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The Role of Plant Growth Regulators for Alleviates Salt-Induced Oxidative Stress in Two Lettuce Cultivars by Enhancing Physiological Studies

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Abstract

This study aimed to explain the role of plant growth regulators-PGRs (AsA & SA -0.5mM) for reducing the effect of salinity stress on two lettuce cultivars (cv. Paris [cv. Paris Island Cos] & cv. Royal). The lettuce seeds for both cultivars (cv. Paris & cv. Royal) soaked in PGRs (AsA & SA -0.5 mM) for 12 hours in the dark at 4°C. Planted seeds in trays of cork contains 218 eye for 14 days, transplanted the seedlings plant to plastic containers each pot containing one plant was irrigated with using NaCl salinity concentrations (0.00, 50; 100; 150 mM) alternative with distilled water until harvest (84 days). The harvest (84 days) data explained that the antioxidant activity enzymes (catalase-CAT, ascorbate peroxidase-APX, glutathione reductase-GR & superoxide dismutase-SOD), proline (Pro) and in-organic nutrient mineral elements in shoots. The catalase enzyme activity (CAT) and Pro contents increased significantly in the present of AsA more than SA under NaCl salinity stress compared with control. While, the ascorbate peroxidase enzyme activity (APX), glutathione reductase enzyme activity (GR), superoxide dismutase enzymes activity (SOD) and the contents of N, P, K, Ca, Mn and B nutrient mineral elements increased with salinity concentrations especially with 50 mM NaCl concentration than control. Whereas, the role of AsA was more effective for both cultivars than SA under NaCl salinity concentrations compared with control. Also, the Mg, Na and Zn contents in shoot of both cultivars tended to increase with increasing NaCl salinity concentrations in the presence or absence of PGRs (AsA & SA) compared with control. The data provide strong support to the hypothesis that the role of exogenous AsA reduces the harmful effects of salinity and increases resistance both cultivars of lettuce plant to salinity stress.

Keywords

Ascorbic Acid, Salicylic Acid, Lettuce, *Lactuca sativa* L., Antioxidant, Proline, Elements, Salinity, Catalase, Ascorbate Peroxidase

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1. Introduction

Salinity is of the most brutal environmental factors limiting growth and the productivity of a wide variety of crops throughout the arid and semi-arid regions of the world, especially those with limited rainfall, high evapotranspiration, and high temperature, salinization is exacerbated by the development of soil sodicity associated with the use of irrigation water containing high concentrations of sodium [1-4]. Soil salinity includes saline, sodic and alkaline soils, characterized by high salt content, high sodium cation (Na^+) content, and high pH, respectively [5]. A progressive increase in salinity has caused degradation of arable land over many hundred years' period cultivated land could be degraded due to salinity during less than 100 years. At present, about 10% of the global land area and 50% of

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irrigated areas are exposed to salinity, causing a loss of about 12 billion US$ in the agricultural sector [6]. Saline soil is characterized by an electrical conductivity (EC) of 4 dSm^{-1} generating an osmotic pressure of about 0.2 MPa [7] and the density of more salt available in soil alter the physiological and metabolic activities in the agricultural crops and reduce the growth and production of crops both qualitative and quantitative ways [8].

Osmotic stresses, such as drought and salt stress, are caused by excessive accumulation of salt in the soil, directly, because of salinization, or indirectly because of water loss. As a consequence, a wide spectrum of physiological and biochemical alterations of plants are induced by salinity, which causes lowered water potential in the soil solution, ionic disequilibrium, specific ion effects, and a higher accumulation of reactive oxygen species (ROS) so, that negatively affects the growth and development of the plant [9-11].

Plant growth regulators (PGRs) play important roles in the regulation of plant developmental processes and signaling networks as they are involved either directly or indirectly in a wide range of biotic and abiotic stress responses and tolerance in plants [12-14]. Ascorbic acid (AsA) or vitamin C is water-soluble vitamin, it is one of the universal non-enzymatic antioxidants having substantial potential to prevent or in minimizing the damage caused by ROS in plants [15-16]. The AsA levels are different among organs and it tends to be more concentrated in leaves than in roots and usually being higher in photosynthetic cells, meristems and some fruits [17-20]. Several studies report the AsA-regulation of antioxidant defense metabolism in different plants grown under salinity stress conditions [21-23]. This has been mainly ascribed to AsA involvement in activation of different antioxidant enzymes [24-25].

Salicylic acid (SA) is a lipophilic monohydroxy benzoic acid and it is one of many phenolic compounds that are synthesized by plants [26-27]. Free SA is a white bitter-tasting powder a crystalline, it is moderately soluble in water and very soluble in polar organic solvents [28]. Biosynthesis of SA occurs in the chloroplast, while its perception by receptor complexes is in the nucleus [29-30]. Salicylic acid (SA) is one of the endogenous PGRs and it plays a crucial role in modulate various metabolic and physiological events during the entire lifespan of the plant [31-32] Ameliorative effect of SA on growth of crop plants under abiotic stress conditions may have been due to its role in influence a range of diverse processes in plants such as nitrogen metabolism, Pro metabolism, production of glycinebetaine, antioxidant defense system, and plant-water relations and thereby provides protection in plants against abiotic stresses [33-37]. In addition, SA influences establishment of seedling, cell growth and expansion and also stimulates the activity of enzymes; synthesis of flavonoid and chlorophyll and photosynthesis process under adverse environmental conditions [38-40].

Due to the importance of the lettuce plant, where it is the leaf vegetable primarily consumed fresh as a salad or in salad mixtures with other kinds of fresh vegetables [41]. Lettuce, *Lactuca sativa* L. has 95% water contain and good source of fiber, iron, folate, lutein, β-carotenoids, and the antioxidants compounds such as vitamins A, C and E and they are the most important health beneficial bioactive compounds in lettuce [42-44]. The objective of the present study was to evaluate the effects of exogenous application of PGRs (AsA & SA) on lettuce seeds before germinated for alleviates salt-induced oxidative stress in two selected lettuce cultivars enhancing antioxidant enzyme activities, proline and inorganic mineral elements contents.

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 and 150 mM).

2.2. The Soil Used

The soil used for cultivated lettuce 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. Plant Growth Regulators (AsA & SA)

2.3.1. Ascorbic Acid (AsA-0.5 mM)

Ascorbic acid obtained from Sigma Chemical Co. UK, was initially dissolved in a little amount of distilled water and the final volume was reached, using distilled water.

2.3.2. Salicylic Acid (SA-0.5 mM)

Salicylic acid; 2-hydroxybenzoic acid, obtained from Sigma Chemical Co. UK (*POLYOXYETHYLENESORBITAN MONOLAURATE, SIGMA CHEMICALS, UK*), were initially dissolved in dimethyl sulfoxide to obtained concentration of 0.5 mM (pH 6.0-6.5) then added 0.02% Tween 20 to help for distributed the SA in media [45].

2.4. The Role of Plant Growth Regulators (AsA & SA) on Lettuce Seeds Germination

Selected of the *Lactuca sativa,* L. for two lettuces cultivar, (cv. Paris & cv. Royal) seeds germination was undertaken in

the greenhouse at Al Taif City, Kingdom of Saudi Arabia, which are obtained from Al Taif City, Saudi Arabia for cultivation. The lettuces seeds for both cultivars are characterized by its earliness, high yield ability, uniform ripening and disease tolerance, intact, homogeneous in size and free from wrinkles. Then soaked the seeds for 12 hours in the dark and leaves in the refrigerator for dormancy the lettuce seeds as follow: (1) 1st group, seeds soaked in distilled water (2) 2nd group, seed soaked in a solution of ascorbic acid (AsA-0.5 mM). (3) 3rd group, seeds soaked in a solution of salicylic acid (SA-0.5 mM). Germinated *Lactuca sativa,* L. seeds (cv. Paris and cv. Royal) after soaking in different PGRs (AsA and SA) in 4 trays of cork (39 cm × 67cm), which containing 218 tray diameter eyes (3cm and depth 6.5 cm) 2 trays for each PGRs treatment. The tray eyes containing an equal amount of peat-moss (*GERMANY*), pH 6.30-5.50, EC 0.6-0.5 mmohs/cm 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 14°C ± 2°C (night)/ 20°C±2°C (day).

