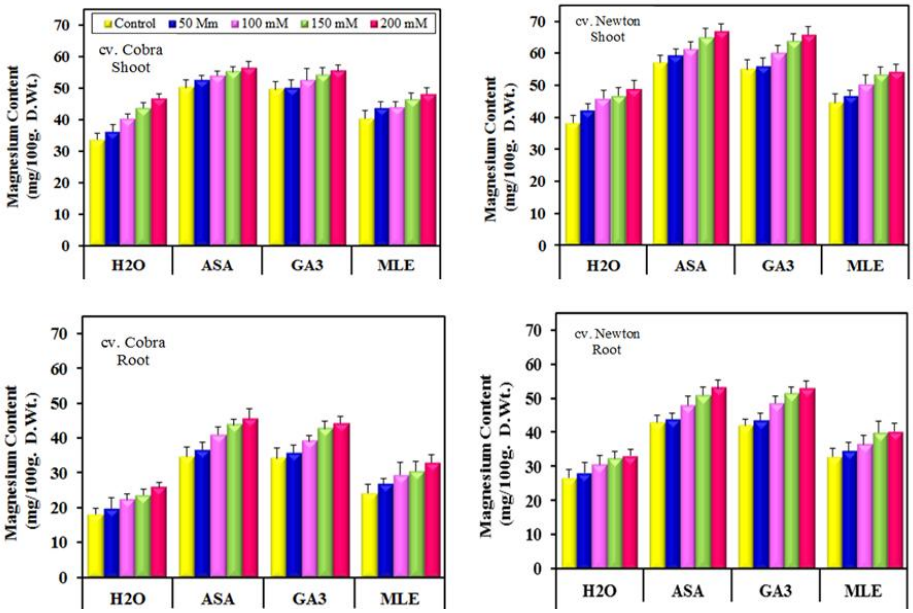

Fig. (8). Influence of ASA, GA3 and MLE on Magnesium (Mg^{+2}) (mg/100g D. Wt.) contents in shoot and root of tomato (*Lycopersicon esculentum*, L. cv. Cobra & cv. Newton) under salinity stress

Table (8). Statistical analysis for influence of ASA, GA3 and MLE on Magnesium (Mg^{+2}) (mg/100g D. Wt.) contents in shoot and root of tomato (*Lycopersicon esculentum*, L. cv. Cobra & cv. Newton) under salinity stress

NaCl Conc. (mM)	Magnesium Nutrient Elements (mg/100g D. Wt.)							
	cv. Cobra				cv. Newton			
Statistical Analysis (*ANOVA*) Shoot (Mg^{+2})	H_2O	ASA	GA3	MLE	H_2O	ASA	GA3	MLE
F	8.704*	1.768	0.941	2.145	2.903	2.783	3.473*	2.939
p	<0.003*	<0.212	<0.479	<0.149	<0.078	<0.086	<0.049*	<0.076
LSD	5.680	5.776	8.210	6.466	7.728	7.601	7.992	7.693
Root (Mg^{+2})								
F	2.572	4.019*	3.692*	1.682	1.373	3.737*	5.655*	1.544
p	<0.103	<0.034*	<0.043*	<0.230	<0.311	<0.041*	<0.012*	<0.263
LSD	6.067	7.401	7.073	8.227	7.354	7.246	6.327	8.223

3.3.2 Micro-minerals nutrient elements (mg/100g Dry Weight)

Manganese, Iron and Boron Content (mg/100g Dry Weight): Overall, the shoot and root micro-nutrient elements (Mn^{+2}, Fe^{+3} & B^{+2}) contents increased highly significant at ($p \leq 0.001$) with increasing salinity concentrations (gradually start with 50 then 100, 150 & 200 mM NaCl) of tomato plant for both cultivars, in the presence or absence of ASA, GA3 and MLE compared with control. The results indicated that the micro-nutrient elements (Mn^{+2}, Fe^{+3} & B^{+2}) contents increased highly significant at ($p \leq 0.001$) especially with 200 mM NaCl concentration compared with control as shown in Figs. (9, 10 & 11) and Tables (9, 10 & 11). The results shown that the effect of ASA was more effective by increasing significantly ($p \leq 0.001$) of shoot and root micro-nutrient elements (Mn^{+2}, Fe^{+3} & B^{+2}) contents for both cultivars under NaCl salinity concentrations than GA3 and MLE. Consequently, the all of these results it has been found the shoot micro-nutrient elements (Mn^{+2}, Fe^{+3} & B^{+2}) contents increased significantly ($p \leq 0.001$) more in shoot than in root especially in the

present of ASA more than GA3 and MLE compared with control respectively. Thereby, the micro-nutrient elements (Mn^{+2}, Fe^{+3} & B^{+2}) contents increased significantly in shoot more than in root for both cultivars compared with control. So, the role of ASA on tomato plant for both cultivars have been alleviated the effect of salinity by increasing the shoot and root Micro-nutrient elements (Mn^{+2}, Fe^{+3} & B^{+2}) contents more than GA3 and MLE compared with control.

