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# Effect of an Eco-friendly Treatment on Dynamic Changes of Barley Plant Metabolites Under Different Doses of Salinity

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**Abstract**

**Salinity is a major abiotic stressor that affects plant cell metabolism and reduces plant yield. Numerous beneficial effects on plants' ability to withstand abiotic stressors have been linked to biostimulants like ascorbic acid (AsA). But not much is known about AsA's effects on important medical compounds (caffeic acid, syringic acid, rosmarinic acid, ellagic acid, ferulic acid, methyl gallate, vanillin, naringenin, rutin, daidzein, and quercetin) of barley grains produced under different doses of salinity. So, in our study we showed the effect of different salinity levels (4000 and 8000 ppm of sodium chloride) in the presence or absence AsA (at 100 and 200 ppm) on phenolic profile in grains of barley plant by high-performance liquid chromatography analysis. Pigments, carbohydrates, protein, proline, phenolic and flavonoid compounds of barley shoot (*Hordeum vulgare* L. (Giza 134) also investigated. AsA at 100 ppm appeared the highest values of caffeic acid, syringic acid, rosmarinic acid, ellagic acid, ferulic acid, methyl gallate, vanillin, naringenin, rutin, daidzein, and quercetin under 4000 ppm of salt compared with control and other treatments, AsA showed significant improvement in contents of pigments, carbohydrates, protein, proline, phenolic, and flavonoids compounds of barley shoot under salinity stress. By employing environmentally safe natural products, the search aims to enhance the metabolites of barley plants, particularly phenolic compounds. This might lead to an improvement in the caliber of significant crops grown in salinized environments. We also have shown that the harmful effects of salt stress on the metabolites of *Hordeum vulgare* plants can be lessened by the exogenous application of AsA. In addition to improving the physiological characteristics of barley shoots, this treatment lowers oxidative stress in plants by increasing phenolic chemicals.**

*Keywords:* Ascorbic acid, Barley plant, Phenolic compounds, Salinity stress

## 1. Introduction

People started cultivating barley more than 10 000 years ago for use in beverages, soups, animal feed, and barley bread [1]. Barley has a relatively high salt tolerance when compared with other cereal crops. However, barley has been the focus of a lot of research on abiotic stress conditions [2,3]. The negative effects of necessary elements like salt and chlorine on the growth and development of plants are referred to as salinity. Furthermore, it is commonly known that salt in the soil inhibits plant growth through osmotic stress, which is followed by ion toxicity [4]. Salinity is the main environmental stressor preventing the growth in demand for food crops since it significantly affects plant productivity and growth [3]. Depending on the degree and duration of the stress, salinity stress impacts several physiological and metabolic functions and ultimately reduces crop yield [5]. Reductions in plant height, shoot fresh weight, dry weight, number of siliques plant, silique length, number of seeds

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silique$^{-1}$, 1000-seed weight, and seed yield plant$^{-1}$ were among the phenotypic responses of rapeseed plants to salt stress [6]. Our previous study [7] observed that reduced growth parameters (plant height, number of tillers/plant, fresh and dry weight of shoot/plant) and decreased yield components (weight of spike, length of spike, number of grains/spike, and weight of 100 grains) of barley plants were the results of salinity stress at 2000, 4000, and 8000 ppm of sodium chloride (NaCl). These reductions were progressively increased with increasing salinity concentration. In the meantime, the presence of salinity led to a considerable increase in the antioxidant enzymes catalase, peroxidase, polyphenol oxidase, and superoxide dismutase. When plants growing under normal conditions or at salt levels received foliar applications of ascorbic acid (AsA) at concentrations of 100 and 200 ppm, all growth metrics, yield and its components, and the activity of antioxidant enzymes were significantly increased. On the other hand, AsA, a nonenzymatic antioxidant found in many plants, plays a critical role in reducing the effects of some oxidative stressors caused by both biotic and abiotic stress [8]. AsA effectively reduces the generation of reactive oxygen species brought on by a variety of abiotic stimuli and fights oxidative stress [9]. Furthermore, AsA protects plants from oxidative stress by removing a range of free radicals, mainly by acting as a substrate for ascorbate peroxidase, an essential enzyme in the ascorbate-glutathione pathway [10]. Furthermore, AsA is a strong contender for possible usage as a chemical in agriculture due to its many positive benefits during salt stress [11]. Exogenous applications of AsA enhance the antioxidant capacity of plant tissue. This may be explained by the fact that AsA functions as a cofactor for several cellular enzymes [12]. There are many studies discuss the effect of salinity on growth, yield, and primary metabolites, but the effect of salinity on the medical important components such as phenolic compounds (Gallic acid, Chlorogenic acid, Catechin, Methyl gallate, Caffeic acid, Syringic acid, Coumaric acid, Vanillin, Ferulic acid, Naringenin, and Kaempferol) in presence or absence of AsA not clear. So the novelty of this search is estimating these important compounds in the presence of natural, eco-friendly products (AsA) under salinity stress. This might potentially lead to an improvement in the quality of significant crops grown in salinized environments. Furthermore, we demonstrated that exogenous AsA treatment can lessen the negative effects of salt stress on the metabolites of *Hordeum vulgare* plants by increasing phenolic compounds; this treatment not only improves physiological characteristics in the plant shoots but also lowers oxidative stress in plants by increasing phenolic compound.

## 2. Materials and methods

### *2.1. Experimental design*

During the winter (November 2020), the current investigation was developed at Faculty of Science (Girls, AL-Azhar University, Cairo, Egypt). To test the effects of foliar spray with two concentrations of AsA at 100 and 200 ppm (these concentrations were selected according to a preliminary experiment which caused the best growth of sunflower plant) on barley plants watered with salt water (NaCl) at 4000 (≈70 mM), and 8000 ppm (≈140 mM), whereas control plants were irrigated with tap water, barley grains (Giza 134) were purchased from the Agriculture Research Centre, Giza, Egypt, and were planted on November 15, 2020, in porcelain pots. After 10 days from emergence, the number of seedlings per pot was decreased to 10. The split plot design of the experiment used three separate replicates, each with five pots filled with loamy soil. The pots were watered with a range of salinities. Three foliar applications of AsA (at 30, 50, and 75 days after sowing) were made. Samples of shoot at 45, 75, and 90 days post-transplantation were dried at 75 °C for 72 h to assess different metabolites (protein, carbohydrates proline, phenol, and flavonoids contents), and fresh leaves were used to asses pigments. Powder of grains after maturity is used to determine phenolic compounds by using high-performance liquid chromatography.

### *2.2. Determination of metabolic contents in shoot*

#### *2.2.1. Pigments contents*

A method for measuring the pigments present in plant leaves was designed by researchers [13]. Using this method, the green tissues were divided into tiny pieces and weighed in sections of one gram. To eliminate the plant colors, 100 ml of acetone 80% was combined with the tissue pieces for 2 min. The mixture was quantitatively transferred and filtered using a Buchner filter with No. 1 filter paper. After being transferred to a 100 ml flask, the filtrate's volume was raised by 80% using acetone. Three separate wavelengths (649, 665, and 470 nm) were used to measure the optical density of the extract using a Carl Zeiss spectrometer. These wavelengths are found in the area that is most densely packed with carotenoids and chlorophyll 'a' and 'b'. To

calculate the amounts of chlorophylls *a*, *b*, and their sum in plants, use the following formulas:

Chlorophyll a (Mg/g tissue) = 11.63 (A665) − 2.39 (A649).

Chlorophyll b (Mg/g leaves) = 20.11 (A649) − 5.18 (A665).

Total chlorophyll a+b (Mg/g tissue) = 6.45 (A665)+ 17.72 (A649).

For the estimation of carotenoid chemical composition, the method described by [14]. is applicable:

Carotenoids (mg/g fresh weight)=(1000 * A470) − (1.82 * chlorophyll a) − (85.02 * chlorophyll b)/198.