The seedling of *Lactuca sativa,* L. seeds should be sown at a depth of soil between 0.6 cm and 1.3 cm in the tray eyes. The optimal temperature for germination is 20°C±2°C. The relative humidity varied between 60-70% and day light from 11 to 12 h. The *Lactuca sativa,* L. seeds watering with distilled water until the emergence of the 4th leaf then transplanted to a pot (diameter 21 cm and depth of 18 cm with perforated bottoms) which containing the sandy soil (washed by diluted hydrochloric acid (1N HCl) and washed thoroughly with distilled water more five times), and peat moss with agricultural perlite (agrolite) as a ration (2:1-v: v).

Transplanting seedling and Irrigation System of lettuce from cork trays to plastic pots, each pot containing one plant, all pots were irrigated with 450 ml distilled water immediately after transplanting. Started treatments with second irrigated for each group with using NaCl salinity concentrations (0.00, 50; 100; 150 mM) alternative with distilled water. Also used fertilizer N: P: K + Trace Elements 20:20:20, European Commission fertilization produced by COMPO Epert GmbH (Germany), (*Agroleaf power*) as 1.5 gl^{-1} was added once every two weeks after the emergence of the 4th leaf for all experiments treatments as shown in Figure 1.

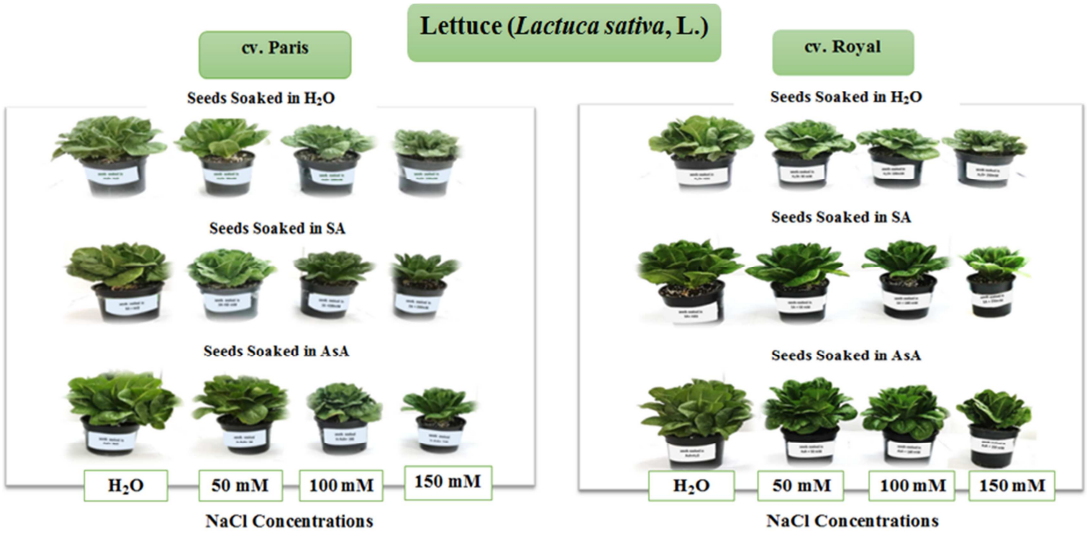

Figure 1. The Role of Plant Growth Regulators (AsA & SA) On Growth of Lettuce (*Lactuca sativa,* L. cv. Paris & cv. Royal) Plant under Salinity Stress.

2.5. Estimation of Enzymes

For catalase (CAT), ascorbate peroxidase (APX), superoxide dismutase (SOD), 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 [46].

2.5.1. Catalase (CAT) Activity Enzyme

Extraction of soluble proteins by a frozen sample of 0.5 g lettuce leaves was homogenized in 8 ml of 50 mM cold phosphate buffer at pH 7.5 modified from Beauchamp and Fridovich [47]. 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 [48].

2.5.2. Ascorbate Peroxidase (APX) Activity Enzyme

Ascorbate peroxidase (APX) activity was measured according to Yoshimura *et al*. [49] 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.

2.5.3. 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 [50]. The absorbance at 412 nm recorded at 25°C over a period of 5 min on a spectrophotometer. For enzyme specific activity = R2X100/ R1.

2.5.4. Superoxide Dismutase (SOD) Activity Enzyme

The plant sample supernatant was used for enzyme activity assay according to Esfandiari *et al.* [46] 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.* [51].

2.6. 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 ºC, 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 Pro and calculated on a dry weight basis as µg proline/100 g dry weight according to the method of Bates *et al.* [52].

2.7. Inorganic Mineral Elements Contents

Cation contents of the milled samples were estimated following the "wet ashing procedure" of the powdered samples as described by Richards [53]. The acid digests of the oven dried samples were analyzed for sodium, potassium, calcium and magnesium. Sodium, potassium and calcium contents were determined photometrically using a corning-400 flam photometer [54-55]. The levels of magnesium and manganese 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 was estimated by the Molybdenum-blue method [55], while nitrogen was estimated by the Automatic MicroKjeldahl method [55].

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 calculated from the equation:

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

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

2.8. Statistical Analysis

Statistical analyses of the data by Kotz *et al.* [56] were fed to the computer and analyzed using IBM SPSS software package version 20.0. (Armonk, NY: IBM Corp) [57]. Quantitative data were described using mean and standard error. Significance of the obtained results was judged at the 5% level. The used tests were as follow:

1) Student t-test: For normally distributed quantitative variables, to compare between two studied groups.

2) F-test (ANOVA): For normally distributed quantitative variables, to compare between more than two groups, and Post Hoc test (*LSD*) for pairwise comparisons [56-57].

3. Results and Discussion

3.1. Antioxidant Enzyme Activity

Catalase Enzymes Activity (CAT- Unite/mg Protein/min): Data presented in Figure 2A and Table 1 indicated that the CAT enzyme activity (unite/mg protein/min) contents in leaves of lettuce (*Lactuca sativa*, L. cv. Paris & cv. Royal) plants tended to increased highly significant at ($p \leq 0.001$), with increasing NaCl salinity concentrations (50,100 & 150 mM), in the presence or absence of PGRs (AsA & SA) compared with control. The role of AsA on lettuce plant for both cultivars have been alleviated the effect of salinity on the CAT enzyme contents in leaves more than SA compared with control. The CAT enzyme activates increased significantly ($p \leq 0.001$) more in cv. Royal S2 than in cv. Paris S1 especially in the present of AsA more than with SA compared with control. Overall the statistical analysis indicated that the two ways analysis of variance (*ANOVA*) between different concentrations of salinity stress, PGRs (AsA & SA) in two cultivars indicated that the *LSD* test highly significant at $P \leq 0.001$. The result agrees with Dolatabadian and Jouneghani [58] they found that the catalase activity in bean plant increased with increasing salt stress. However, there

was not significant effect in non-stressed plant, total CAT activity mostly increased with salt stressed plants, but it was higher at 400 mM NaCl than at 100 mM NaCl.