Consequently, the all of this results it has been found that the contents of Mn^{+2}, Fe^{+3} & B^{+2}increased significantly ($p \leq 0.001$) in tomato shoot and root more in cv. Newton than in cv. Cobra especially in the present of ASA more than GA3 and MLE respectively compared with control. Overall, the statistical analysis indicated that the two ways analysis of variance (*ANOVA*) between different concentrations of salinity stress, ASA, GA3 and MLE in two tomato cultivars indicated that the *F test* and *LSD test* highly significant at $P \leq 0.001$. Fertilizers with microelements such as manganese (Mn^{+2}), iron (Fe^{+3}) and Boron (B^{+2}) have been shown to be convenient for field use, have a good effectiveness and very rapid plant response [86]. Also, it helps plant to avoid toxicity symptoms that may occur after soil application of the same microelements [87].

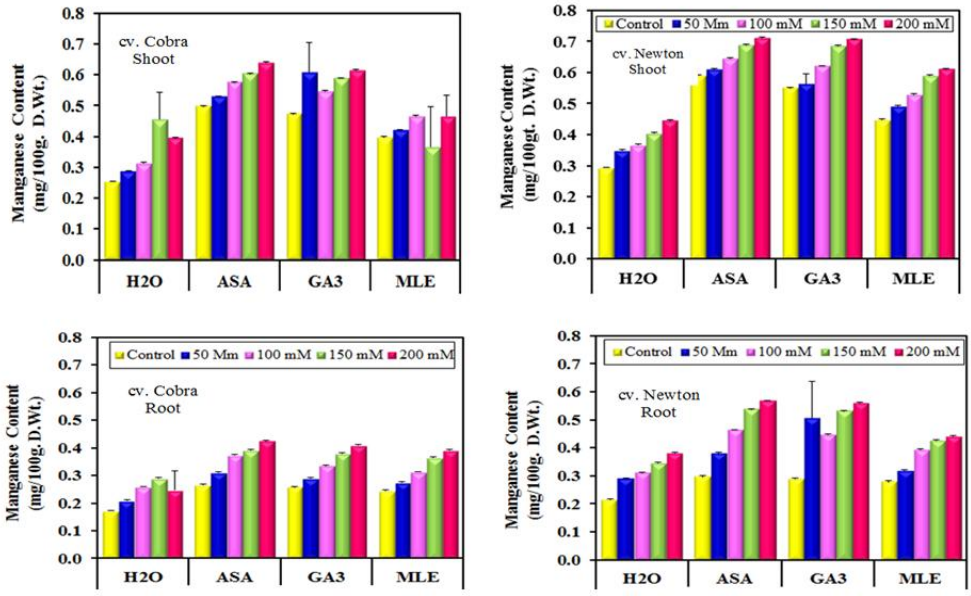

Fig. (9). Influence of ASA, GA3 and MLE on Manganese (Mn^{+2}) (mg/100g D. Wt.) contents in shoot and root of tomato (*Lycopersicon esculentum*, L. cv. Cobra & cv. Newton) under salinity stress

Table (9). Statistical analysis for influence of ASA, GA3 and MLE on manganese (Mn^{+2}) (mg/100g D. Wt.) Contents in shoot and root of tomato (*Lycopersicon esculentum*, L. cv. Cobra & cv. Newton) under salinity stress

NaCl Conc. (mM)	Manganese Nutrient Elements (mg/100g D. Wt.)							
	cv. Cobra				cv. Newton			
Statistical Analysis (*ANOVA*) Shoot (Mn^{+2})	H_2O	ASA	GA3	MLE	H_2O	ASA	GA3	MLE
F	4.529*	446.243*	1.796	0.429	550.548*	404.180*	25.099*	773.500*
p	0.024*	<0.001*	0.206	0.785	<0.001*	<0.001*	<0.001*	<0.001*
LSD	0.123	0.008	0.138	0.209	0.008	0.008	0.044	0.008
Root (Mn^{+2})								
F	2.181	627.705*	653.730*	792.579*	544.444*	1067.992*	3.436	588.546*
p	0.145	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	0.052	<0.001*
LSD	0.097	0.008	0.008	0.007	0.008	0.011	0.183	0.009