Note that '(A)' denotes the optical density in these calculations.

*2.2.2. Soluble carbohydrates and proteins*

Utilizing the methodology of [15], the total carbohydrates in the shoot were estimated. The procedures of [16] were used to evaluate the contents of the total proteins of the shoot.

*2.2.3. Proline content*

To ascertain the proline content in the shoot, 0.5 g from fresh leaves was homogenized in 10 ml aqueous sulfosalicylic acid (3%). The filtrate was combined with 2 ml of acid ninhydrin reagent and 2 ml of glacial acetic acid, and the mixture was left at 100 °C for 1 h. After the liquid cooled, 4 ml of toluene were added to extract the proline. Toluene was used as a blank in a spectrophotometer (VEB Carl Zeiss) to measure the absorbance at 520 nm [17].

*2.2.4. Total phenolic and flavonoid contents*

The method outlined by [18] was used to measure total phenolic of shoot (mg/100 g of dry weight) as follows: A 25 ml volumetric flask containing 9 ml of distilled water was filled with an aliquot (1 ml) of extracts or a standard solution of gallic acid (20, 40, 40, 60, 80, and 100 μg/ml). Distilled water was used to create a blank for the reagent. After adding 1 ml of Folin-Ciocalteu phenol reagent to the mixture, it was shaken. A 10 ml of a 7% $Na_2 CO_3$ solution were added to the mixture after 5 min. Subsequently, the volume was adjusted appropriately. Using a UV/Vis spectrophotometer, the absorbance against the reagent blank was measured at 550 nm following a 90 min room temperature incubation period. The amount of total phenolics was reported in milligrams of gallic acid equivalents.

Total flavonoids of the shoot determined by using the aluminum chloride colorimetric method [19] as follows. In a 10 ml volumetric flask with 4 ml of distilled water, an aliquot (1 ml) of quercetin extracts or standard solutions (20, 40, 60, 80, and 100 μg/ml) was added. 0.30 ml of 5% $NaNO_2$ and 0.3 ml of 10% $AlCl_3$ were added to the flask over 5 min. The volume was increased to 10 ml with distilled water after adding 2 ml of 1 M NaOH after 5 min. After mixing the solution, absorbance was calculated in relation to a 510 nm blank. In milligrams of quercetin equivalents (QE), the total flavonoid content was reported.

*2.2.5. Determination of sodium and chloride contents*

Sodium and chloride contents were determined at the atomic spectroscopy laboratory, arid land agricultural research and services center, faculty of agriculture, Ain Shams University, Cairo, Egypt.

*2.2.6. Detection of phenolic compounds of barley yield (grains) by using HPLC*

After spike maturation, for traditional extraction, which was accomplished in a sonicate at 4 °C for 30 min, 1 g of powdered dried barely grains was soaked in 50 ml (85%) of methanol in a stoppered container. The powder was then allowed to stand at room temperature for 24 h. To produce crude extract, the extract was filtered and concentrated using a rotary evaporator at 40 °C while under vacuum. This was accomplished using high-performance liquid chromatography equipment from the Agilent 1260 series. The separation was carried out using the Eclipse C18 column (4.6 mm × 250 mm internal diameter, 5 m particle size). The mobile phase contained both water (A) and acetonitrile with 0.05% trifluoroacetic acid (B), supplied at a flow rate of 0.9 ml/min. We measured the flow rate in milliliters per minute. For the mobile phase, the following linear gradient was created: 8 min (82% A), 5–10 min (80% A), 5–12 min (60% A), 12–15 min (82% A), 15–16 min (82% A), and 16–20 min (82% A). A multi-wavelength detector that was calibrated to 280 nm helped with the detection process. For each sample solution injection, a volume of 5 μl was utilized. A constant temperature of 40 °C was maintained within the column.

*2.2.7. Statistical evaluation*

Analysis of variance was mostly used to determine the mean differences between treatments. Tukey's test (truly significant difference) was used for post hoc comparisons at a significance level of *P* less than 0.05. The data from barley seeds was subjected to a hierarchical two-way cluster analysis to determine

the concentration of phenolic chemicals. In this work, the centered technique for normalized data was employed.

## 3. Results

### 3.1. Photosynthesis pigments

The effects of salinity concentrations (4000 and 8000 ppm NaCl) on the amount of photosynthetic pigments (carotenoids, chlorophyll *a*, and chlorophyll *b*) in barley plant leaves during the stages under study are displayed in Table 1. Data showed that elevating NaCl concentrations resulted in a notable and gradual decrease in the amount of carotenoids, chlorophyll *a*, and *b*. Irrigation plants with water containing 4000 ppm NaCl decreased chlorophyll *a*, *b*, and carotenoids by 28.57, 31.91, and 18.75%, respectively, and by 31.43, 36.17, and 31.25%, respectively, at 8000 ppm of NaCl during the first stage compared with control plants. The results were in the same trend for the second and third stages. Also, data showed the interaction effect between NaCl concentrations in irrigation water and the application of AsA at 100 and 200 ppm on photosynthetic pigments of barley plants of three taken samples during the growth season. It can be found that chlorophyll contents significantly increased in salinized plants treated with AsA compared with unsprayed plants. These increments were true through growth stages. In plants irrigated with 8000 ppm NaCl and sprayed with high level of AsA (200 ppm) treatment, the content of chlorophyll *a* increased by 29.16, 40, and 47.06%, respectively, over the control ones of the first, second, and third stages. Interestingly, the same trend was detected in the case of chlorophyll *b* and carotenoid content.

### 3.2. Carbohydrates, protein, and proline contents

Data in Table 2. showed the effects of salinity stress at 400 ppm and 8000 ppm in the presence or absence AsA at 100 and 200 ppm on carbohydrates and protein contents in the shoot system at three different stages (45, 75, and 90 days after transplantation). Compared with untreated salinized plants, it is evident that employing both AsA doses boosted the total carbohydrate contents in the shoot system of barley plants growing in saline circumstances. For instance, plants irrigated with 8000 ppm NaCl saline water and treated with a low concentration of AsA (100 ppm) increased total carbohydrate contents of shoot by 5.93, 6.36, and 4.04%, respectively, at three growth stages compared with control treatments. On the other hand, the high

*Table 1. Pigments contents in fresh leaves of Barley plants during different growth stages under interaction between salinity at 4000 ppm and 8000 ppm of sodium chloride and ascorbic acid at 100 and 200 ppm.*

| Treatments | Chl a | | | Chl b | | | Carotenoids | | |
|---|---|---|---|---|---|---|---|---|---|
| | First sample | Second sample | Third sample | First sample | Second sample | Third sample | First sample | Second sample | Third sample |
| Control | 0.35 ± 0.02a | 0.37 ± 0.005a | 0.34 ± 0.040a | 0.47 ± 0.075a | 0.56 ± 0.020a | 0.31 ± 0.018a | 0.16 ± 0.030a | 0.26 ± 0.025a | 0.27 ± 0.000a |
| S1 | 0.25 ± 0.006cd | 0.30 ± 0.035d | 0.20 ± 0.020d | 0.32 ± 0.021e | 0.42 ± 0.061bc | 0.21 ± 0.015c | 0.13 ± 0.031d | 0.18 ± 0.025de | 0.18 ± 0.000e |
| S2 | 0.24 ± 0.006d | 0.25 ± 0.020b | 0.17 ± 0.020e | 0.30 ± 0.015f | 0.32 ± 0.087c | 0.17 ± 0.015d | 0.11 ± 0.005d | 0.12 ± 0.015e | 0.17 ± 0.001f |
| S2+AS1 | 0.26 ± 0.012c | 0.39 ± 0.025a | 0.25 ± 0.006ab | 0.34 ± 0.020d | 0.62 ± 0.065a | 0.31 ± 0.021b | 0.18 ± 0.006c | 0.34 ± 0.012a | 0.20 ± 0.015a |
| S2+AS2 | 0.29 ± 0.006b | 0.42 ± 0.025a | 0.26 ± 0.006a | 0.36 ± 0.015c | 0.80 ± 0.017a | 0.33 ± 0.006a | 0.19 ± 0.010b | 0.35 ± 0.015a | 0.23 ± 0.020a |
| S2+AS1 | 0.26 ± 0.015c | 0.31 ± 0.010b | 0.20 ± 0.010d | 0.32 ± 0.01de | 0.43 ± 0.045bc | 0.22 ± 0.026c | 0.16 ± 0.020d | 0.19 ± 0.020bc | 0.18 ± 0.010e |
| S2+AS2 | 0.31 ± 0.015ab | 0.35 ± 0.025a | 0.25 ± 0.040c | 0.34 ± 0.010d | 0.48 ± 0.020a | 0.31 ± 0.040c | 0.17 ± 0.015bc | 0.22 ± 0.015ab | 0.21 ± 0.014cd |
| LSD | 0.02 | 0.03 | 0.02 | 0.02 | 0.03 | 0.05 | 0.02 | 0.03 | 0.02 |