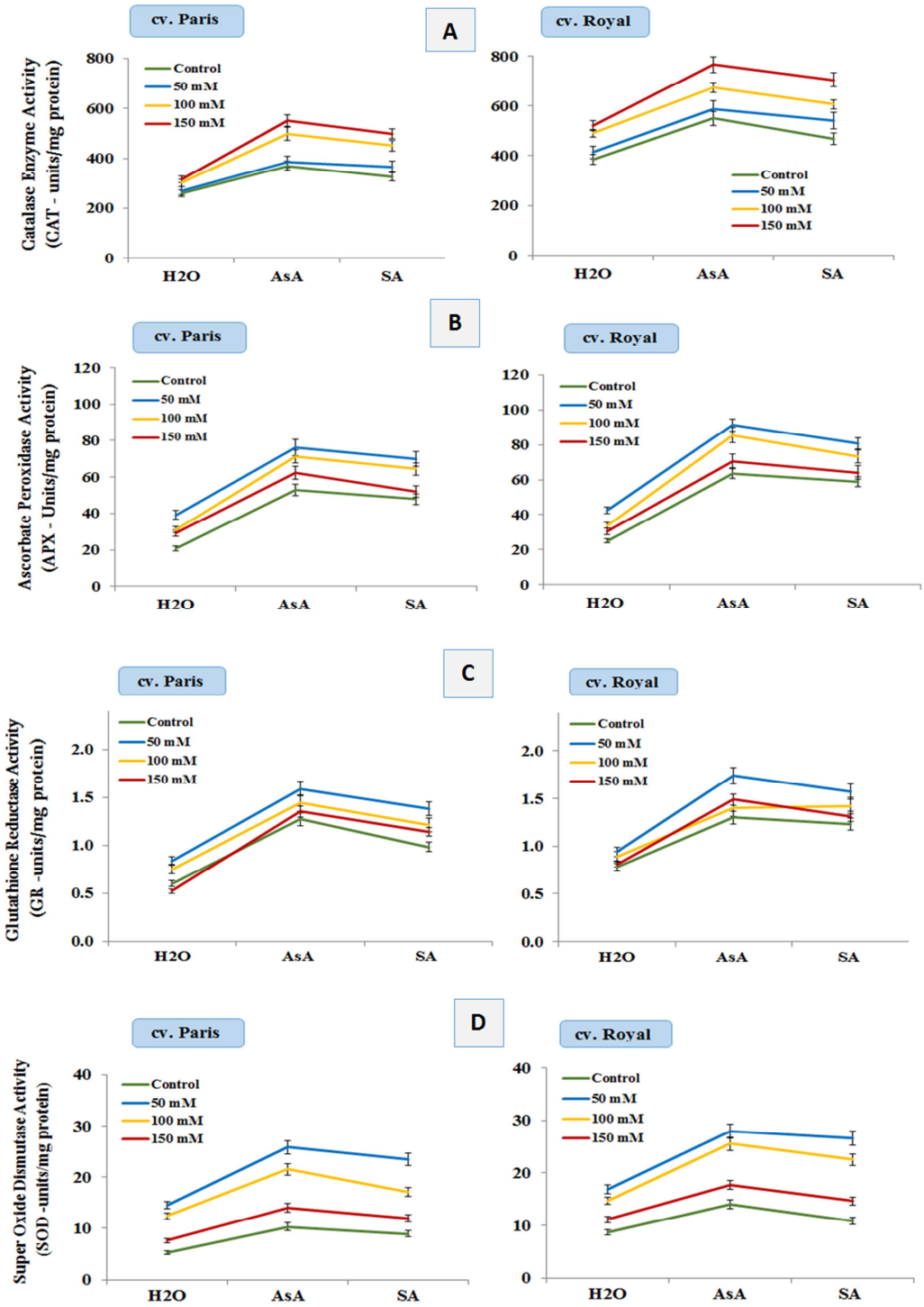

Figure 2. The Role of Plant Growth Regulators (AsA & SA) On Shoot (A)-Catalase (CAT), (B)- Ascorbate Peroxidase (APX), (C)- Glutathione Reductase (GR), (D)- Super Oxide Dismutase (SOD) Activities (Units/mg protein) of Lettuce (*Lactuca sativa,* L. cv. Paris & cv. Royal) Plant under Salinity Stress.

Ascorbate Peroxidase Enzyme Activity (APX), Glutathione Reductase Enzyme Activity (GR), Superoxide Dismutase Enzymes Activity (SOD) (Unite/mg Protein/min): Overall, the data presented in Figures (2 B, C & D) and Table 1 indicated that the APX, GR & SOD enzymes activity contents in leaves of lettuce (*Lactuca sativa*, L cv. Paris & cv. Royal) plant increased highly significant at ($p \leq 0.001$) with decreased salinity concentrations (gradually start with 50 then 100 & 150mM NaCl) in the presence or absence of PGRs (AsA & SA) compared with normal control. The all of APX, GR &

SOD enzymes activity increased significant ($p \leq 0.001$) especially with 50 mM NaCl concentration than control. The effect of AsA was more effective by increasing significantly ($p \leq 0.001$) the all of APX, GR & SOD enzymes activity for both cultivars under NaCl salinity concentrations compared with the effect of SA. So, the role of AsA on lettuce plant for both cultivars have been alleviated the effect of salinity by increasing the all of APX, GR & SOD enzymes activity more than SA compared with control. Consequently, the all of this results it has been found that the all of APX, GR & SOD enzymes activity increased significantly ($p \leq 0.001$) more in cv. Royal S2 than in cv. Paris S1 especially in the present of AsA more than SA compared with control. Overall the statistical analysis indicated that the two ways analysis of variance (*ANOVA*) between different concentration of salinity stress, PGRs (AsA and SA) in two cultivars indicated that the *LSD* test highly significant at $P \leq 0.001$. The all of APX, GR & SOD enzymes activity is a part of enzymatic and non-enzymatic antioxidant defense system and thus contributes to ROS neutralization. AsA induces the expression dismutase H_2O_2 to water and molecular oxygen using AsA as an electron source under salt stress [25]. Mittal *et al.* [59] found the increase in total antioxidant capacity in turnip plant under salinity after the application of AsA could be due to the regenerative nature of ascorbate which plays a key role in quenching intermediate/excited reactive forms of molecular oxygen either directly or through enzymatic catalysis [60].

Table 1. The Role of Plant Growth Regulators (AsA & SA) On Shoot Catalase (CAT), Ascorbate Peroxidase (APX), Glutathione Reductase (GR), Super Oxide Dismutase (SOD) Activities (Units/mg protein) of Lettuce (*Lactuca sativa,* L. cv. Paris & cv. Royal) Plant under Salinity Stress.

NaCl Conc. (mM)	Lettuce (*Lactuca sativa* L.)					
	cv. Paris			cv. Royal		
	H_2O	AsA	SA	H_2O	AsA	SA
Catalase Activity (CAT-Units/mg protein)						
Control	$260.3^{d} \pm 1.03$	$369.5^{d\#} \pm 0.47$	$325.9^{d\#@} \pm 0.70$	$385.8^{d} \pm 1.06$	$549.5^{d\#} \pm 1.08$	$467.6^{d\#@} \pm 1.05$
50	$269.7^{c} \pm 1.16$	$385.5^{c\#} \pm 2.23$	$367.1^{c\#@} \pm 1.55$	$413.3^{c} \pm 1.23$	$586.5^{c\#} \pm 1.26$	$540.0^{c\#@} \pm 1.52$
100	$302.0^{b} \pm 1.24$	$498.2^{b\#} \pm 1.31$	$450.3^{b\#@} \pm 0.85$	$489.8^{b} \pm 0.27$	$672.1^{b\#} \pm 1.85$	$605.5^{b\#@} \pm 2.15$
150	315.1a ±1.37	$551.2^{a\#} \pm 1.48$	$497.2^{a\#@} \pm 0.83$	$520.7^{a} \pm 1.36$	$765.1^{a\#} \pm 1.08$	$704.3^{a\#@} \pm 2.29$
F	466.066*	3411.412*	5622.896*	3514.595*	5037.277*	3052.136*
p	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*
LSD	3.798	4.749	3.270	3.368	4.268	5.741
Ascorbate Peroxidase Activity (APX-Units/mg protein)						
Control	$20.71^{d} \pm 0.35$	$53.02^{d\#} \pm 0.63$	$47.75^{d\#@} \pm 0.31$	$24.90^{d} \pm 0.52$	$63.67^{d\#} \pm 0.78$	$58.80^{d\#@} \pm 0.21$
50	$39.13^{a} \pm 0.57$	$76.13^{a\#} \pm 0.72$	$69.82^{a\#@} \pm 0.39$	$42.50^{a} \pm 0.44$	$91.32^{a\#} \pm 0.22$	$80.68^{a\#@} \pm 0.83$
100	$31.16^{b} \pm 0.41$	$71.26^{b\#} \pm 0.53$	$64.29^{b\#@} \pm 0.52$	$33.75^{b} \pm 0.80$	$85.63^{b\#} \pm 0.50$	$73.41^{b\#@} \pm 0.81$
150	$29.04^{c} \pm 0.17$	$62.22^{c\#} \pm 0.79$	$51.89^{c\#@} \pm 0.84$	$30.39^{c} \pm 0.61$	$70.48^{c\#} \pm 0.48$	$64.16^{c\#@} \pm 0.58$
F	353.638*	228.886*	352.533*	147.658*	586.774*	220.925*
p	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*
LSD	1.269	2.128	1.737	1.914	1.676	2.061
Glutathione Reductase Activity (GR-Units/mg protein)						
Control	$0.60^{d} \pm 0.01$	$1.27^{d\#} \pm 0.01$	$0.98^{d\#@} \pm 0.02$	$0.78^{c} \pm 0.01$	$1.30^{d\#} \pm 0.01$	$1.23^{d\#@} \pm 0.02$
50	$0.84^{a} \pm 0.01$	$1.59^{a\#} \pm 0.02$	$1.38^{a\#@} \pm 0.01$	$0.94^{a} \pm 0.01$	$1.74^{a\#} \pm 0.01$	$1.\ 57^{a\#@} \pm 0.01$
100	$0.75^{b} \pm 0.01$	$1.44^{b\#} \pm 0.01$	$1.21^{b\#@} \pm 0.01$	$0.89^{b} \pm 0.01$	$1.40^{b\#} \pm 0.01$	$1.42^{b\#} \pm 0.01$
150	$0.52^{c} \pm 0.02$	$1.35^{c\#} \pm 0.02$	$1.14^{c\#@} \pm 0.01$	$0.81^{c} \pm 0.01$	$1.49^{c\#} \pm 0.01$	$1.31^{c\#@} \pm 0.01$
F	199.135*	89.849*	160.575*	44.813*	277.761*	159.807*
p	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*
LSD	0.032	0.046	0.041	0.034	0.036	0.037
Super Oxide Dismutase Activity (SOD-Units/mg protein)						
Control	$5.20^{d} \pm 0.63$	$10.29^{d\#} \pm 0.55$	$8.94^{d\#} \pm 0.45$	$8.64^{d} \pm 0.28$	$14.06^{d\#} \pm 0.23$	$10.84^{d\#@} \pm 0.25$
50	$14.57^{a} \pm 0.22$	$25.86^{a\#} \pm 0.55$	$23.48^{a\#@} \pm 0.49$	$16.90^{a} \pm 0.23$	$27.91^{a\#} \pm 0.31$	$26.63^{a\#@} \pm 0.19$
100	$12.43^{b} \pm 0.37$	$21.52^{b\#} \pm 0.26$	$17.04^{b\#@} \pm 0.37$	$14.67^{b} \pm 0.39$	$25.53^{b\#} \pm 0.37$	$22.47^{b\#@} \pm 0.50$
150	$7.57^{c} \pm 0.22$	$14.08^{c\#} \pm 0.33$	$11.95^{c\#@} \pm 0.45$	$11.08^{c} \pm 0.17$	$17.66^{c\#} \pm 0.24$	$14.62^{c\#@} \pm 0.32$
F	140.570*	310.669*	209.023*	209.448*	555.688*	509.254*
p	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*
LSD	1.245	1.400	1.388	0.885	0.918	1.059

