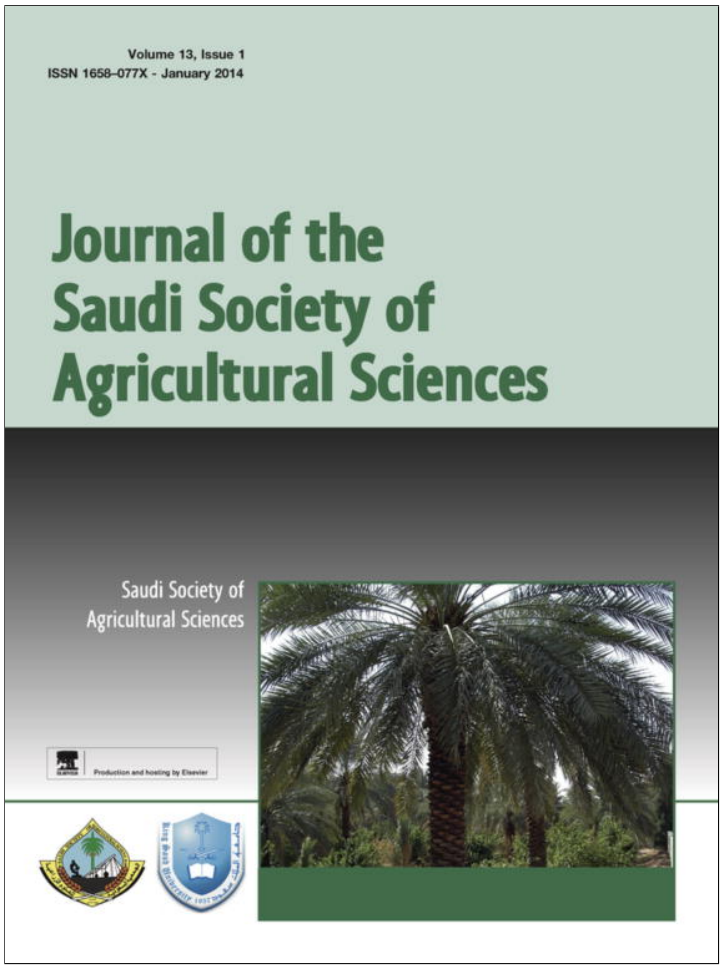

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FULL LENGTH ARTICLE

# Improving drought and salinity tolerance in barley by application of salicylic acid and potassium nitrate

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**Abstract** Growth and physiological activities of barley (*Hordeum vulgare* L. cv. Gustoe) grown in soil cultures were evaluated to recognize the ameliorative role of salicylic acid (SA) and $KNO_3$ against the negative effects of salt and water deficit stresses. Barley plants were subjected to three levels of NaCl (50, 100 and 150 mM), three levels of water stress (80%, 70% and 50% of the soil water content (SWC) and the combination of 150 mM NaCl + 50 μM SA, 150 mM NaCl + 10 mM $KNO_3$, 50% SWC + 50 μM SA and 50% SWC + 10 mM $KNO_3$ for two weeks. Salt and water deficit stresses reduced the shoot growth, leaf photosynthetic pigments, $K^+$ contents and provoked oxidative stress in leaves confirmed by considerable changes in soluble carbohydrate, proline, malondialdehyde (MDA), total phenolic compounds, antioxidant activity and $Na^+$ contents. Leaf soluble protein of salt and water deficit treated plants was unaffected. The $Na^+/K^+$ ratio increased with increasing salt and water deficit treated plants. Application of 50 μM SA or 10 mM $KNO_3$ to150 mM NaCl and/or 50% SWC treated plants improved these attributes under salt and water stresses. Soluble carbohydrates in stressed plants may have a significant role in osmotic adjustment. It can be concluded that the addition of SA or $KNO_3$ can ameliorate the oxidative stress in barley stressed plants. This ameliorative effect might be maintained through low MDA contents and decreased $Na^+/K^+$ ratio in leaves. This study also provided evidence for the ability of barley cultivation in salt and water deficit soils due to its capacity for osmotic adjustment.