Control = irrigation with tap water, S1 = salinity at 4000 ppm, S2 = salinity at 8000 ppm. S1+As1 = salinity at 4000 ppm + ascorbic acid at 100 ppm and S1+As2 = salinity at 4000 ppm + ascorbic acid at 200 ppm, S2+As1 = salinity at 8000 ppm + ascorbic acid at 100 ppm and S2+As2 = salinity at 8000 ppm + ascorbic acid at 200 ppm.

*Table 2. Effect of interaction between salinity and ascorbic acid on carbohydrates and protein contents of barley shoot.*

| Treatments | Total soluble carbohydrate | | | Total soluble protein | | |
|---|---|---|---|---|---|---|
| | First sample | Second sample | Third sample | First sample | Second sample | Third sample |
| Control | 5.40 ± 0.021g | 6.50 ± 0.025e | 7.31 ± 0.054f | 0.90 ± 0.026B | 0.99 ± 0.021C | 1.10 ± 0.017C |
| S1 | 7.33 ± 0.065f | 9.20 ± 0.178d | 11.4 ± 0.145e | 1.03 ± 0.064bcd | 1.05 ± 0.042ef | 1.12 ± 0.010ef |
| S2 | 8.60 ± 0.081c | 10.37 ± 0.065c | 13.60 ± 0.085b | 1.06 ± 0.042abc | 1.09 ± 0.015bcd | 1.14 ± 0.015cd |
| S1+AS 1 | 8.31 ± 0.185e | 9.88 ± 0.136c | 11.98 ± 0.042d | 1.06 ± 0.006abc | 1.08 ± 0.015cd | 1.15 ± 0.010c |
| S1+AS 2 | 9.14 ± 0.251d | 10.20 ± 0.247c | 12.25 ± 0.036c | 1.08 ± 0.010abc | 1.10 ± 0.010bc | 1.17 ± 0.015ab |
| S2+AS 1 | 9.11 ± 0.213b | 11.03 ± 0.189b | 14.15 ± 0.159b | 1.08 ± 0.055ab | 1.11 ± 0.015ab | 1.16 ± 0.001bc |
| S2+AS 2 | 9.62 ± 0.021a | 12.25 ± 013a | 14.82 ± 0.247a | 1.11 ± 0.015a | 1.13 ± 0.014a | 1.18 ± 0.016a |
| LSD | 0.21 | 0.44 | 0.56 | 0.03 | 0.02 | 0.01 |

Control = irrigation with tap water, S1 = salinity at 4000 ppm, S2 = salinity at 8000 ppm. S1+As1 = salinity at 4000 ppm + ascorbic acid at 100 ppm and S1+As2 = salinity at 4000 ppm + ascorbic acid at 200 ppm, S2+As1 = salinity at 8000 ppm + ascorbic acid at 100 ppm and S2+As2 = salinity at 8000 ppm + ascorbic acid at 200 ppm.

concentration of AsA (200 ppm) increased total carbohydrate contents at the same salinization by 11.63, 18.13, and 8.97%, respectively, at the same growth stages compared with control. The highest values of carbohydrates content showed in presence AsA at 200 ppm under 8000 ppm of NaCl by 78.15, 88.46, and 102.94% at 45, 75, and 90 days after transplantation, respectively. Salinity at 4000 ppm caused improvement in carbohydrates contents by 35.74, 41.53, and 55.95%, while at 8000 ppm appeared enhancement by 59.26, 59.54, and 86.05% at 45, 75, and 90 days after transplantation, respectively.

Table 2 also, illustrated the effect of interaction between salinity levels (2000, 4000, and 8000 ppm NaCl) and AsA at 100 and 200 ppm on total protein content in the shoot system of barley plants at three studied stages, results showed a significant decrease in protein contents in response to a different level of salinity, but the highest drop showed at 8000 ppm of NaCl. It is evident that applying AsA to plants cultivated in saltier environments considerably enhanced their total protein contents when compared with plants grown in salt without any treatment. The highest increment values were detected in plants grown under 4000 ppm NaCl and treated with 200 ppm by 4.85, 4.76, and 4.46%, respectively, at three growth stages. The interactive effect between salinity concentrations in irrigation water and AsA on proline contents at three studied stages in the shoot system of barley plants are shown in Fig. 1. Data showed that proline contents were significantly increased in salinized plants treated with AsA. For instance, the increment percentage of proline contents for plants irrigated with 8000 ppm

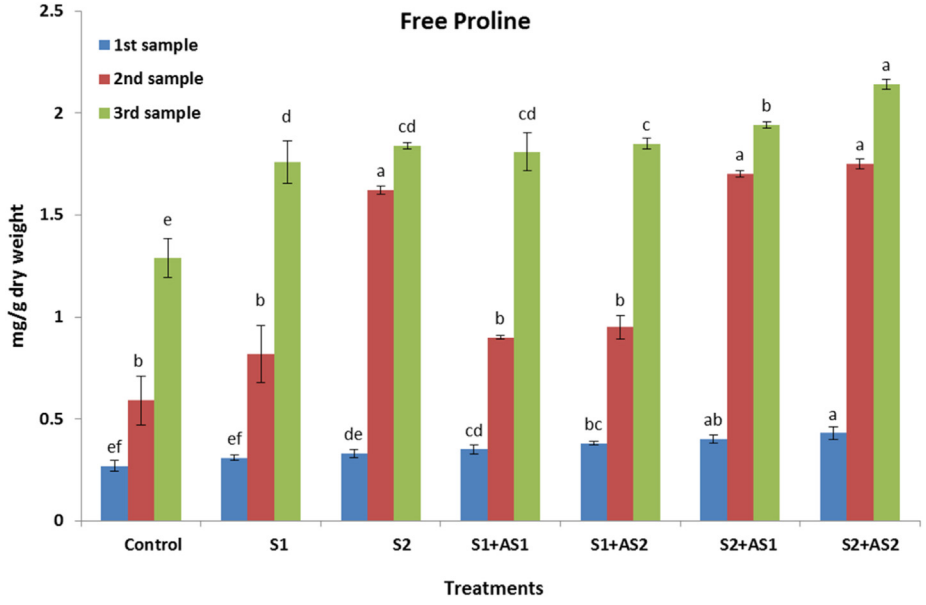


*Fig. 1. Effect of interaction between salinity and ascorbic acid on proline contents of barley plant. Control = irrigation tap water, S1 = salinity at 4000 ppm, S1+As1 = salinity at 4000 ppm + ascorbic acid at 100 ppm, S1+As2 = salinity at 4000 ppm + ascorbic acid at 200 ppm S2 = salinity at 8000 ppm, S2+As1 = salinity at 8000 ppm + ascorbic acid at 100 ppm and S2+As2 = salinity at 8000 ppm + ascorbic acid at 200 ppm.*

saline water and treated with a low concentration of AsA (100 ppm) was 7.32, 7.41, and 6.01%, respectively, at three growth stages compared with salinize untreated plants. Also, the increment of proline contents in plants grown under the same salinization being 12.20, 9.88, and 16.94% as a result of the application of the high concentration of AsA, respectively, at the same stages compared with salinized untreated plants.