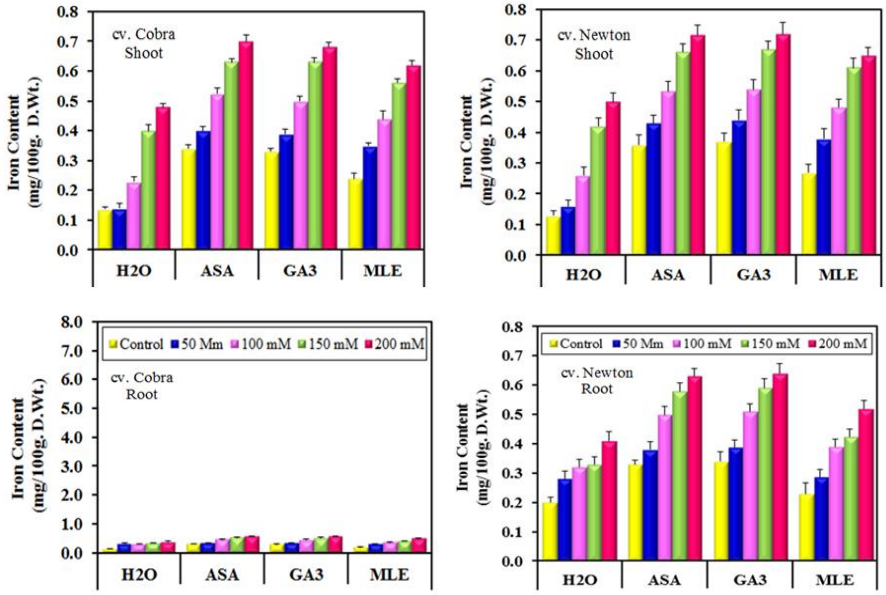

Fig. (10). Influence of ASA, GA3 and MLE on Iron (Fe^{+3}) (mg/100g D. Wt.) contents in shoot and root of tomato (*Lycopersicon esculentum*, L. cv. Cobra & cv. Newton) under salinity stress

Table (10). Statistical analysis for influence of ASA, GA3 and MLE on Iron (Fe^{+3}) (mg/100g D. Wt.) contents in shoot and root of tomato (*Lycopersicon esculentum*, L. cv. Cobra & cv. Newton) under salinity stress

NaCl Conc. (mM)	Iron Nutrient Elements (mg/100g D. Wt.)							
	cv. Cobra				cv. Newton			
Statistical Analysis (*ANOVA*)	H_2O	ASA	GA3	MLE	H_2O	ASA	GA3	MLE
Shoot (Fe^{+3})								
F	112.510*	91.710*	105.609*	79.067*	47.133*	24.920*	22.418*	29.964*
p	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*
LSD	0.046	0.050	0.046	0.055	0.074	0.095	0.099	0.091
Root (Fe^{+3})								
F	20.695*	35.459*	25.926*	35.408*	8.549*	26.918*	18.431*	15.603*
p	<0.001*	<0.001*	<0.001*	<0.001*	0.003*	<0.001*	<0.001*	<0.001*
LSD	0.065	0.064	0.073	0.060	0.083	0.078	0.094	0.091

Manganese (Mn^{+2}) is very important for the growth of higher plants was determined by McHargue [88]. Manganese is essential role is in water splitting and O_2 evolution in photosynthesis and involve respiration and as a cofactor or component of numerous enzymes [89-90]. A deficiency of Mn^{+2} for plants occurs in soils low in Mn^{+2} minerals and especially in alkaline and calcareous soils or of high redox status that often also results in Fe, Cu and Zn deficiencies [91-92]. Iron (Fe^{+3}) mineral element, as micronutrient, plays different roles in the structure of various enzymes as well as a regulating role of cofactors in the metabolism of carbohydrates, proteins, and cellular photosynthesis [93]. Plant leaves absorb some nutrients better than soil application, so, application of micronutrient elements such as Fe leads to an increased yield of crops [94-95]. Boron (B^{+2}) roles in plants include effects on the germination of pollen grains, the elongation of pollen tube, fruit set and yield, and is also indirectly responsible for the activation of dehydrogenase enzymes, sugar translocation, nucleic acids and plant hormones[96-97]. Boron deficiency is a common micronutrient problem in agriculture, which results in yield reductions and impaired crop quality [98].

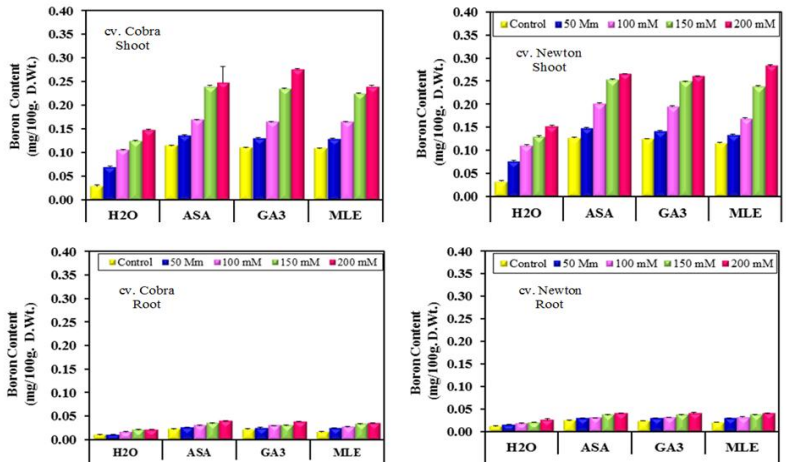

Fig. (11). Influence of ASA, GA3 and MLE on Boron (B^{+2}) (mg/100g D. Wt.) contents in shoot and root of tomato (*Lycopersicon esculentum*, L. cv. Cobra & cv. Newton) under salinity stress