F: F for *ANOVA* test, Pairwise comparison between each 2 groups were done using Post Hoc Test (*LSD*), p: p value for comparing between the studied groups, Means in the same column with Common letters are not significant (i.e. Means with Different letters are significant), #: Statistically significant with H_2O, @: Statistically significant with AsA, *: Statistically significant at $p \leq 0.05$, Data was expressed using Mean ± SE.

Also, the same results with Ebrahimian and Bybordi [61] they explained that the increasing in SOD activity of sunflower due to salinity stress, whereas, the results disagree with our results in the present of AsA, the application of AsA into nutrition solution significantly decreased lipid peroxidation content and SOD activity under salinity stress conditions. Generally, AsA prevented lipid peroxidation by oxygen free radicals, whether under salt stress or normal conditions. Ascorbic acid (AsA) is known to be an effective antioxidant in ROS scavenging, so decease in antioxidant enzyme activity may be due to low level

of ROS in plant tissues.

The results of this study agree with the findings by Athar *et al.* [15, 62] they reported that an increase in antioxidant enzyme activities in wheat plants after AsA application. Some works suggested that an increase in the activity of antioxidant enzymes helps the plants to maintain their growth under stress conditions and may be regarded as an indicator of salinity tolerance [63-64]. Plants containing higher concentrations of antioxidants show more resistance to the oxidative damage caused by salt stress [65]. Likewise, activities of antioxidant enzymes peroxidase (POD) and SOD in sugarcane plants were increased under salt stress as well as after AsA application. A considerable increase in the POD activity was observed with AsA application. Higher levels of antioxidant enzymes might be attributed to their property to help develop the plant's resistance against oxidative damage [66].

The results of this study confirmed with findings by Yusuf *et al*. [67] where they reported that SA enhanced the level of antioxidant system (SOD and CAT) both under stress and stress-free conditions. However, the influence of SA on antioxidant system was more pronounced under stressful condition, therefore, suggesting that the elevated level of antioxidant system might be responsible for increased tolerance of *Brassica juncea* plants to NaCl stress. Similarly, SA-pretreatment was evidenced to alleviate the adverse effects of salinity stress on photosynthesis and growth in *V. radiata* through enhancing the activities of antioxidant enzymes including SOD, CAT, APX, and GR [35, 68]. Salicylic Acid (SA) application (at 0.5 mM) increased activity of enzymes of AsA–GSH pathway resulted in the increased tolerance of *B. juncea* to salinity stress [69]. Exogenous SA (0.5 mM) significantly improved salinity (250 mM NaCl) tolerance in *T. aestivum* by markedly increasing the pools of AsA and glutathione (GSH) [70].

The data presented here similar to the results by Ma *et al.* [71] they found that the SOD and CAT enzymes activities in the D. *superbus* were significantly increased by under the 0.3 and 0.6 NaCl conditions and the SA treatment enhanced SOD and CAT enzymes activities compared with the non-SA-treated plants under salt stress. However, when the plants were grown under severe salt stress (0.9% NaCl condition), there was no significant difference in oxidant enzyme activity between SA-treated and non-SA-treated. The results of foliar application of SA treatments on two lettuce cultivars were enhanced antioxidant enzyme activity under salinity stress conditions, [72-74].

Likewise, the results presented here agree with the results obtained by Siddiqui *et al*. [75] they found that under NaCl stress, the addition of AsA to the growth medium enhanced the activity of antioxidant enzymes (SOD, CAT, and APX) and organic solutes under salt stress, which may have caused inhibition of lipid peroxidation and membrane deterioration by decreasing the accumulation of malondialdehyde and H_2O_2, and electrolyte leakage; including minimizing degradation of chlorophyll (Chl) and other photosynthetic pigments. Also, foliar applied AsA has been up-regulating the activities of some key enzymes of oxidative defense system in a halophyte *Lymonium stocksii* under saline stress [76].

3.2. Proline Contents (µg/100g Dry Weight)

Shoots Proline Content: Overall, the proline contents in shoot lettuce (*Lactuca sativa*, L. cv. Paris & cv. Royal) plant tended to increased highly significant at ($p \leq 0.001$), with increasing NaCl salinity concentrations in the presence or absence of PGRs (AsA & SA) compared with control as shown in Figure 3 & 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 both cultivars under NaCl salinity compared with SA. While, the role of AsA have been alleviated the effect of salinity on lettuce plant for both cultivars by increasing proline contents in shoot more than with SA compared with control. So, the all of this results it has been found that the proline contents in shoot increased significantly ($p \leq 0.001$) more in cv. Royal S2 than in cv. Paris S1 especially in the present of AsA compared with control. Overall the statistical analysis indicated that the two ways analysis of variance (*ANOVA*) between different concentration of salinity stress and PGRs (AsA & SA) in two cultivars indicated that the *LSD* test highly significant at $P \leq 0.001$.

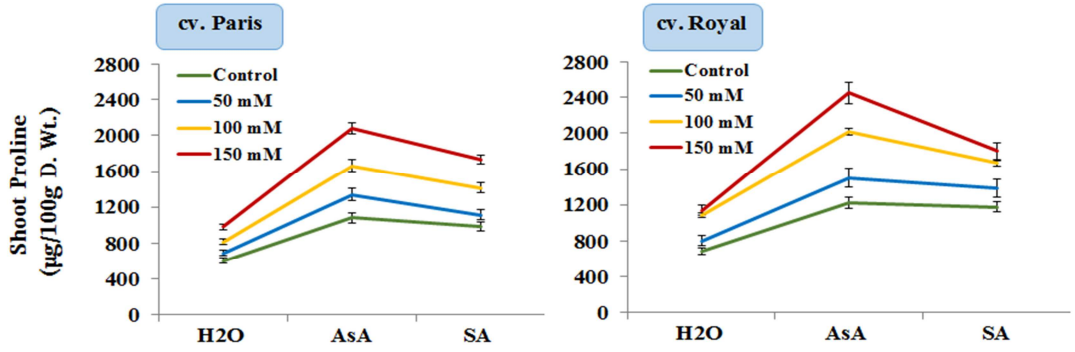

Figure 3. The Role of Plant Growth Regulators (AsA & SA) on Shoot Proline (µg/100g D. Wt.) Contents of Lettuce (*Lactuca sativa,* L. cv. Paris & cv. Royal) Plant under Salinity Stress.

Table 2. The Role of Plant Growth Regulators (AsA & SA) On Shoot Proline (µg/100g D. Wt.) Contents of Lettuce (*Lactuca sativa,* L. cv. Paris & cv. Royal) Plant under Salinity Stress.