### 3.3. Phenolic and flavonoid compounds in shoot

Data in Table 3. portrays the effects of salinity stress at 400 ppm and 8000 ppm in the presence or absence AsA at 100 and 200 ppm on phenolic and flavonoid content in shoot system at three different stages (45, 75, and 90 days after transplantation). All treatment caused improvement in these contents compared with control groups. Plants irrigated with 4000 ppm NaCl and treated with a low concentration of AsA (100 ppm) recorded an increment of total phenols content by 1.05, 4.66, and 7.53%, respectively, at three growth stages Meanwhile, high concentrations of AsA for same salinized plants increased total phenols content by 1.83, 5.59, and 8.56%, respectively, at the same growth stages. Salinity levels at 4000 ppm NaCl and AsA at 200 ppm markedly increased total flavonoids content by 26.18, 9.44, and 9.70%, respectively, at three growth stages. While, salinity at 8000 ppm NaCl and AsA at 200 ppm caused a great increment in total flavonoids content represented by 17.07, 11.11, and 27.27%, respectively, at three growth stages compared with salinized untreated plants.

### 3.4. Mineral contents

#### 3.4.1. Sodium content

Data recorded in Table 4 show the effect of NaCl concentrations in irrigated water on sodium content in the presence or absence of AsA of shoot of barley plants at three growth samples during the studied season. Results reveal that the sodium content of barley plants was significantly increased by increasing NaCl concentrations in the growth media. NaCl at 4000 ppm NaCl caused an increment of sodium content being 15.45, 8.28, and 10.24, respectively, at three growth samples relative to control treatment. It could be observed that using the concentrations of AsA decreased sodium content of plants grown under saline conditions compared with salinized unsprayed plants during the three studied samples.

#### 3.4.2. Chloride content

The effect of salinity concentrations on chloride content in presence or absence of AsA of barley plant is shown in Table 4. A significant and progressive increase in chloride content was detected as a result of increasing NaCl concentration in the growth medium. The increment of chloride content was true for the three tested samples, during growth season. The increment percent of chloride content for the first stage caused by 4000 and 8000 ppm NaCl was 36.54 and 59.23%, respectively, relative to check plants. Meanwhile, the increment of chloride content of the second sample was 64.90 and 76.92%, respectively, as affected by 4000 and 8000 ppm NaCl treatments throughout the first stage growth season. It is obvious from Table 4 that the chloride content of barley plants grown under salinity concentration and treated with AsA was significantly reduced compared with salinized unsprayed ones. For instance, the reduction percentage of chloride content of plants grown under 4000 ppm NaCl salinity and treated with low concentration of AsA (100 ppm) was 28.17, 25.66, and 8.45%, respectively, at three growth samples compared with check plants.

*Table 3. Total phenols and flavonoids contents in shoots of Barley plants during different growth stages under interaction between salinity at 4000 ppm and 8000 ppm of NaCl and ascorbic acid at 100 and 200 ppm.*

| Treatments | Phenols | | | Flavonoids | | |
|---|---|---|---|---|---|---|
| | First | Second | Third | First | Second | Third |
| Control | 3.02 ± 0.537d | 2.20 ± 0.100c | 2.15 ± 0.017e | 1.58 ± 0.029e | 1.49 ± 0.036f | 1.20 ± 0.100g |
| S1 | 3.82 ± 0.855c | 3.22 ± 0.215c | 2.92 ± 0.835d | 1.91 ± 0.458d | 1.80 ± 0.200e | 1.65 ± 0.000f |
| S2 | 4.11 ± 0.017b | 3.35 ± 0.100b | 3.22 ± 0.755b | 2.05 ± 0.350c | 1.98 ± 0.425c | 1.87 ± 0.026c |
| S1+AS1 | 3.86 ± 0.510c | 3.27 ± 0.285b | 3.14 ± 0.855c | 2.03 ± 0.350c | 1.90 ± 0.350d | 1.73 ± 0.126e |
| S1+AS2 | 3.89 ± 0.301c | 3.29 ± 0.110b | 3.17 ± 0.805c | 2.41 ± 0.126a | 1.97 ± 0.388c | 1.81 ± 0.200d |
| S2+AS1 | 4.28 ± 0.400a | 3.41 ± 0.144ab | 3.25 ± 0.250b | 2.31 ± 0.090b | 2.10 ± 0.150b | 2.11 ± 0.150b |
| S2+AS2 | 4.37 ± 0.200a | 3.48 ± 0.371a | 3.41 ± 0.312a | 2.40 ± 0.100a | 2.20 ± 0.100a | 2.38 ± 0.225a |
| LSD | 0.13 | 0.09 | 0.06 | 0.02 | 0.04 | 0.06 |

Control = irrigation with tap water, S1 = salinity at 4000 ppm, S2 = salinity at 8000 ppm. S1+As1 = salinity at 4000 ppm + ascorbic acid at 100 ppm and S1+As2 = salinity at 4000 ppm + ascorbic acid at 200 ppm, S2+As1 = salinity at 8000 ppm + ascorbic acid at 100 ppm and S2+As2 = salinity at 8000 ppm + ascorbic acid at 200 ppm.

*Table 4. Sodium and chlorine contents in shoots of Barley plants during different growth stages under interaction between salinity at 4000 ppm and 8000 ppm of sodium chloride and ascorbic acid at 100 and 200 ppm.*

| Treatments | Na | | | Cl | | |
|---|---|---|---|---|---|---|
| | First | Second | Third | First | Second | Third |
| Control | 1.23 ± 0.02e | 1.57 ± 0.14c | 1.27 ± 0.05d | 2.60 ± 0.12c | 2.08 ± 0.24c | 1.30 ± 0.05e |
| S1 | 1.42 ± 0.04c | 1.70 ± 0.03b | 1.40 ± 0.18b | 3.55 ± 0.15b | 3.43 ± 0.32a | 2.13 ± 0.16b |
| S2 | 1.59 ± 0.12a | 1.80 ± 0.21a | 1.51 ± 0.24a | 4.14 ± 0.17a | 3.68 ± 0.28a | 2.48 ± 0.08a |
| S1+AS1 | 1.32 ± 0.21d | 1.62 ± 0.13c | 1.31 ± 0.17c | 2.55 ± 0.15c | 2.55 ± 0.19b | 1.95 ± 0.18c |
| S1+AS2 | 1.20 ± 0.03e | 1.59 ± 0.16c | 1.28 ± 0.25cd | 2.25 ± 0.08c | 2.19 ± 0.08bc | 1.86 ± 0.25c |
| S2+AS1 | 1.50 ± 0.15b | 1.73 ± 0.14b | 1.37 ± 0.09b | 3.60 ± 0.04b | 2.90 ± 0.24b | 1.50 ± 0.19d |
| S2+AS2 | 1.45 ± 0.02b | 1.69 ± 0.06b | 1.32 ± 0.07bc | 3.26 ± 0.23b | 2.70 ± 0.29b | 1.45 ± 0.08d |
| LSD | 0.07 | 0.06 | 0.10 | 0.52 | 0.75 | 0.11 |

Control = irrigation with tap water, S1 = salinity at 4000 ppm, S2 = salinity at 8000 ppm. S1+As1 = salinity at 4000 ppm + ascorbic acid at 100 ppm and S1+As2 = salinity at 4000 ppm + ascorbic acid at 200 ppm, S2+As1 = salinity at 8000 ppm + ascorbic acid at 100 ppm and S2+As2 = salinity at 8000 ppm + ascorbic acid at 200 ppm.