Table (11). Statistical analysis for influence of ASA, GA3 and MLE on Boron (B^{+2}) contents (mg/100g D. Wt.) in shoot and root of tomato (*Lycopersicon esculentum*, L. cv. Cobra & cv. Newton) under salinity stress

NaCl Conc. (mM)	Boron Nutrient Elements (mg/100g D. Wt.)							
	cv. Cobra				cv. Newton			
Statistical Analysis (*ANOVA*)	H_2O	ASA	GA3	MLE	H_2O	ASA	GA3	MLE
Shoot (B^{+2})								
F	672.092*	15.396*	1825.500*	1491.618*	684.948*	1334.023*	1602.000*	1630.259*
p	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*
LSD	0.006	0.049	0.005	0.005	0.006	0.005	0.005	0.006
Root (B^{+2})								
F	18.420*	22.500*	21.879*	30.430*	12.857*	23.060*	14.446*	34.620*
p	<0.001*	<0.001*	<0.001*	<0.001*	0.001*	<0.001*	<0.001*	<0.001*
LSD	0.004	0.005	0.004	0.004	0.005	0.004	0.006	0.004

4. CONCLUSION

Generally, this study concluded that the antioxidant enzyme activities (Super Oxide Dismutase (SOD), Catalase (CAT), Ascorbate Peroxidase (APX), Glutathione Reductase (GR) & nitrogenous components (proline and total amino acids), inorganic components (macro- and micro- mineral nutrient elements) contents in tomato plant for b cultivars (cv. Cobra and cv. Newton) increased significantly in the present of ASA (0.75 mM), GA3 (0.05 mM) and MLE (5%) respectively more than in the absence under NaCl all salinity concentrations (0.0, 50, 100, 150 and 200 mM) compared with control. Also, the results indicated that the antioxidant enzyme activity, nitrogenous component and inorganic macro- and micro minerals nutrient elements increased in shoot more than in root for both cultivars under salinity stress in the presence or absence of ASA, GA3 and MLE compared with control. Generally, the role of ASA, GA3 & MLE were one of the main mechanisms used by the plant to raise its efficiency to bear the salt stress compared to the control. Therefore, should be pre-treatment (soaking) of tomato seeds for both cultivars in ASA (0.75 mM); GA3 (0.05 mM) and MLE (5%) before germinated for gave the best results and more effective for overcoming the harmful impacts of salinity stress and produced new strain adapted to salinity stress.

COMPETING INTEREST

Authors have declared that no competing interests exist.

REFERENCES

1. Mridha MAU. Prospects of moringa cultivation in Saudi Arabia. Journal of

Applied Environmental and Biological Sciences. 2005; 5(3):39-46.
2. Sudhakar C, Lakshmi A, Giridarakumar S. Changes in the antioxidant enzyme efficacy in two high yielding genotypes of mulberry (*Morus alba*, L.) under NaCl salinity. Plant Science. 2001; 16:613-619.
3. Mohamed HI, Gomaa EZ. Effect of plant growth promoting *Bacillus subtilis* and *Pseudomonas fluorescens* on growth and pigment composition of radish plants (*Raphanus sativus*) under NaCl stress. *Photosynthetica*. 2012; 50(2); 263- 272.
4. Klaya I, Gouiaa BS, Liuc M, Milaal Khoudib H, Bernadaca A, Bouzayena M, Pirrelloa J. Ethylene response factors (ERF) are differentially regulated by different abiotic stress types in tomato plants. Journal of Plant Science. 2018; 274:137-145. Available:www.elsevier.com/locate/plantsci
5. Foyer CH, Noctor CH. Ascorbate and glutathione: the heart of the redox Hub. Plant Physiology. 2011; 155(1):2-18.
6. El Sayed, Hameda El Sayed Ahmed Baziad S, Basaba R. Application of exogenous ascorbic acid on tomato (*Solanum lycopersicum* L.) seeds under NaCl salinity stress. International Journal of Current Research in Biosciences and Plant Biology. 2005;2(5):33-46.
7. El Sayed, Hameda El Sayed. Ahmed; Baziad, Salih A. M.; Basaba, Reem A. A. Alleviated effect of salinity stress by exogenous application of ascorbic acid on the antioxidant catalase enzymes and inorganic mineral nutrient elements contents on tomato plant. Journal of Life Sciences. 2016; 4(4):467-490.
8. Minguet EG, Alabadí D, Blázquez MA. Gibberellin implication in plant growth and stress responses. In: Tran LSP, Pal S. (Eds.), Phytohormones: A window to metabolism, signaling and biotechnological applications. Springer, New York. 2014; 119-161.
9. Colebrook EH, Thomas SG, Phillips AL, Hedden P. The role of gibberellin signaling in plant responses to abiotic stress. Journal of Experimental Botany; 2014.
10. Maggio A. Miyazaki S, Veronese P, Fujita T, Ibeas JI, Damsz B, Narasiman ML, Hasegawa PM, Joly RJ, Bressan RA. Does praline accumulation play an active role in stress induced growth reduction? Plant Journal. 2010; 31:699-712.
11. Miceli A, Moncada A, Sabatino L, Vetrano-Agronomy F. Effect of gibberellic acid on growth, yield, and quality of leaf tomato and rocket grown in a floating system. Agronomy. 2019; 382(9):1-22.
12. Akhtar T, Akhtar H, Tariq MI, Iqbal S, Sultana N, Ahmad M. Optimization of biodiesel production through base-catalyzed methanolysis of cantaloupe seed oil. Pakistan Journal of Science. 2019; 69(2):174-178.
13. Moyo B, Masika PJ, Hugo A, Muchenje V. Nutritional characterization of Moringa (*Moringa oleifera* Lam.)Leaves. African Journal of Biotechnology. 2011; 10:12925-12933.
14. Desoky EM, Elrys AS, Rady MM. Integrative moringa and licorice extracts application improves *Capsicum Annuum* fruit yield and declines its contaminant contents on a heavy metals-contaminated saline soil. Ecotoxicology and Environmental Safety. 2019; 169:50-60.
15. Lee KY, Yang HJ, Song KB. Application of a puffer fish skin gelatine film containing *Moringa oleifera* Lam. leaf extract to the packaging of Gouda cheese. Journal of Food Science and Technology -Mysore. 2016; 53(11):3876-3883.
16. Yameogo CW, Bengaly MD, Savadogo A, Nikiema PA, Traore SA. Determination of chemical composition and nutritional values of *Moringa oleifera* leaves. Pakistan Journal of Nutrition. 2011; 10(3):264- 68.
17. Abd El-Rahman SS, Mohamed I. Application of benzothiadiazole and *Trichoderma harzianum* to control faba bean chocolate spot disease and their effect on some physiological and biochemical traits. Acta Physiologiae Plantarum. 2014; 36(2):343- 354.
18. Shabala S, Cuin TA. Potassium transport and plant salt tolerance. Physiologia Plantarum. 2014; 133(4):651- 669. Available:https://doi.org/10.1111/j.1399-3054.2007.01008.x
19. Zayton A, El-Shafei A, Allam K, Mourad M. Effect of water salinity and potassium fertilizer levels on tomato productivity and water consumption in Siwa Oasis. Misr Journal of Agricultural Engineering. 2019; 26 (1):107-131.
20. Colla G, Rouphaelb Y, Leonardic C, Bied Z. Review Role of grafting in vegetable crops grown under saline conditions. *Scientia Horticulturae*. 2010; 127(2):147-155.
21. Ajayi AA, Adejuwon OA, Olutiola PO. Partial purification of polygalacturonase