NaCl Conc. (mM)	Lettuce (*Lactuca sativa* L.)					
	cv. Paris			cv. Royal		
	Proline (µg/100g D. Wt.) Contents					
	H_2O	AsA	SA	H_2O	AsA	SA
Control	602.8^{c}±3.23	$1080.5^{d\#}$ ±3.11	$990.9^{d\#@}$ ±2.16	688.6^{d}± 2.35	$1224.4^{c\#}$±2.81	$1175.4^{d\#}$± 4.12
50	693.1^{b} ± 4.10	$1337.8^{c\#}$± 2.18	$1115.4^{c\#}$± 3.1	804.7^{c} ± 2.14	$1497.5^{b\#}$ ± 3.12	$1384.8^{c\#@}$ ± 2.6
100	816.7^{a} ± 2.29	$1658.6^{b\#}$ ± 4.07	$1410.5^{b\#}$± 2.4	1083.2^{b} ± 4.08	$2013.6^{b\#}$ ± 3.12	$1663.9^{b\#}$± 3.2
150	982.5^{a} ± 2.83	$2075.2^{a\#}$± 3.17	$1734.3^{a\#}$± 3.6	1137.3^{a} ± 3.02	$2450.9^{a\#}$ ± 4.07	$1801.7^{a\#}$± 3.6
F	343.486*	157.658*	104.670*	174.503*	69.963*	120.736*
p	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*
LSD	0.426	0.749	0.945	0.636	1.378	0.807

Nowadays it is known that Pro has multifunctional roles in plants [77]. In addition, proline being an osmoprotectant, can act as a potent non-enzymatic antioxidant. Results of a recent study by Bulgari *et al*. [78] on lettuce plant supports our findings in this study; they observed an increase in Pro content when increasing salt concentration. Proline (Pro) can play an important role in the osmotic adjustment and may participate to the scavenging of reactive oxygen species.

Salt and water deficit both contribute to the accumulation of proline in the plants. Proline (Pro) stabilizes cell membrane and acts to prevent NaCl from disrupting the cell membrane [79]. Proline (Pro) may also act as a signaling molecule to activate multiple processes essential for the adaptation process [80]. Results of a recent study by Ahmed *et al.* [81] on lettuce plant supports our findings in this study; they found a positive correlation between Pro accumulations in lettuce plant with increasing salt concentrations. Proline (Pro) concentration was also found increased on rice, tomato and mangrove (*Bruguiera cylindrica* L.) plants due to the salt stress [82-84].

Production and accumulation of free amino acids, especially proline by plant tissue during salt and water stress is an adaptive response. Proline (Pro) has been proposed to act as a compatible solute that adjusts the osmotic potential in the cytoplasm [85]. The results presented here agree with the results obtained by Bartha *et al*. [86] they found that the presence of 100 mM NaCl in the growth medium caused a significant increase in the free proline content in all of the five lettuce cultivars. In the Paris Island cultivar, the proline content increased twenty-five times as compared to the control, which is a very pronounced metabolic reaction related to an effective osmoregulation with involvement of this compatible solute. Similar data were presented about the involvement of proline accumulation in salt stress tolerance of some other lettuce cultivars [87].

The data presented here disagree with results obtained by Sakr *et al*. [88] they found that the all salinity stress levels slightly increased Pro content and total soluble sugars in shoots or fruits of pepper plants. On the other hand, the applied protectants AsA decreased Pro content in both shoots and fruits of pepper plant. The application of AsA was effective to mitigate the adverse effects of salt stress on plant growth due to increased leaf area, improved Chl and carotenoids contents, enhanced Pro accumulation and decreased H_2O_2 content, as reported by Azzedine *et al.* [89]. The results presented here agree with the results obtained by El-Afry *et al.* [90] they found that in shoots of flax plant, proline accumulation was significantly increased that observed in all salinity stressed plants. These results are in agreement with those reported by Rady *et al.* [91] and Saeidnejad *et al.* [92] on several plant species. While foliar spraying of AsA caused significant increases in Pro contents of flax cultivars.

The data presented here disagree with results obtained by Hussain *et al.* [93] they found that the Pro concentrations were statistically equal in all treatments under NaCl stress and NaCl + SA in violet plants. Contrary to its generally accepted role in many other plant species, Pro did not seem to play an important role in the mechanism of salt tolerance. The significance of Pro accumulation in osmotic adjustment is still debated and varies according to the plant species.

Researches have well documented that SA is involved in increasing Pro metabolism and decreasing losses in biomass under abiotic stresses [34, 94-95]. Salicylic acid (0.5 mM) significantly induced activity of Pro biosynthesis enzymes (such as pyrroline-5-carboxylate reductase and γ-glutamyl kinase) under salinity stress along with the increased Pro content. This increase in Pro metabolism was attributed to salinity stress tolerance in *Lens esculenta* [94]. Misra and Misra [96] they have shown that up-regulation of Pro biosynthesis enzymes (such as pyrroline-5-carboxylate reductase and γ-glutamyl kinase) and down-regulation of Pro oxidase activity were responsible for increased Pro level. In turn, the increased Pro level was advocated to the maintenance of the cell turgor in *R. serpentina* under salinity stress.

Proline (Pro) acts as an antioxidant by scavenging hydroxyl radical and protecting the structure and function of DNA,

protein and membranes [97]. Sayyari *et al*. [98] they found SA treatment increased proline levels in lettuce leaves plants under drought stress conditions. A highest amount of Pro was achieved in application of 1.5 mM SA and lowest amounts were observed in non- treated plants. These results are in agreement with those of Delavari *et al*. [99] who found that SA treatment increases the Pro content in basil plant under salinity stress.

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

3.3.1. Macro-Elements (mg/100g Dry Weight)

Potassium, Nitrogen, Phosphorous and Calcium Contents (mg/100g Dry Weight): Overall, the shoot K^+, N^{+3}, P^{+3} & Ca^{+2}contents increased highly significant at ($p \leq 0.001$) with decreased salinity concentrations (gradually 50 then 100 & 150 mM NaCl) of lettuce (*Lactuca sativa*, L. cv. Paris & cv. Royal) plant, in the presence or absence of PGRs (AsA & SA) compared with control. The results indicated that the shoot K^+, N^{+3}, P^{+3} & Ca^{+2} contents increased highly significant at ($p \leq 0.001$) especially with 50 mM NaCl concentration compared with control as shown in Table 3.

The results shown that the impact of AsA was more effective increasing significantly ($p \leq 0.001$) of shoot K^+, N^{+3}, P^{+3} & Ca^{+2} contents for both cultivars under NaCl salinity concentrations than SA. So, the role of AsA on lettuce plant for both cultivars have been alleviated the effect of salinity by increasing the shoot K^+, N^{+3}, P^{+3} & Ca^{+2} contents more than SA compared with control. Consequently, the all of this results it has been found that the shoot K^+, N^{+3}, P^{+3} & Ca^{+2} contents increased significantly ($p \leq 0.001$) more in cv. Royal S2 than in cv. Paris S1 especially in the present of AsA more than SA compared with control. Overall the statistical analysis indicated that the two ways analysis of variance (*ANOVA*) between different concentrations of salinity stress, PGRs (AsA & SA) in two cultivars indicated that the *LSD* test highly significant at $P \leq 0.001$. The present result agrees with Hniličková *et al.* [100] they found that in case of common purslane (*Portulaca oleracea* L.) K content decreased with increasing concentrations of NaCl. Bartha *et al*. [86] they demonstrate that selected cultivars of lettuce have shown evident differences in potassium content between 50 and 100 mmol/L NaCl concentrations. Also, Yousif *et al.* [101] reported insignificant differences in K content in *T. tetragonoides* between 50, 100 and 200 mmol/L NaCl concentrations.

Naz *et al*. [102] they explained in *Capsicum annuum* plants treated with different NaCl concentrations (60 mM-100 mM NaCl) exhibited significant increase in Na^+ concentration as compared to non-saline, one of the most important effects of high concentrations of Na^+ is the displacement of K^+ from target sites within the cell because both ions show great chemical similarity. The first point of interaction between Na^+ and K^+ is the entry of these ions to the root symplast. However, high concentrations of Na^+ inhibit directly the transport systems for K^+. Furthermore, Na^+ causes depolarization of the membrane electrical potential, which decreases the absorption of K^+. Accordingly, in salt stress conditions, K^+ deficiency may occur as has been observed in corn, melon and pepper [103-104].