### *3.5. Phenolic compounds in barley grains by using HPLC analysis*

Here, phenolic compounds in barley grains under salt stress were identified and quantified using nine phenolic acids, three phenols, and seven flavonoid standards at 4000 and 8000 ppm in the presence or absence of AsA at 100 and 200 ppm. Nine phenolic acids, two phenols, and six flavonoids were found in both control and treated plants, according to the results in Table 5 and Fig. 2a−g. However, rutin was only found in plants that received AsA treatment at concentrations of 100 and 200 ppm under 4000 ppm of NaCl. Data in Table 5 and Fig. 2a−c. appeared that salinity stress at 4000 and 8000 ppm of NaCl caused a decrease in Gallic acid by 25.46 and 29.81%, Chlorogenic acid by 41.17 and 52.55%, Catechin by 47.18 and 41.33%, Methyl gallate by 38.56 and 28.06%, Caffeic acid by 22.70 and 19.74%, Syringic acid by 34.97 and 49.78%, Coumaric acid by 27.38 and 33.56%, Vanillin by 32.15 and 46.97%, Ferulic acid by 25.19 and 27.65%, Naringenin by 6.38 and 15.82%, and Kaempferol by 19.46 and 42.19, respectively, compared with control groups. Data also, appeared that the high decrease in gallic acid, chlorogenic acid, syringic acid, ellagic acid, coumaric acid, ferulic acid, methyl gallate, vanillin, catechin, and kaempferol showed at 8000 ppm of salinity. The highest increase of gallic acid appeared in prescene AsA at 100 ppm under salinity 4000 ppm by 19.50% compared with control. AsA at 100 ppm under 4000 ppm of salinity appeared the highest

*Table 5. Phenolic and flavonoids compounds in the different treatments of barley grains by using high-performance liquid chromatography analysis.*

| Phenolic compounds | Conc. (μg/g) | | | | | | |
|---|---|---|---|---|---|---|---|
| | Control | S1 | S2 | S1+As1 | S1+As2 | S2+As1 | S2+As2 |
| Gallic acid | 2029.28 | 1512.58 | 1424.29 | 1942.63 | 2425.06 | 2057.78 | 2119.69 |
| Chlorogenic acid | 1246.63 | 733.38 | 591.49 | 1035.22 | 1310.95 | 1099.95 | 1192.44 |
| Coffeic acid | 107.18 | 82.84 | 86.02 | 199.39 | 131.31 | 119.44 | 115.98 |
| Syringic acid | 207.30 | 134.79 | 104.09 | 235.20 | 221.60 | 189.42 | 194.14 |
| Rosmarinic acid | 80.22 | 236.99 | 78.93 | 946.30 | 60.72 | 45.41 | 66.92 |
| Cinnamic acid | 2.25 | 2.88 | 2.49 | 5.28 | 2.96 | 8.22 | 1.53 |
| Ellagic acid | 9.44 | 32.35 | 7.80 | 126.96 | 27.53 | 10.95 | 15.68 |
| Coumaric acid | 52.80 | 38.34 | 35.08 | 67.58 | 51.24 | 39.98 | 42.34 |
| Ferulic acid | 57.74 | 43.19 | 41.77 | 84.40 | 63.65 | 54.36 | 52.32 |
| Methyl gallate | 29.61 | 18.19 | 21.30 | 44.59 | 38.38 | 32.14 | 33.61 |
| Vanillin | 64.91 | 44.04 | 34.42 | 74.99 | 51.72 | 37.84 | 36.90 |
| Pyro catechol | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| Catechin | 487.14 | 257.29 | 285.76 | 143.91 | 372.72 | 464.41 | 408.29 |
| Naringenin | 57.96 | 54.26 | 48.79 | 83.13 | 56.14 | 38.83 | 42.31 |
| Rutin | 0.00 | 0.00 | 0.00 | 59.63 | 29.50 | 0.00 | 0.00 |
| Daidzein | 8.27 | 12.05 | 8.66 | 40.26 | 14.99 | 22.02 | 5.21 |
| Querectin | 11.15 | 71.60 | 53.30 | 147.51 | 62.52 | 55.24 | 46.07 |
| Kaempferol | 139.19 | 112.10 | 80.46 | 222.91 | 117.37 | 42.08 | 109.04 |
| Hesperetin | 12.91 | 14.00 | 15.85 | 17.35 | 24.81 | 22.00 | 13.50 |

Control = irrigation with tap water, S1 = salinity 4000 ppm of NaCl, S2 = 8000 ppm of NaCl, S1+As1 = salinity 4000 ppm of NaCl + Ascorbic at 100 ppm, S1+As2 = salinity 4000 ppm of NaCl + Ascorbic at 200 ppm, S2+As1 = salinity 8000 ppm of NaCl + Ascorbic at 100 ppm, S2+As2 = salinity 8000 ppm of NaCl+.

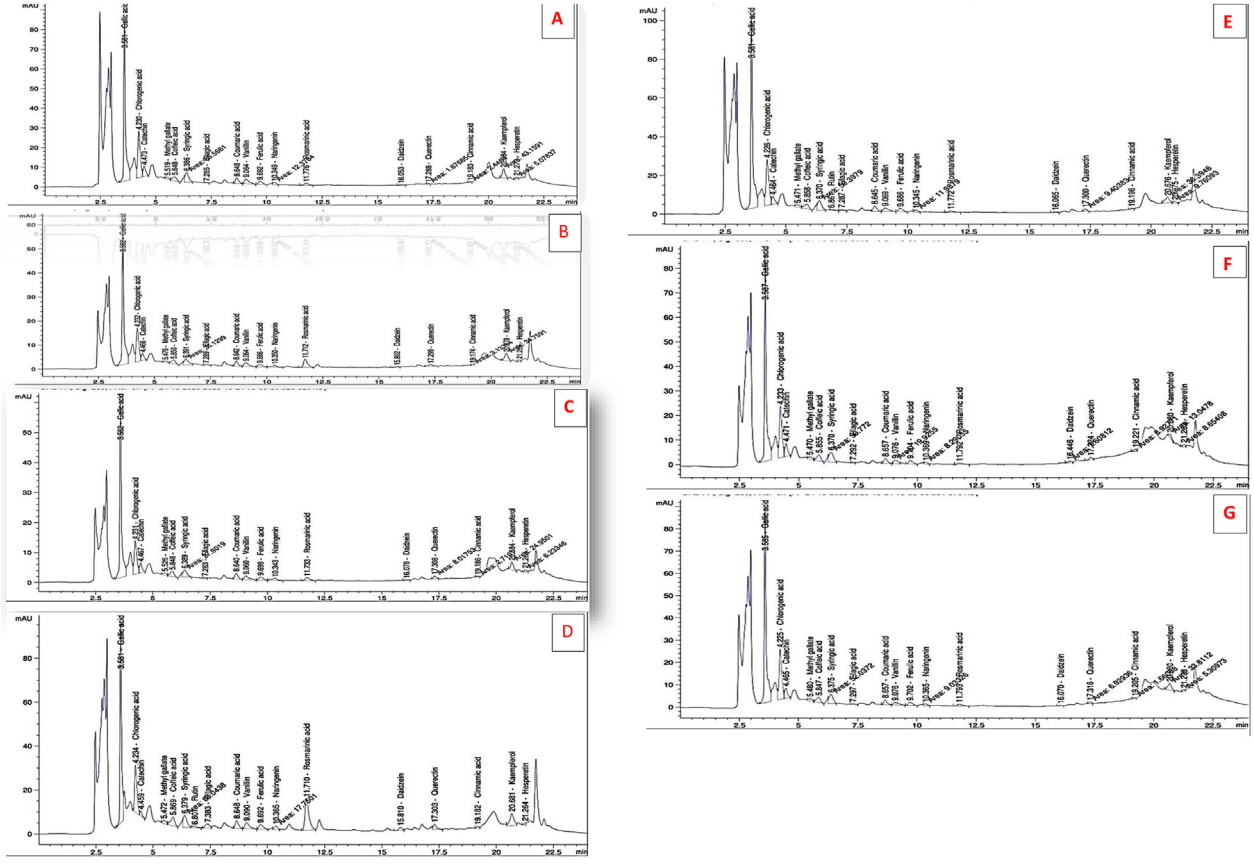


*Fig. 2. Phenolic compounds in barley grains under different treatments. Which A = Control, B = salinity at 4000 ppm, C = salinity at 8000 ppm, D = salinity at 4000 ppm + ascorbic acid at 100 ppm, E = salinity at 4000 ppm + ascorbic acid at 200 ppm, F = salinity at 8000 ppm + ascorbic acid at 100 ppm and G = salinity at 8000 ppm + ascorbic acid at 200 ppm.*

value of Caffeic acid, Syringic acid, Rosmarinic acid, Ellagic acid, Ferulic acid, Methyl gallate, Vanillin, Naringenin, Rutin, Daidzein, and Querectin compared with control and other treatment.