from tomato fruits infected by *Rhizopus arrhizus fisher*. Journal of Plant Sciences. 2007; 2(2):216- 221.

22. Chourasiya PK, Lal AA Simon S. Effect of certain fungicides and botanicals against early blight of Tomato caused by *Alternaria solani* (*ellis and martin*) under Allahabad uttar pradesh, india conditions. International Journal of Agricultural Science and Research (IJASR). 2013; 3(3):151-156.
23. Singha R, Upadhyaya AK, Chandrab P, Singha DP. Sodium chloride incites reactive oxygen species in green algae *Chlorococcum humicola* and *Chlorella vulgaris*: Implication on lipid synthesis, mineral nutrients and antioxidant system. Bioresource Technology. 2018; 270:489-497.
24. Bashir Abubakar, Abdulkadir Wilson, Danbature, Fai Y. Yirankinyuki Buhari, Magaji Muhammad, Muzakkir M. In situ transesterification of rubber seeds (*Hevea brasiliensis*). Greener Journal of Physical Sciences. 2014;4(3):038-044.
25. Hogland DR, Arnon II. The water culture method for growing plants without soil. Journal Circular. California Agricultural Experiment Station. 2nd Edition. 1950; 347:32.
26. Esfandiari E, Shekari F, Fariborz S, Manouchehr E. The effect of salt stress on antioxidant enzymes' activity and lipid peroxidation on the wheat seedling. Notulae Botanicae Horti Agrobotanici Cluj-Napoca. 2007;35(1).
27. Gupta AS, Webb RP, Holaday AS, Allen RD. Overexpression of Superoxide Dismutase Protects plants from oxidative stress (induction of ascorbate peroxidase in superoxide dismutase - overexpressing plants). Environmental and Stress Physiology. 1993; 103:1067-1073. Available:https://doi.org/10.1104/pp.103.4.1067
28. Beauchamp C., Fridovich I. Superoxide dismutase: improved assays and an assay applicable to acrylamide gels. Analytical Biochemistry. 1971; 44(1):276-87.
29. Aebi Hugo B. Isolation, purification, characterization, and assay of antioxygenic enzymes; Catalase *In Vitro.* Methods in Enzymology. Volume 1984; 105:121-126.
30. Yoshimura K, Yabuta Y, Ishikawa T, Shigeoka S. Expression of spinach ascorbate peroxidase isoenzymes in response to oxidative stresses. Plant Physiology. 2000; 123:223-234.
31. Sairam RK, Rao V, Srivastava GC. Differential response of wheat genotypes to long term salinity stress in relation to oxidative stress, antioxidant activity and osmolyte concentration. *Plant Science.* 2002; 163(5):1037-1046.
32. Bates LS, Waldern RP, Teare ID. Rapid determination of free proline for water stress studied. Plant and Soil. 1973;3:205-207.
33. Ya PL, Tunekazu T. An improved colorimetric determination of amino acids with the use of ninhydrin. Analytical Biochemistry. 1966;23(14):71-77.
34. Richards LA. Diagnosis and improvement of saline alkali soils. USDA Handbook No. 60. USDA, Washington, D.C; 1954.
35. Jonson CM, Ulich A. *Amaivtical mertfhods* for use in piant ayss. U.S. Department of Agriculture. Cali Uin Agriculture. 1959;756.
36. Allen S, Grimshay HM, Parkin Son JA, Quarmby C. Chemical analysis of ecological materials. Blackwell Scientific Publications, Osney, Oxford, London. 1974;565.
37. Sharma SK, Greetham M, Schippers JC. Adsorption of iron onto filter media. Journal of Water SRT - Aqua. 1999;48(3):84-91.
38. Leslie E, Geoffrey J, James M. Statistical analysis. In: Interpretation and uses of medical statistics (4th ed). Oxford Scientific Publications (pub). 1991;411-6.
39. Kirkpatrick LA, Feeney BC. A simple guide to IBM SPSS statistics for version 20.0 student ed. Belmont, Calif., Wadsworth, Cengage Learning. 2013;x:115.
40. Scandalios JG. Oxygen stress and superoxide dismutases. plant physiology. 1993;101:7-12.
41. De Gara L, De Pinto MC, Tommasi F. The antioxidant systems vis-à-vis reactive oxygen species during plant–pathogen interaction. Plant Physiology and Biochemistry. 2003; 41:863-870.
42. Candan N, Tarhan L. The correlation between antioxidant enzyme activities and lipid peroxidation levels in *Mentha pulegium* organs grown in Ca^{2+}, Mg^{2+}, Cu^{2+}, Zn^{2+} and Mn^{2+} stress conditions. Plant Science. 2003; 163:769-779.
43. Implay JA. Pathways of oxidative damage. Annual Review of Microbiology. 2003; 57:395-418.