## 1. Introduction

Salinity and drought are the major abiotic stresses that reduce plant growth and crop productivity worldwide. Water scarcity resulting from global climate change is accompanied by more frequent and more severe summer droughts in many regions (Hamdy et al., 2003; Munns, 2005). Abiotic stresses resulting from excessive salinity or water deficit led to reduction in photosynthesis, transpiration and other biochemical processes associated with plant growth, development and crop productivity (Shannon, 1997; Tiwari et al., 2010). Furthermore, abiotic stress lead to oxidative stress in the plant cell

*Abbreviations*: Car, carotenoid; Chl, chlorophyll; SWC, soil water content; MDA, malondialdehyde; ROS, reactive oxygen species

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resulting in a higher leakage of electrons towards $O_2$ during photosynthetic and respiratory processes which leading to enhancement of reactive oxygen species (ROS) generation (Asada, 2006). Much of the injury on plants under abiotic stress is linked to oxidative damage at the cellular level leading to cell death (Mittler, 2002). During optimal growth conditions, balance between ROS formation and consumption is tightly controlled by plant antioxidant defense system (Hameed et al., 2011). Plants containing high activities of antioxidant enzymes have shown considerable resistance to oxidative damage caused by ROS (Gapinska et al., 2008). Higher plants have developed different adaptive mechanisms to reduce oxidative damage resulting from salt stress, through the biosynthesis of a cascade of antioxidants. General metabolic adaptation which enables plants to cope with water or osmotic stress, involves an increased synthesis of osmoprotectants, such as proline and soluble sugar. Compatible osmolytes not only contribute to osmoregulation but they may also protect the structure of different biomolecules and membranes (Yancey et al., 1982; Hare et al., 1998) or act as free-radical scavengers that protect DNA from damaging effects of ROS (Ashraf and Foolad, 2007). Proline accumulation has been reported to counter balance the deleterious effects of water deficit and salinity. It may also serve as an organic nitrogen reserve that can be utilized during recovery from stress (Sairam and Tyagi, 2004). In addition, proline is widely distributed in plants and it accumulates in larger amounts than other amino acids in drought stressed plants (Irigoyen et al., 1992). Soluble sugar accumulation has been reported with salt and drought stresses (Pinheiro et al., 2004; Munns, 2005). Phenolic compounds also play an important role in scavenging free radicals and protect plants against the damaging effects of increased ROS levels due to salt and drought stresses (Petridis et al., 2012).

Salicylic acid (SA) is considered as a hormone-like substance, which plays an important role in photosynthetic rate, stomatal conductance and transpiration (Khan et al., 2003; Arfan et al., 2007), increasing antioxidative protection (Xu et al., 2008), and inhibiting $Na^+$ and $Cl^-$ accumulation (Gunes et al., 2007). Several lines of evidence demonstrate the alleviating role of SA during salinity (Shakirova et al., 2003) and drought (Singh and Usha, 2003). However, in the case of salt stress, the effect of SA on plant growth and metabolism is still a matter of controversy in consideration of different plant species, salt stress intensity and duration, as well as SA doses applied (Horváth et al., 2007). Exogenous SA could regulate the activities of antioxidant enzymes and increase plant tolerance to abiotic stresses (He et al., 2002; Erasalan et al., 2007).

Potassium plays an important role in balancing membrane potential and turgor, activating enzymes, regulating osmotic pressure, stoma movement, and membrane polarization (Maathuis and Sanders, 1996; Kaya et al., 2007). Previous studies revealed that supplying low levels of $KNO_3$ could alleviate the NaCl induced decreases in seed germination of certain grass species (Neid and Biesboer, 2005). As the $K^+$ is involved in multiple previous plant activations, the $K^+/Na^+$ ratio has been proposed as an effective indicator for salinity tolerance in wheat (Zheng et al., 2008). However, overproduction of reactive oxygen species (ROS) caused by salinity usually leads to lipid peroxidation and induces $K^+$ leak from the cell by activating $K^+$ efflux channels (Demidchik et al., 2003; Cuin and Shabala, 2007).

The present study aimed to evaluate and assess whether SA and $KNO_3$ can alleviate the negative effects of salt and water deficit stresses on barley leaves through monitoring the shoot growth, photosynthetic pigments, proline, carbohydrate, protein, MDA, phenolic compounds and total antioxidant activity. In addition, the change in $K^+/Na^+$ ratio due to salt and water deficit treatments was also evaluated in barley leaves.