Data in Table 5 and Fig. 2d and e also, appeared that AsA at 100 and 200 ppm caused an increase of Gallic acid by 28.43 and 60.32%, Chlorogenic acid by 41.15 and 78.75%, Methyl gallate by 145.13 and 110.99%, Caffeic acid by 140.69 and 58.51%, Syringic acid by 74.49 and 64.40%, Coumaric acid by 76.26 and 33.64%, Vanillin by 70.27 and 17.43%, Ferulic acid by 95.41% and 47.37%, Naringenin by 53.206 and 3.464, Daidzein by 234.10% and 24.39%, Cinnamic acid by 83.33 and 2.77%, Kaempferol by 98.84 and 4.70%, and Hesperetin by 23.92 and 77.21%, respectively, under 4000 ppm of salinity compared with salinity alone. Data in Table 3 and Fig. 2f and g appeared that AsA at 100 and 200 ppm caused increase in Gallic acid by 44.47 and 48.82%, Chlorogenic acid by 85.96 and 101.59%, Catechin by 62.51 and 42.87%, Methyl gallate by 50.89 and 57.79%, Caffeic acid by 38.85 and 34.82%, Syringic acid by 81.97 and 86.51%, Ellagic acid by 40.38 and 101.02%, Coumaric acid by 13.96 and 20.69%, Vanillin by 9.93 and 7.20%, Ferulic acid by 30.14 and 25.25%, respectively, under 8000 ppm of salinity. Figure 3 presents the content and percentages of different phenolic compounds in two groups: the control

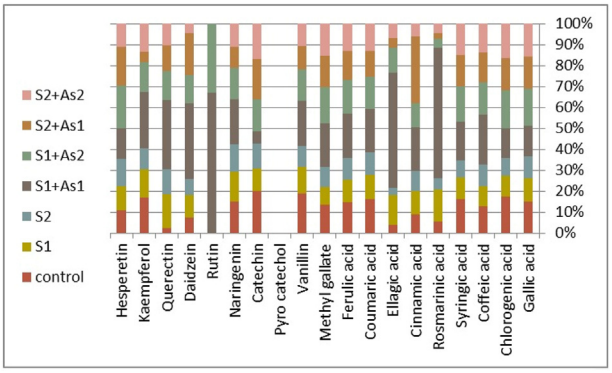


*Fig. 3. Percentages of phenolic compounds based on high-performance liquid chromatography analysis in the presence and absence of ascorbic acid at 100 and 200 ppm under salinity stress treatments (4000 and 8000 ppm of NaCl).*

group and the other treatments (S1, S2, S1+AS1, S1+AS2, S2+AS1, and S2+AS2). Nineteen phenolic chemicals were tested, and it was discovered that the quantities varied depending on the treatment. This shows that the plant samples, which showed the highest value of these phenolic compounds in the majority of cases, were affected by the application of AsA at 100 ppm under 4000 ppm of salt.

## 4. Discussion

A serious abiotic stressor, soil salinization impairs plant growth and development, resulting in physiological anomalies and ultimately endangering the world's food supply. The ailment is brought on by an overabundance of salt buildup in the soil, which is mostly caused by human activities including over fertilization, incorrect land usage, and irrigation [20]. Data in our study showed that, salinity caused a drop of chlorophyll *a*, *b*, and carotenoids content but it appeared significant increment in sodium and Chloride contents. Accumulation of Na and Cl ions under salinity stress in the presence AsA reported by [6] which found that exogenous application of Asc (1 mM) of rapeseed plants under salt stress in (75 mM and 150 mM NaCl) resulted in the higher accumulation of sodium ions ($Na^+$). Application of AsA appeared to improve chlorophyll *a*, *b*, and carotenoids content. In this concept [21], found that salt stress (90 mM NaCl) decreased the total chlorophyll, chlorophyll *a*, and chlorophyll *b* of maize (Malika and DTC hybrid varieties). Also [22], showed that salinity levels at 150 Mm NaCl decreased photosynthetic pigments of barley Plants. Overly high concentrations of $Na^+$, $Cl^-$, and other related ions in the soil can interfere with the normal functioning of plant cells and change vital metabolic processes like photosynthesis, severely damaging plant tissues and, in the worst cases, even killing them [20]. The vital role of AsA on chlorophyll under salinity stress is reported by many studies, for instance, In a pot experiment [23], illustrated the positive effect of foliar spray with AsA at 100 and 300 ppm on barley plants grown in pots under saline conditions (9.3 and 14 ds/$m^{-1}$NaCl) compared with salinized plants. Applications of AsA outside of plants increase plant tissue's antioxidant capability which stops degradation of chlorophyll molecules under salinity stress. Also, the fact that AsA serves as a coenzyme for a number of biological processes could help to explain this. It is well known that AsA (AsA) shields cells and organelles from reactive oxygen species, which over proliferate as a result of oxidative damage brought on by stress, it modifies plant sensing, regulates cell division and growth, and is involved in photosynthesis, hormone biosynthesis, and antioxidant renewal. It also functions as a cofactor for numerous enzymes [24].

In our study, contents of carbohydrates, protein, proline, phenolic, and flavonoid contents showed significant improvement in response to salinity at 4000 and 8000 ppm alone or in presence ascorbic 100 and 200 ppm concurrently with this [25], on Chia (*Salvia hispanica* L.) found that, under salt stress (10, 25, 50, 75, 100, and 150 mM) shown that as NaCl levels grew, so did the accumulation of total carbohydrate, phenolic, flavonoid compounds and proline contents in dried plant sprouts. Additionally [26], found that exposed barely plants (*H. vulgare* L. genotype B-14011) to 200 mM NaCl caused increased total soluble protein content in leaves. In regards to proline content [27], found an improvement in the proline content of Okra (*Abelmoschus esculentus* L.) leaves grown with increasing salinity levels (80, 100, and 160 mM NaCl). Also [21], showed that salt stress (at 90 mM of NaCl) appeared significantly increase proline contents in two varieties of maize (DTC (hybrid) and Malika). Proline and soluble sugars were also found to be more abundant in compatible solutes, in alfalfa plants that were stressed by salt and given a 1 mM AsA treatment [28]. In regards to phenol and flavonoid contents under salinity stress [29], on cucumber and tomato plants at different NaCl concentrations at (25, 50, 100, and 200 mM), found that, both treated plants had higher phenol and flavonoid concentrations due to salinity stress. Additionally [30], on *Amaranthus tricolor* plants in a pot experiment found that total polyphenolic and flavonoid contents dramatically increased with increasing salinity. On the contrary [31], on Soybean plants found that salinity stress led to reduction in content of soluble proteins in fresh shoot samples. The vital role of AsA on carbohydrates, proline and crud protein contents under salinity conditions portrays in many studies. for instance [32], investigated that, salinity of NaCl (at 50, 100, and 150 mM) showed a significant improvement in carbohydrates content in seeds soaking in AsA (100 ppm) of two *Vicia faba* L. cultivars (Giza843 and Giza 716). Also [23], showed that exogenous application of AsA (100 and 300 ppm) on barley plants (*H. vulgare* L.) grown under saline conditions (9.3 and 14 ds/$m^-$) appeared significantly increment of total carbohydrates, proline, and crud protein content compared with salinized untreated plants.