44. Beak KH, Skinner DZ. Alteration of antioxidant enzyme gene expression during cold acclimation of near-isogenic wheat lines. Plant science. 2003; 165:1221-1227.
45. Khalid A, Aftab F. Effect of exogenous application of IAA and GA3on growth, protein content , and antioxidant enzymes of *Solanum tuberosum*, L. grown in vitro under salt stress. Vitro Cellular & Developmental Biology-Plant. 2020; 56:377-389.
46. Keles Y, Oncel L. Change of superoxide dismutase activity in wheat seedling exposed to natural environmental stresses. Communications Faculty *of Sciencesb* University *of Ankara* Series C-Biology. 2000; 18:1-8.
47. Edwards K. The Interplay of Affect and Cognition in Attitude Formation and Change. Journal of Personal and Social Psychology. 1990;59(2):202-216.
48. Baisak R, Rana D, Acharya BB, Kar M. Alterations in the Activities of Active Oxygen Scavenging Enzymes of Wheat Leaves Subjected to Water Stress. Plant and Cell Physiology, 1994; 35(3):489-495.
49. Castillo FJ, Penel C, Gasper TH, Greppin H. Plant peroxidases. Topics and detailed literature on molecular. Biochemical, and Physiological Aspects, University of Geniva. 1992; 187-203.
50. Hernandez JA, Olmos E, Corpas FJ, Sevilla F, Del Rio LA. Salt induced oxidative stress in chloroplasts of pea plants. Plant Science. 1995; 105:151-167.
51. Azevedo AM, Martins VC, Prazeres DM, Vojinovic V, Cabral JM, Fonseca LP. Horseradish peroxidase: a valuable tool in biotechnology. Biotechnol Annual Reviews. 2003; 9:199-247.
52. Chaparzadeh N, Amico ML, Nejad RK, Izzo R, Izzo FN. Antioxidative responses of Calendula officinalis under salinity conditions. Plant Physiology *and* Biochemistry. 2004; 42:695-701.
53. Aggarwal M, Sharma S, Kaur N, Pathania D, Bhandhari K, Kaushal Kaur N, Singh RK, Srivastava A, Nayyar H. Exogenous proline application reduces phytotoxic effects of selenium by minimising oxidative stress and improves growth in bean (*Phaseolus vulgaris* L.) seedlings. Biology Trace Element Research. 2011; 140:354-367.
54. Azevedo NAD, Prico JT, Eneas FJ, Braga ACE, Gomes FE. Effect of salt stress on antioxidative enzymes and lipid peroxidation in leaves and roots of salt-tolerant and salt-sensitive maize genotypes. Environmental and Experimental Botany. 2006; 56:235-241.
55. Koca H, Bor M, Özdemir F, Türkan D. The effect of salt stress on lipid peroxidation, antioxidative enzymes and proline content of sesame cultivars. Environmental journal of experimental botany. 2007;60:344-351.
56. Hameed BH, Mahmoud DK, Ahmad AL. Equilibrium modeling and kinetic studies on the adsorption of basic dye by a low-cost adsorbent: coconut (*Cocos nucifera*) bunch waste. Journal of Hazardous Materials. 2008; 158:65-72.
57. Monica Q; Consuelo G, Miguel AB, Araceli B, Iraida A, Marıa IM, Francisco JA, Silvia Milrad DF, Horacio T, Victoriano V. A tomato peroxidase involved in the synthesis of lignin and suberin. Plant Physiology. 2000; 122:1119-1127.
58. DenHerder J, Lievens S, Rombauts S, Hol M, Sters Goormachtig S. A symbiotic plant peroxidase involved in bacterial invasion of the tropical Legume Sesbania rostrate. Plant Physiology. 2007; 144:717-772.
59. Sakhabutdinova AR, Fatkhutdinova DR, Bezrukova MV, Shakirova FM. Salicylic acid prevents the damaging action of stress factors on wheat plants. Journal of Plant Physiology. 2003; 29(Special Issue):314-319.
60. Sivakumar P, Sharmila P, Parthasaradhi P. Proline alleviates salt stress induced enhancement in rubulose-1,5-bisphosphate oxygenase activity. Biochemical and Biophysical Research Communications. 2000; 279(2):512-515.
61. Teixeira J, Fidalgo F. Salt stress affects glutamine synthetase activity and mRNA accumulation on potato in an organ – dependent manner. Biochemistry. 2009;47(9):807-813.
62. Kumar SG, Madhusudhan KV, Sreenivasulu N, Sudhakar C. Stress responses in two genotypes of mulberry (*Morus alba* L.) under NaCl salinity. Indian Journal of Experimental Biology. 2000;38(2):192-195.
63. Ramajulu S, Sudkakar C. Proline metabolism during dehydration in two mulberry genotypes with contrasting drought tolerance. Journal of Plant Physiology. 2000; 157 (1):81-85.
64. Ramajulu S, Sudkakar C. Alleviation of NaCl salinity stress by calcium is partly