The results of the present study agree with Farahat *et al.* [105] they found the highest N in shoot resulted from AsA (200 ppm) and salinity (3000 ppm) level. Also, Barakat [106] found that the AsA has effects on many physiological processes under saline conditions including increasing physiological availability of water and nutrient. Also, the results of the previous study were confirmed by Turhan *et al.* [107] they observed that the highest NO_3^-N (nitrate) contents in lettuce plants were found in response to control and 1.2 dS m^{-1} seawater treatments, while, there was a considerable decrease in NO_3^-N concentration as salinity stress increased. A negative linear relationship was observed between salinity of irrigation water and NO_3^-N content. Miceli *et al.* [108] reported that lettuce plants under salt stress conditions may decrease their water uptake and change the absorption ratio of nutrients. Additionally, Turhan *et al*. [109] indicated that NO_3^- N concentration significantly decreased in salt-stressed spinach (*Spinacia oleracea* L.) plants.

Magnesium and Sodium Content (mg/100g Dry Weight): Overall, the magnesium and sodium contents (Mg^{2+}& Na^+) in shoot of lettuce (*Lactuca sativa*, L. cv. Paris & cv. Royal) plant tended to increased highly significant at ($p \leq 0.001$), with increasing NaCl salinity concentrations in the presence or absence of PGRs (AsA & SA) compared with control as shown in Tables 3. The results *shown* that the impact of AsA on the Mg^{2+}& Na^+ contents in shoot tended to increased highly significantly ($p \leq 0.001$) for both cultivars under NaCl salinity compared with SA. While, the role of AsA on lettuce plant for both cultivars have been alleviated the effect of salinity on the Mg^{2+}& Na^+ contents in shoot more than SA compared with control. So, the all of this results it has been found the Mg^{2+}& Na^+ contents in shoot increased significantly ($p \leq 0.001$) more in cv. Royal S2 than in cv. Paris S1 especially in the present of AsA more than SA compared with control. Overall the statistical analysis indicated that the two ways analysis of variance (*ANOVA*) between different concentration of salinity stress, PGRs (AsA and SA) in two cultivars indicated that the *LSD* test highly significant at $P \leq 0.001$.

Table 3. The Role of Plant Growth Regulators (AsA & SA) On Shoot Minerals Nutrient Elements (mg/100g D. Wt.) Contents of Lettuce (*Lactuca sativa,* L. cv. Paris) Plant under Salinity Stress.

NaCl Conc.	Macro &Micro-Nutrient Minerals Elements (mg/100g Dry Weight)				
mM	**N**	**P**	**K**	**Na**	**Mg**
	Shoot-Lettuce (*Lactuca sativa*, L. cv. Paris)				
Seeds Pre-king in Distilled Water Before Germination					
Control	7.42d ± 0.01	25.18c ± 0.43	28.70d ± 0.39	38.97d ± 0.38	12.17c ± 0.29
50	11.96a ± 0.16	30.12a ± 0.48	36.04a ± 0.23	41.81c ± 0.24	13.72b ± 0.14
100	10.08b ± 0.29	29.29b ± 0.40	33.98b ± 0.11	45.79b ± 0.47	14.07b ± 0.14
150	8.60c ± 0.18	26.20c ± 0.20	30.35c ± 0.36	49.15a ± 0.21	16.14a ± 0.47
F	251.125*	61.942*	128.782*	309.583*	46.541*
p	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*
LSD	0.611	1.281	0.963	1.119	0.782
Seeds Pre-soaking in AsA Before Germination					
Control	$16.79^{d\#}$ ± 0.18	$31.71^{d\#}$ ± 0.25	$36.92^{d\#}$ ± 0.19	$62.28^{d\#}$ ± 0.13	$16.01^{d\#}$ ± 0.53
50	$22.80^{a\#}$ ± 0.34	$40.70^{a\#}$ ± 0.35	$48.70^{a\#}$ ± 0.24	$67.61^{c\#}$ ± 0.60	$19.03^{c\#}$ ± 0.14
100	$21.95^{b\#}$ ± 0.28	$36.12^{b\#}$ ± 0.11	$43.91^{b\#}$ ± 0.40	$75.84^{b\#}$ ± 0.29	$21.60^{b\#}$ ± 0.13
150	$20.60^{c\#}$ ± 0.26	$33.61^{c\#}$ ± 0.42	$41.78^{c\#}$ ± 0.54	$78.93^{a\#}$ ± 0.14	$24.75^{a\#}$ ± 0.18
F	253.113^{*}	162.551^{*}	175.083^{*}	481.035^{*}	172.292^{*}
p	$<0.001^{*}$	$<0.001^{*}$	$<0.001^{*}$	$<0.001^{*}$	$<0.001^{*}$
LSD	0.887	0.996	1.206	1.140	0.926
Seeds Pre-soaking in SA Before Germination					
Control	$14.16d^{\#@}$ ± 0.18	$30.46c^{\#@}$ ± 0.04	$34.25d^{\#@}$ ± 0.27	$56.67d^{\#@}$ ± 0.48	14.98d# ± 0.24
50	$20.64a^{\#@}$ ± 0.15	$36.91a^{\#@}$ ± 0.19	$46.78a^{\#@}$ ± 0.26	$61.14c^{\#@}$ ± 0.12	$17.02c^{\#@}$ ± 0.18
100	19.09b@ ± 0.52	35.97b# ± 0.57	$41.89b^{\#@}$± 0.38	$70.44b^{\#@}$± 0.16	$19.45b^{\#@}$ ± 0.18
150	$17.14c^{\#@}$ ± 0.11	$31.25c^{\#@}$ ± 0.32	$38.67c^{\#@}$ ± 0.23	$74.98a^{\#@}$ ± 0.14	$22.68a^{\#@}$ ± 0.19
F	222.409*	123.980*	382.088*	890.820*	966.496*
p	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*
LSD	0.951	1.105	0.952	0.870	0.348

Table 3. Continued.

NaCl Conc.	Macro &Micro-Nutrient Minerals Elements (mg/100g Dry Weight)			
mM	**Ca**	**Mn**	**Zn**	**B**
	Shoot-Lettuce (*Lactuca sativa*, L. cv. Paris)			
Seeds Pre-king in Distilled Water Before Germination				
Control	39.18d ± 0.53	0.224d ± 0.003	0.341c ± 0.019	0.105a ± 0.002
50	43.94a$ ± 0.40	0.356a ± 0.006	0.395b ± 0.012	0.196d ± 0.003
100	42.60b$ ± 0.31	0.331b ± 0.009	0.421b ± 0.014	0.158c ± 0.004
150	40.45c$ ± 0.30	0.257c ± 0.003	0.466a ± 0.001	0.121b ± 0.002
F	53.802*	134.123*	15.471*	2.968
p	<0.001*	<0.001*	0.001*	<0.001*
LSD	1.126	0.018	0.043	0.009
Seeds Pre-soaking in AsA Before Germi nation				
Control	$47.33^{d\#}$$ ± 0.46	$0.385^{d\#}$ ± 0.003	$0.490^{c\#}$ ± 0.038	$0.250^{c\#}$ ± 0.002
50	$53.98^{a\#}$$ ± 0.16	$0.573^{a\#}$ ± 0.005	$0.550^{bc\#}$ ± 0.002	$0.362^{a\#}$ ± 0.003
100	$52.50^{b\#}$$ ± 0.25	$0.492^{b\#}$ ± 0.005	$0.626^{ab\#}$ ± 0.041	$0.319^{ab\#}$ ± 0.014
150	$49.12^{c\#}$$ ± 0.34	$0.447^{c\#}$ ± 0.003	$0.682^{a\#}$ ± 0.006	$0.306^{bc\#}$ ± 0.003
F	175.892^{*}	357.276^{*}	9.169^{*}	5.517^{*}
p	$<0.001^{*}$	$<0.001^{*}$	0.006^{*}	0.024^{*}
LSD	1.054	0.014	0.091	0.008
Seeds Pre-soaking in SA Before Germin ation				
Control	45.69d#$ ± 0.29	$0.337d^{\#@}$ ± 0.002	0.411d ± 0.011	$0.213b^{\#@}$± 0.003
50	$52.87a^{\#@}$$ ± 0.22	$0.521a^{\#@}$ ± 0.007	$0.518c^{\#@}$ ± 0.010	$0.325a^{\#@}$± 0.002
100	$50.03b^{\#@}$$ ± 0.33	0.468b# ± 0.011	0.576b# ± 0.006	$0.290a^{\#@}$± 0.002
150	$47.57c^{\#@}$$ ± 0.21	$0.391c^{\#@}$± 0.007	$0.615a^{\#@}$± 0.011	$0.244ab^{\#@}$± 0.003
F	474.139*	118.181*	85.759*	4.637*
p	<0.001*	<0.001*	<0.001*	0.037*
LSD	0.546	0.024	0.031	0.009