Our results appeared showed that, gallic acid, chlorogenic acid, caffeic acid, syringic acid, catechin, and kaempferol are the major phenolic compounds detected in treated plants and control. Also,

appeared that salinity stress in the presence of AsA showed improvement of phenolic compound especially salinity at 4000 ppm + ascorbic at 100 ppm. In this trend [33], showed that the phenol content of sweet pepper (presoaked seeds in AsA) was considerably increased by AsA (50 ppm) and salt stress at 50, 100, 150, 200, and 250 mM NaCl. Furthermore [34], on geranium plants cultivated in AsA (50, 100, and 150 ppm) and salt levels (1000, 3000, and 5000 ppm) mediums, revealed that, in comparison to salinized plants, the phenolic and flavonoid content was raised as a result of the interactive treatment between salinity and AsA. In our study salinity at high dose of salinity showed highly decrease in gallic acid, chlorogenic acid, syringic acid, ellagic acid, coumaric acid, ferulic acid, methyl gallate, vanillin, catechin, and kaempferol compared with control and other treatments. A study on leaves of wheat [35], found that, p-coumaric acid dropped under salt stress. On the contrary, in *Amaranthus gangeticus* at 100 and 50 mM of salinity concentrations, polyphenolic profiles were much rose than those of the control [36]. Also in a study on *Glaux maritima* L. under various salinity levels (25−500 mM in the form of NaCl) showed that the maximum values of protocatechuic acid, catechin, astragalin, hyperoside, rutin, isoquercitrin, and apigenin derivative, were found to be at 100−300 mM NaCl [37]. Data in our results showed that, AsA at 100 ppm in group subjected to 4000 ppm of salinity appeared the highest values of coffeic acid, syringic acid, rosmarinic acid, ellagic acid, ferulic acid, methyl gallate, vanillin, naringenin, rutin, daidzein, and quercetin compared with control and other treatment. In the pharmaceutical and medical fields, these phenolic compounds and flavonoids have the potential to replace bioactive chemicals in order to improve human health and prevent and treat a variety of disorders. In our research, two separate groups could be formed from the various treatments, according to a two-way cluster analysis (Fig. 4). The first cluster included AsA at 100 ppm under 4000 ppm of NaCl treatment, while the second cluster encompassed two main groups, the first group included S1 (salinity at 4000 ppm) and S2 (salinity at 4000 ppm) but the second group included other treatment. This clustering was likely due to the high content of phenolic compounds in presence AsA at 4000 ppm level of salinity. Specifically, gallic acid, chlorogenic acid, caffeic acid, Syringic acid, Rosmarinic acid, Ellagic acid, Rutin, Daidzein, quercetin, and Kaempferol exhibited comparable concentrations in these two groups, explaining their grouping together.

### 4.1. Conclusion and recommendation

(a) The potential advantages of phenolic compounds for human health have also garnered attention. Certain chemicals, such as the antioxidants and flavonoids present in fruits, vegetables, and seeds, may be advantageous to human health. Additionally, they may aid in the prevention of some types of cancer and heart disease, among other chronic illnesses. Consequently, there is significant economic significance to AsA's ability to raise the concentration of these phenolic compounds in barley grain plants.
(b) Salinity at 4000 and 8000 ppm appeared significant decrease of pigments contents but showed

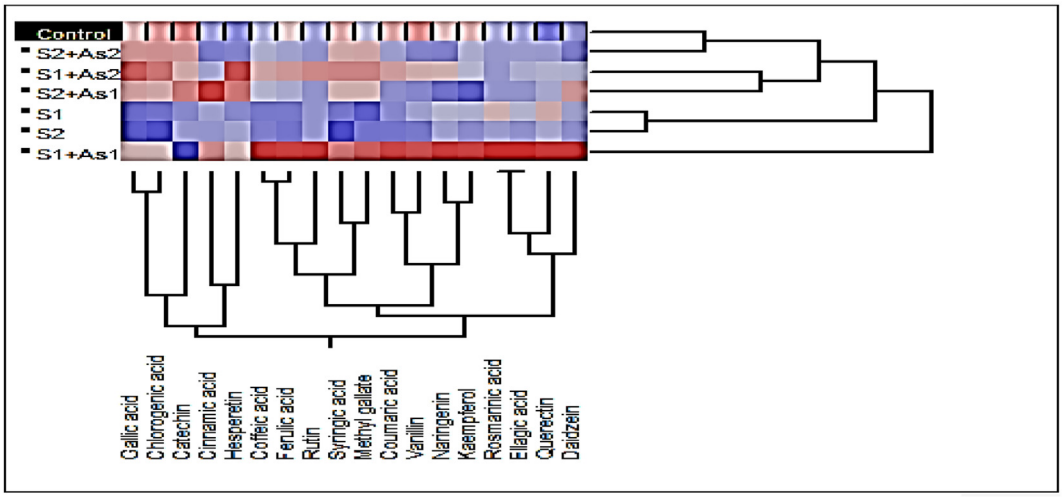


*Fig. 4. Two-way cluster analysis to group the different treatments into several clusters based on the observed values of the compounds and their percentages existing in the grains of barly plant treated or not with ascorbic acid (100 and 200 ppm).*

significant increase in carbohydrates protein, proline, total phenol, and flavonoid contents.

(c) AsA at 100 and 200 ppm portrays improvement in carbohydrates,protein, proline, total phenol and flavonoid contents under saline condition

(d) AsA at 100 in saline soil at 4000 ppm appeared highly increased in medical important compounds (Caffeic acid, Syringic acid, Rosmarinic acid, Ellagic acid, Ferulic acid, and Methyl gallate).

(e) AsA at 200 in saline soil at 4000 ppm appeared highly increased in Gallic acid and Chlorogenic acid and Hesperetin

(f) We recommended that application AsA at 100 ppm or 200 ppm can alleviate the harmful effects of salinity stress at high concentrations in many economic plants

## Availability of data and material

The datasets analyzed during the current study are available from the corresponding author on reasonable request.

## Authors' contributions

K.A.H. design and performed experiments. A.E.M. design and prepared experiments. H.M.S. revision of the manuscript. S.M.I. prepared of the manuscript. M.A.A. analyzed data and compiled figures.

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## Ethics information

Compliance with ethical standards.

## Conflicts of interest

The authors declare that they have no competing interests.

## References

[1] Aksakal O. Assessment of paraquat genotoxicity on barley (Hordeum vulgare L.) seedlings using molecular and biochemical parameters. Acta Physiol Plant 2013;35:2281−7.

[2] Ullah HA, Javed F, Wahid A, Sadia B. Alleviating effect of exogenous application of ascorbic acid on growth and mineral nutrients in cadmium stressed barley (Hordeum vulgare) seedlings. Int J Agric Biol 2015;18:73−9.

[3] Hassan A, Fasiha Amjad S, Hamzah Saleem M, Yasmin H, Imran M, Riaz M, et al. Foliar application of ascorbic acid enhances salinity stress tolerance in barley (Hordeum vulgare L.) through modulation of morpho-physio-biochemical attributes, ions uptake, osmo-protectants and stress response genes expression. Saudi J Biol Sci 2021;28:4276−90.

[4] Dawood MG. Alleviation of salinity stress on Vicia faba L. plants via seed priming with melatonin. Acta Biol Colomb 2014;20:2.