related to the increased proline accumulation in mulberry (*Morus alba* L.) callus. Journal of Plant Biology. 2001; 28 (2):203-206.
65. Kaur G, Asthir B. Proline: a key player in plant abiotic stress tolerance. Biology Plant. 2015; 59 (4):609-619.
66. Machado R, Serralheiro R. Soil salinity: effect on vegetable crop growth management practices to prevent and mitigate soil Salinization. *Horticulturae*. 2017; 3:30.
67. Woldemariam SH, Lal S, Zelelew DZ, Solomon MT. Effect of potassium levels on productivity and fruit quality of tomato (*Lycopersicon esculentum,* L.). Journal of Agricultural Studies. 2018;6(1):104-117.
68. Labrada FP, Vargas LER, Ortiz HO, Pliego GC, Mendoza AB, Maldonado AJ. Responses of tomato plants under saline stress to foliar application of copper nanoparticles. Plants Journal. 2019; 8 (151):1-17.
69. Sharma S, Villamor JG, Verslues PE. Essential role of tissue-specific proline synthesis and catabolism in growth and redox balance at low water potential. Plant Physiology. 2011; 157: 292-304.
70. Qureshi M, Abdin M, Ahmad J, Iqbal M. Effect of long-term salinity on cellular antioxidants, compatible solute and fatty acid profile of sweet Annie (*Artemisia annua* L.). Phytochemistry. 2013; 95:215-223.
71. Pottosin I, Velarde-Buendía AM, Bose J, Zepeda-Jazo I. Shabala S, Dobrovinskaya O. Cross-talk between reactive oxygen species and polyamines in regulation of ion transport across the plasma membrane: implications for plant adaptive responses. Journal of Experimental Botany. 2014; 65(5):1271-1283.
72. Hildebrandt Tatjana M, Nesi Adriano. Nunes, Arau´jo Wagner L, Braun, Hans - Peter. Amino Acid Catabolism in Plants. Molecular Plant. 2015; 1-17.
73. Kahlaoui B, Hachicha M, Misle E, Fidalgo F, Teixeira J. Physiological and biochemical responses to the exogenous application of proline of tomato plants irrigated with saline water. Journal of Saudi Society of Agricultural Sciences. 2018; 17 (1):17-23.
74. Rady MM, Sadak MSh, El-Bassiouny H. MS, Abd El Monem AA. Alleviation the adverse effects of salinity stress in sunflower cultivars using nicotinamide and α-tocopherol. Australian j basic applied sciences. 2011; 5(10):342-355.
75. Sadak MSH, Abd El-Monem AA, El-Bassiouny HMS, Badr NB. Physiological response of sunflower (*Helianthus annuus* L.) to exogenous arginine and putrescine treatments under salinity Stress. Journal of Applied Sciences Research. 2012; 8(10):4943- 4957.
76. Sadak MSh, Abd Elhamid EM. Physiological response of flax cultivars to the effect of salinity and salicylic acid. Journal of Applied Sciences Research. 2013; 9(6):3573-3581.
77. Tasir S, Per A, Nafees A, Khan A, Masood, Mirza Hasanuzzaman, M. Iqbal R. Khan, Naser A. Anjum. Approaches in modulating proline metabolism in plants for salt and drought stress tolerance: Phytohormones, mineral nutrients and transgenics. Plant Physiology and Biochemistry. 2017; 115:126-40.
78. Farahat MM, Azza AM, Mona HM, Sahar MZ. Salt tolerance in grevillea robusta seedlings via foliar application of ascorbic acid. Middle-East Journal of Scientific Research. 2013; 14(1):09-15.
79. Shao HB, Chu LY, Zhao HL, Kang C. Primary antioxidant free radical scavenging and redox signaling pathways in higher plant cells. International Journal of Biological Sciences. 2008; 4(1):8-14.
80. Ahanger MA, Begum N, Qin C, Raza S, Khan MI, Ashraf M, Ahmed N, Zhang L. Role of arbuscular mycorrhizal fungi in plant growth regulation: implications in abiotic stress tolerance. Frontiers in Plant Science. 2019; 10:1-15.
81. Soltani Nezhad F, Ehsanpour AA, Hosseini SM. Effect of salt stress on acid phosphatase activity and phosphorus content of *lycopersicon peruvianum,* L. under in vitro culture. Malays. Applied Biological Chemistry. 2011;40(1):7-11.
82. McLaughlin SB, Wimmer R. Transley reviews No.104- Calcium physiology terrestrial ecosystem processes. New phytologist. 1999; 142: 373-417.
83. Bidel LP, Chomicki G, Ming F, Zhan X, Wang Y, Baissac Y, Allemand C, Renner S. The velamen protects photosynthetic orchid roots against UV-B damage, and a large dated phylogeny implies multiple gains and losses of this function during the Cenozoic. New Phytologist. 2007; 205:1330-1341.