The results presented here agree with results reported by Bartha *et al*. [86] they found that all lettuce cultivars growth in saline conditions showed an in-crease in Na^+ concentration, the highest amount of Na^+ was found in the cultivar Paris Island. Therefore, two main strategies of salt stress tolerance can be considered, i.e. salt exclusion and salt se-questration, the latter one is used by lettuce cultivars. This is why the marketable biomass gets a slight salty taste. In

another study on different cultivars of barley, Shabala *et al.* [110] conclude that after one week of salt treatment (320 mM NaCl), shoot Na^+ content of the tolerant variety was about 20 % higher than in the sensitive genotype. In the first phase of the salt stress the rapidly accumulating Na^+ is an osmolite with low energy cost in the leaf vacuoles for the adjustment of cell turgor, and ultimately of tissue growth under the hyperosmotic stress condition imposed by salinity [110-111]. Salt stress disturbs the uptake of essential mineral nutrients such as K^+ and Ca^{2+}, as Na^+ competitively inhibits K^+ and Ca^{2+} transport through membranes [112].

The results of Al-Erwy *et al*. [113] on wheat plant, agree with our results, as well, they mention that salinity caused a significant increase in sodium concentrations in plant shoots and roots. This increase was accompanied by a decline in the K concentration, especially in shoots, indicating an apparent antagonism between K and Na. This antagonism may be due to the direct competition between K and Na at a site of ion uptake in the plasmalemma [114]. Sodium may also enhance the efflux of K into the growth medium, because of disturbed membrane integrity [115]. It is well known that, salinity stress affects the availability of nutrients in the soil by its effects on the solubility and precipitation of salt, and alters physiological processes within the plant, including nutrient uptake and translocation [116]. The distribution of Na^+ varies among the organs of the plant, due to this variation, accumulation of Na^+ in different parts of plant differs [117]. Generally, the sodium ions in shoots and roots were gradually accumulated and increased in salt-stressed condition but the increasing rate depends on salt concentrations [118].

Table 3. Continue: The Role of Plant Growth Regulators (AsA & SA) On Shoot Minerals Nutrient Elements (mg/100g D. Wt.) Contents of Lettuce (*Lactuca sativa,* L. cv. Royal) Plant under Salinity Stress.

NaCl Conc.	Macro &Micro-Nutrient Minerals Elements (mg/100g Dry Weight)				
mM	**N**	**P**	**K**	**Na**	**Mg**
	Shoot-Lettuce (*Lactuca sativa*, L. cv. Royal)				
Seeds Pre-soaking in Distilled Water Before Germination					
Control	7.21d ± 0.34	27.48d ± 0.30	29.51d ± 0.16	41.83d ± 0.23	14.97c ± 0.19
50	11.90a ± 0.12	33.83a ± 0.12	40.31a ± 0.45	44.51c ± 0.18	15.18c ± 0.32
100	11.39b ± 0.15	31.98b ± 0.09	38.33b ± 0.05	50.05b ± 0.15	17.42b ± 0.24
150	9.77c ± 0.07	29.41c ± 0.49	31.98c ± 0.48	54.67a ± 0.21	19.67a ± 0.19
F	245.266*	90.628*	237.898*	1222.225*	95.341*
p	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*
LSD	0.646	0.959	1.000	0.631	0.739
Seeds Pre-soaking in AsA Before Germination					
Control	$19.31^{d\#} \pm 0.06$	$35.32^{c\#} \pm 0.64$	$39.02^{d\#} \pm 0.12$	$64.16^{d\#} \pm 0.48$	$19.32^{d\#} \pm 0.13$
50	$24.09^{a\#} \pm 0.22$	$43.82^{a\#} \pm 0.05$	$51.95^{a\#} \pm 0.24$	$72.20^{c\#} \pm 0.22$	$21.89^{c\#} \pm 0.29$
100	$21.86^{b\#} \pm 0.07$	$42.74^{a\#} \pm 0.29$	$50.08^{b\#} \pm 0.46$	$79.94^{b\#} \pm 0.15$	$24.41^{b\#} \pm 0.35$
150	$20.73^{c\#} \pm 0.30$	$39.55^{b\#} \pm 0.16$	$46.21^{c\#} \pm 0.20$	$83.89^{a\#} \pm 0.22$	$26.97^{a\#} \pm 0.20$
F	433.956^{*}	110.834^{*}	470.798^{*}	446.268^{*}	258.876^{*}
p	$<0.001^{*}$	$<0.001^{*}$	$<0.001^{*}$	$<0.001^{*}$	$<0.001^{*}$
LSD	0.622	1.184	0.934	0.960	0.667
Seeds Pre-soaking in SA Before Germination					
Control	$16.91d^{\#@} \pm 0.33$	34.16d# ± 0.05	$38.25d^{\#@} \pm 0.23$	$60.05d^{\#@} \pm 0.09$	$18.10d^{\#@} \pm 0.15$
50	$22.51a^{\#@} \pm 0.30$	$41.70a^{\#@} \pm 0.70$	$51.71a^{\#@} \pm 0.12$	$66.58c^{\#@} \pm 0.27$	$20.54c^{\#@} \pm 0.17$
100	$20.16b^{\#@} \pm 0.62$	$40.30b^{\#@} \pm 0.25$	$47.29b^{\#@} \pm 0.18$	$70.61b^{\#@} \pm 0.44$	$23.38b^{\#@} \pm 0.18$
150	$17.72c^{\#@} \pm 0.13$	$37.20c^{\#@} \pm 0.09$	$40.42c^{\#@} \pm 0.22$	$76.25a^{\#@} \pm 0.53$	$24.34a^{\#@} \pm 0.39$
F	112.898*	78.240*	676.869*	230.830*	196.277*
p	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*
LSD	1.259	1.223	0.628	1.218	0.660

Table 3. Continued.

NaCl Conc.				
mM	**Ca**	**Mn**	**Zn**	**B**
Seeds Pre-soaking in Distilled Water Before Germination				
Control	41.25c ± 0.57	0.253d ± 0.004	0.360c ± 0.016	0.138c ± 0.002
50	45.82a$ ± 0.44	0.430a ± 0.004	0.420b ± 0.013	0.212a ± 0.001
100	44.32b$ ± 0.24	0.362b ± 0.009	0.438b ± 0.011	0.181ab ± 0.002
150	42.50c ± 0.33	0.279c ± 0.004	0.489a ± 0.008	0.167bc ± 0.001
F	39.689*	214.255*	18.784*	7.563*
p	<0.001*	<0.001*	0.001*	0.010*
LSD	1.254	0.018	0.040	0.006
Seeds Pre-soaking in AsA Before Germination				
Control	$50.56^{d\#}\$ \pm 0.27$	$0.438^{c\#} \pm 0.004$	$0.545^{d\#} \pm 0.010$	$0.272^{a\#} \pm 0.003$
50	$57.10^{a\#}\$ \pm 0.13$	$0.596^{a\#} \pm 0.010$	$0.634^{c\#} \pm 0.010$	$0.396^{a\#} \pm 0.005$
100	$53.07^{b\#}\$ \pm 0.44$	$0.554^{ab\#} \pm 0.004$	$0.716^{b\#} \pm 0.008$	$0.360^{a\#} \pm 0.004$

NaCl Conc. mM	Ca	Mn	Zn	B
150	$51.83^{c\#}\$ \pm 0.23$	$0.491^{bc\#} \pm 0.009$	$0.821^{a\#} \pm 0.011$	$0.314^{a\#} \pm 0.004$
F	147.156^{*}	6.634^{*}	106.006^{*}	2.121
p	$<0.001^{*}$	0.015^{*}	$<0.001^{*}$	$<0.001^{*}$
LSD	0.949	0.088	0.032	0.013
Seeds Pre-soaking in SA Before Germination				
Control	$47.82d^{\#@}\$ \pm 0.43$	$0.398d^{\#@} \pm 0.002$	$0.486d^{\#@} \pm 0.006$	$0.260a^{\#@} \pm 0.005$
50	$55.53a\#\$ \pm 0.52$	$0.567a^{\#@} \pm 0.003$	$0.576c^{\#@} \pm 0.010$	$0.352a^{\#@} \pm 0.004$
100	$53.10b\#\$ \pm 0.36$	$0.520b^{\#@} \pm 0.005$	$0.670b^{\#@} \pm 0.009$	$0.339a^{\#@} \pm 0.003$
150	$49.98c^{\#@}\$ \pm 0.33$	$0.476c^{\#@} \pm 0.004$	$0.723a^{\#@} \pm 0.007$	$0.281a^{\#@} \pm 0.005$
F	139.537*	386.472*	158.485*	1.489
p	<0.001*	<0.001*	<0.001*	<0.001*
LSD	1.061	0.012	0.027	0.014