[5] Kazemi R, Ronaghi A, Yasrebi J, Ghasemi-Fasaei R, Zarei M. Effect of shrimp waste−derived biochar and arbuscular mycorrhizal fungus on yield, antioxidant enzymes, and chemical composition of corn under salinity stress. J Soil Sci Plant Nutr 2019;19:758−70.

[6] Hasanuzzaman M, Raihan MRH, Alharby HF, Al-Zahrani HS, Alsamadany H, Alghamdi KM, et al. Foliar application of ascorbic acid and tocopherol in conferring salt tolerance in rapeseed by enhancing K+/Na+ homeostasis, osmoregulation, antioxidant defense, and glyoxalase system. Agronomy 2023;13:361.

[7] Hamid KA, Mustafa AE, Salem HM, Ismaiel SM, Amin MA. Effect of salinity and ascorbic acid treatments on growth, yield and antioxidant enzymes activity of barley plants. Al-Azhar J Agric Res 2024;49(1):117−29.

[8] Sharma R, Bhardwaj R, Thukral AK, Al-Huqail AA, Siddiqui MH, Ahmad P. Oxidative stress mitigation and initiation of antioxidant and osmoprotectant responses mediated by ascorbic acid in Brassica juncea L. subjected to copper (II) stress. Ecotoxicol Environ Saf 2019;182: 109436.

[9] Foyer CH, Noctor G. Ascorbate and glutathione: the heart of the redox hub. Plant Physiol 2011;155:2−18.

[10] Bilska K, Wojciechowska N, Alipour S, Kalemba EM. Ascorbic acid—the little-known antioxidant in woody plants. Antioxidants 2019;8:645.

[11] Chen X, Zhou Y, Cong Y, Zhu P, Xing J, Cui J, et al. Ascorbic acid-induced photosynthetic adaptability of processing tomatoes to salt stress probed by fast OJIP fluorescence rise. Front Plant Sci 2021;12:594400.

[12] Zhou X, Gu Z, Xu H, Chen L, Tao G, Yu Y, et al. The effects of exogenous ascorbic acid on the mechanism of physiological and biochemical responses to nitrate uptake in two rice cultivars (oryza sativa L.) under aluminum stress. J Plant Growth Regulat 2016;35:1013−24.

[13] Vernon LP, Seely GR. The chlorophylls. Cambridge, MA, USA: Academic Press; 1966.

[14] Abada E, Modafer Y, Mashraqi A, Shater ARM, Al MA, Amin MA, et al. Ameliorative effect of micro-algal and medicinal plants on some biochemical properties of bean plants under salinity stress. Bioresources 2023;18: 6142−54.

[15] Umbreit WW, Burris RH, Stauffer JF. Manometric techniques. Minneapolis, USA: Burgess Pub. Co.; 1945.

[16] Lowry OH, Nira JR, A Lewis F, Rose JR. Protein measurement with the Folin phenol reagent. J Biol Chem 1951;193: 265−75.

[17] Bates LS, Waldren RP, Teare ID. Rapid determination of free proline for water-stress studies. Plant Soil 1973;39: 205−7.

[18] Diaz DH, George CM. Peach seed dormancy in relation to endogenous inhibitors and applied growth substances 1. J Am Soc Hortic Sci 1972;97:651−4.

[19] Chang CC, Yang MH, Wen HM, Chern JC. Estimation of total flavonoid content in propolis by two complementary colometric methods. J Food Drug Anal 2020;10:3.

[20] Balasubramaniam T, Shen G, Esmaeili N, Zhang H. Plants' response mechanisms to salinity stress. Plants 2023;12:2253 [Internet].

[21] Perveen S, Hussain SA. Methionine-induced changes in growth, glycinebetaine, ascorbic acid, total soluble proteins and anthocyanin contents of two Zea mays L. varieties under salt stress. J Anim Plant Sci 2020;31:1.

[22] Hassan A, Saleem MH, Parveen A, Mobeen M. Oxidative stress alleviation as indicated by enzymatic and nonenzymatic antioxidants and osmoregulators in barley (Hordeum vulgare L.) under (NaCl) stress by ascorbic acid. Pakistan J Bot 2022;54:1.

[23] Seleem EA, Saad M, Taha ZK. Exogenous application of ascorbic acid and putrescine: a natural eco-friendly potential for alleviating NaCl stress in barley (Hordeum vulgare). Emirates J Food Agric 2021;33(8):657−70.
[24] Alamri SA, Siddiqui MH, Al-Khaishany MY, Nasir Khan M, Ali HM, Alaraidh IA, et al. Ascorbic acid improves the tolerance of wheat plants to lead toxicity. J Plant Interact 2018;13:409−19.
[25] Izadi Y, Moosavi SA, Gharineh MH. Salinity affects eco-physiological aspects and biochemical compositions in chia (Salvia hispanica L.) during germination and seedling growth. Sci Hortic 2022;306:111461.
[26] Noreen S, Sultan M, Akhter MS, Shah KH, Ummara U, Manzoor H, et al. Foliar fertigation of ascorbic acid and zinc improves growth, antioxidant enzyme activity and harvest index in barley (Hordeum vulgare L.) grown under salt stress. Plant Physiol Biochem 2020;158:244−54.
[27] Ding J, Mtolera I, She D, Ma T, Chen K, Yu S. Effects of saline water irrigation and Biochar amendment on Okra growth and nutrient leaching in coastal saline soils. Commun Soil Sci Plant Anal 2020;52:651−65.
[28] El Moukhtari A, Lamsaadi N, Farissi M. Biostimulatory effects of ascorbic acid in improving plant growth, photosynthesis-related parameters and mitigating oxidative damage in alfalfa (Medicago sativa L.) under salt stress condition. Biologia 2024;79:2375−85.
[29] Abdel-Farid IB, Marghany MR, Rowezek MM, Sheded MG. Effect of salinity stress on growth and MetabolomicProfiling of Cucumis sativus and Solanum lycopersicum. Plants 2020; 9:1626.
[30] Sarker U, Islam MdT, Oba S. Salinity stress accelerates nutrients, dietary fiber, minerals, phytochemicals and antioxidant activity in Amaranthus tricolor leavesLightfoot DA, editor. PLoS One 2018;13:e0206388.
[31] Osman MS, Badawy AA, Osman AI, Abdel Latef AAH. Ameliorative impact of an extract of the halophyte arthrocnemum macrostachyum on growth and biochemical parameters of soybean under salinity stress. J Plant Growth Regul 2020;40:1245−56.
[32] Loutfy N, Azooz M, Hassanein A, Bassiony A. Potassium synergize the positive effect of ascorbic acid on some morpho-physiological parameters of salt stressed faba bean cultivars. Egypt J Bot 2019.
[33] Al Othaimen HS. Improve the salinity stress by using ascorbic acid on germination, growth parameters, water relations, organic and inorganic components of sweet pepper (Capsicum annuum, L.) plant. J Adv Agric 2015;4:331−49.
[34] Shay JW, Homma N, Zhou R, Naseer MI, Chaudhary AG, Al-Qahtani M, et al. Abstracts from the 3rd international genomic medicine conference (3rd IGMC 2015). BMC Genom 2016;17(Suppl 6):487.
[35] Kiani R, Arzani A, Mirmohammady Maibody SAM. Polyphenols, flavonoids, and antioxidant activity involved in salt tolerance in wheat, aegilops cylindrica and their amphidiploids. Front Plant Sci 2021;12:646221.
[36] Sarker U, Nazmul Hossain MD, Oba S, Erċõşlõ S, Marc RA, Golokhvast KS. Salinity stress ameliorates pigments, minerals, polyphenolic profiles, and antiradical capacity in lal-shak. Antioxidants 2023;12. 173−3.
[37] Pungin A, Lartseva L, Violetta L, Vladislav S, Popova E, Liubov S, et al. Effect of salinity stress on phenolic compounds and antioxidant activity in halophytes Spergularia marina (L.) Griseb. And Glaux maritima L. Cultured in vitro. Plants 2023;12:1905−5.