84. Bubu D, Motterlini R, Lefebvre R. CO and CO -releasing molecules (CO-RMs) in acute gastrointestinal inflammation. *Pharmacology of the Gasotransmitters*. 2012; 172(6):1557-1573.
85. Sivakumar V, Ponnusami V. Influence of spacing and organics on plant nutrient uptake of Solanum nigrum. Agricultural Science Digest. 2011; 11 (1):431-434.
86. Fernández V, Sotiropoulos T, Brown PH. Foliar fertilization. In scientific principles and field practices. International Fertilizer Industry Association, Paris; 2013.
87. Obreza TA, Zekri M, Hanlon EA, Morgan K, Schumann A, Rouse R. Soil and leaf tissue testing for commercial citrus production. University of Florida Extension Service SL. 2010; 4: 253.
88. McHargue. The role of manganese in plants. Journal of the American Chemical Society. 1922; 44: 1592-1598.
89. Millaleo R, Reyes- Diaz, M.; Ivanov, A. G.; Mora, M. L.; Alberdi, M. (2010): Manganese as essential and toxic element for plants: transport, accumulation and resistance mechanisms. *Journal of Soil Science and Plant Nutrition*. Volume 10; Pages 470-481.
90. Broadley M, Brown P, Cakmak I, Rengel Z, Zhao F. Function of nutrients: micronutrients. In: Marschner P. Marschner's mineral nutrition of higher plants, 3rd edition. San Diego. Academic Press. 2012; 249-269.
91. Cakmak I. Plant nutrition research: priorities to meet human needs for food in sustainable ways. Plant and Soil. 2002;247:3-24.
92. Schjoerring JK, Cakmak I, White PJ. Plant nutrition and soil fertility: synergies for acquiring global green growth and sustainable development. Plant and Soil. 2019; 434:1-6.
93. Marschner H. Mineral nutrition of higher plants. 2ed edition. New York: Academic Press; 1995.
94. Karim MR, Zhang YQ, Zhao RR, Chen X. P, Zhang FS, Zou CQ. Alleviation of drought stress in winter wheat by late foliar application of zinc, boron, and manganese. Journal of Plant Nutrition and Soil Science. 2012; 175:142-51. DOI:10.1002/jpln.201100141.
95. Kutman U, Yildiz B, Ozturk L, Cakmak I. Biofortification of durum wheat with zinc through the soil and foliar applications of nitrogen. Cereal Chemistry. 2010;87: 1-9.
96. El-Sheikh MH, Khafgy SA, Zaied SS. Effect of foliar application with some micronutrients on leaf mineral content, yield and fruit quality of Florida prince desert red peach trees. Journal *of Agriculture* and Biological Sciences. 2007; 3: 309-315.
97. Marschner H. Mineral nutrition of higher plants. Academic Press Limited Harcourt Brace and Company, Publishers, London. 2012;347-364. ISBN: 978-0-12-384905-2.
98. Barker AV, Pilbeam DJ. Handbook of Plant Nutrition. CRC Press; 2006. ISBN 9780824759049.

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