In a recent study, Hniličková *et al.* [100] they found that the Na^{+} content increased in all monitored species with increasing concentrations of NaCl in irrigation water. In lettuce (*Lactuca sativa* L. cv. Orion), the Na^{+} content in leaves at the level of 300 mmol/L NaCl was 62.4 mg/g DW, which is five times more than the control group. Increased Na^{+} content along with increasing salt concentrations were reported for purslane [119], this results agree with this studies results. High Na^{+} content inhibits the uptake of K^{+} ions which is an essential element for growth and development [120]. According to Tester and Danenport [121] the key mechanism of salt tolerance is the ability of plants to regulate Na^{+} uptake from soil.

3.3.2. Micro-Elements (mg/100g Dry Weight)

Manganese and Boron Content (mg/100g Dry Weight): Overall, the shoot Mn^{+2} & B^{2+} contents increased highly significant at ($p \leq 0.001$) with decreased salinity concentrations (gradually start with 50 then 100 & 150 mM NaCl) of lettuce (*Lactuca sativa*, L. cv. Paris & cv. Royal) plant, in the presence or absence of PGRs (AsA & SA) compared with control. The results indicated that the shoot Mn^{+2} & B^{2+} contents increased highly significant at ($p \leq 0.001$) especially with 50mM NaCl concentration compared with normal control as shown in Table 3. The results shown that the effect of AsA was more effective by increasing significantly ($p \leq 0.001$) of shoot Mn^{+2} & B^{2+} contents for both cultivars under NaCl salinity concentrations than SA. So, the role of AsA on lettuce plant for both cultivars have been alleviated the effect of salinity by increasing the shoot Mn^{+2} & B^{2+} contents more than SA compared with control. Consequently, the all of this results it has been found the shoot Mn^{+2} & B^{2+} contents increased significantly ($p \leq 0.001$) more in cv. Royal S2 than in cv. Paris S1 especially in the present of AsA more than SA compared with control. Overall the statistical analysis indicated that the two ways analysis of variance (*ANOVA*) between different concentration of salinity stress, PGRs (AsA and SA) in two cultivars indicated that the *LSD* test highly significant at $P \leq 0.001$.

Zinc Content (mg/100g Dry Weight): Overall, the zinc contents (Zn^{2+}) in shoot of lettuce (*Lactuca sativa*, L. cv. Paris & cv. Royal) plant tended to increased highly significant at ($p \leq 0.001$), with increasing NaCl salinity concentrations in the presence or absence of PGRs (AsA & SA) compared with control as shown in Table 3. The results shown that the effect of AsA on shoot Zn^{2+} contents tended to increased highly significantly ($p \leq 0.001$) for both cultivars under NaCl salinity than with SA. While, the role of AsA on lettuce plant for both cultivars have been alleviated the effect of salinity on the Zn^{2+}contents in shoot more than SA compared with control. So, the all of this results it has been found the Zn^{2+}contents in shoot increased significantly ($p \leq 0.001$) more in cv. Royal S2 than in cv. Paris S1 especially in the present of AsA more than SA compared with control. Overall the statistical analysis indicated that the two ways analysis of variance (*ANOVA*) between different concentration of salinity stress, PGRs (AsA and SA) in two cultivars indicated that the *LSD* test highly significant at $P \leq 0.001$.

Numerous studies have demonstrated that NaCl salinity increased Na content in plant tissue of vegetable crops [122-123]. Recently, it has been reported that ascorbate plays a crucial role in protection against various environmental stresses such as, drought [124-125], salinity [59, 126]. According to El-Hifny and El-Sayed [127] they found that the application of AsA increased N, P, K, Ca and reduced Na and Cl values in pepper leaves relative to their untreated plants (control). The highest values of N, P, K and Ca and the lowest values of Na and Cl were obtained by the highest ascorbic acid concentrations. The obtained results could be explained on the basis that ascorbic acid application significantly increased photosynthetic pigments. This will increase the gained energy and enhancing the photosynthetic process efficiency and increasing carbohydrate accumulation in plant tissues. These results are in agreement with those obtained by Raafat and Radwan [128] on wheat grains.

Gunes *et al.* [129] and Gunes *et al.* [130] they determined that SA supply inhibited Na accumulation, but stimulated N, P, K, Mg, Fe, Mn, and Cu uptake by salt stressed maize

plants compared to non-treated ones. They explained the positive effect of SA on growth of salt stressed maize plants could be attributed to decreasing levels of Na and Cl concentrations and increasing antioxidant activity. Also, the results findings by Talaat [131] detected that foliar application of AsA increased the content of macronutrients (N, P, K) of sweet pepper plant growing under saline conditions. Other investigators found similar results on the stimulatory effect of AsA on other plants such as potato [132], eggplant [133] and sweet pepper [134-135].

According to Al-Erwy *et al*. [113] they found that the N, P, Ca and Mg concentrations in shoots and roots of stressed wheat plants decreased with increasing salinity stress to reach their lowest values at 40% salinity level. Calcium is strongly competitive with Mg. The binding sites on the root plasma membrane appear to have less affinity for the highly hydrated Mg than for Ca [136]. Salinity conditions can disorder nutrient/ion activities, causing plants to be susceptible to osmotic and specification injury, as well as to nutritional disorders that result in reduced yield and crop quality [137]. Khalifa *et al.* [72] exogenous application of SA improved the plant nutrient content of the salt-stressed lettuce plants. These results reinforce the results obtained by Syeed *et al.* [138] treatment of NaCl decrease N, P, K, and Ca content in two mustard (*Brassica juncea* L.) cultivars. Application of 0.5 mM SA on NaCl grown plants completely alleviated the NaCl effects and increased the nutrients content in both the cultivars.

Our current findings agree with the data obtained by Sadak *et al*. [139] they found that the contents of K, Ca, Mg, P and N were decreased with increased Na^+ contents in chickpea plants irrigated with diluted seawater levels, the mineral ion contents decreased but Na^+ content increased with salinity. Similarly, the enhancement of Na^+ content under salinity stress in addition to the reduction of other mineral contents was confirmed by Karlidag *et al*. [140] in strawberry plant, Sadak and Abd Elhamid [141] in flax plant and Rady *et al.* [142] on soybean. Munns [143] concluded that, salinity stress could affect plant by three means, namely water deficiency stress through decreasing water potential in the root, phytotoxicity of Na^+ and Cl^- and nutrient imbalances resulted from their improper uptake. In addition, Na^+ ions compete with K^+ ions for the binding sites necessary for biochemical activities. The increased levels of Na^+ with the reductions in K^+ contents, in response to salinity stress, results in marked reductions in K^+/Na^+. These could lead to disturbances in the accumulation of Na^+ in plant organs [142].

4. Conclusion

Generally, this study concluded that the CAT enzyme activity and Pro contents increased significantly in the present of AsA (0.5 mM) more than in the present of SA under NaCl salinity stress. Whilst, activity enzymes (APX, GR & SOD) and the contents of N, P, K, Ca, Mn and B nutrient mineral elements increased with salinity concentrations especially with 50 mM NaCl concentration than control. Also, the results indicated that the Mg, Na and Zn contents in shoot for both cultivars increased under salinity stress in the presence or absence of PGRs (AsA & SA) compared with control. Generally, the role of AsA was one of the main mechanisms used by the plant to raise its efficiency to bear the salt stress compared to the control.

Abbreviations

Plant growth regulators (PGRs), Ascorbic acid (AsA), Salicylic acid (SA), cv. Paris Island Cos (cv. Paris), Catalase (CAT), Ascorbate peroxidase (APX), Superoxide dismutase (SOD), Glutathione reductase (GR), Proline (Pro), Electrical conductivity (EC), Reactive oxygen species (ROS), Peroxidase (POD), Glutathione (GSH) and Chlorophyll (Chl).

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