## 2. Materials and methods

### 2.1. Plant materials and treatments

Grains of barley (*Hordeum vulgare* L. cv. Gustoe) were obtained from Tabuk Agriculture Company, KSA. Grains were surface sterilized with 0.2% $HgCl_2$ solution for 5 min with frequent shaking and thoroughly washed many times with distwater to remove $HgCl_2$. Grains were sown in each pot containing 2 kg of soil mixture composed of soil, sand and potmos at 2:1:1 v/v ratio in clean plastic pots ($20 \times 25$ cm in diameter and depth, respectively). The soil mixture having pH, 7.2; EC, 1.65 ds $m1^{-1}$; and 55 ppm of available $K^+$. All pots were watered to the soil water content with ground water up to three weeks after sowing. The pots were placed in a greenhouse under natural light and temperature conditions during the growth season of 2010 with an average day/night temperature of $22/16 \pm 2$ °C. After three weeks, the plants of barley that showed the same growth were divided into 12 groups. The identification of plant group treatments was as follows:

| Group | Treatment |
|---|---|
| 1 | Control plants |
| 2 | Plants sprayed with 50 μM SA |
| 3 | Plants treated with 50 mM NaCl |
| 4 | Plants treated with 100 mM NaCl |
| 5 | Plants treated with 150 mM NaCl |
| 6 | Plants treated with 150 mM NaCl + sprayed with 50 μM SA |
| 7 | Plants treated with 150 mM NaCl + 10 mM $KNO_3$ |
| 8 | Plants treated with 80% SWC |
| 9 | Plants treated with 70% SWC |
| 10 | Plants treated with 50% SWC |
| 11 | Plants treated with 50% SWC + sprayed with 50 μM SA |
| 12 | Plants treated with 50% SWC + 10 mM $KNO_3$ |

The soil water content of experimental soil was calculated when water is no longer leaving the soil sample. The plants were treated for two weeks with the various treatments mentioned above according to the soil water content of the experimental soil. Each group consists of three replicates (each replicate is 1 pot containing 10 plants). The levels of soil water content were controlled by weighting the pots daily. The soil water content of the control plants was kept at 85% SWC. The soil water content (SWC) of experimental soil was calculated according to the following equation: [(wet soil-dry soil)/dry soil] $\times$ 100. Where wet soil and dry soil are weight of soil after water drinage and oven dried soil, respectively.

### 2.2. Estimation of shoot fresh weight and height

Three replicates of the control and treated plants (three plants of each) were harvested and the shoot of each plant was

collected separately for estimation of shoot fresh weight and height. The shoot fresh weight and height were expressed as g plant$^{-1}$ and cm shoot$^{-1}$, respectively.

### 2.3. Determination of photosynthetic pigment contents

Contents of Chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*) and total carotenoids were spectrophotometrically (Jenway 6300 spectrophotometer, UK) determined according to Metzner et al. (1965). The photosynthetic pigment content of plants was extracted from a known fresh weight of leaves in 85% (v/v) aqueous acetone. The extract was centrifuged at 4000*g* for 10 min. The supernatant was then taken and diluted by 85% aqueous acetone to a suitable concentration for spectrophotometric measurements. The absorbance was measured against a blank of pure 85% aqueous acetone at three wavelengths of 452, 644 and 663 nm using the following equations:

$$\text{Chlorophyll } a = 10.3^{*}E_{663} - 0:918^{*}E_{644} = \mu\text{g/mL}$$

$$\text{Chlorophyll } b = 19.7^{*}E_{644} - 3.87^{*}E_{663} = \mu\text{g/mL}$$

$$\text{Total carotenoid} = 4.2^{*}E_{452} - \{(0.0264^{*}\text{Chl } a) + (0.426 * \text{Chl } b)\} = \mu\text{g/mL}.$$

The photosynthetic pigments were expressed as mg g$^{-1}$ FW.

### 2.4. Determination of proline content

Proline content of leaves was determined according to Bates et al. (1973). A known dry weight (0.1 g) of leaves was extracted in 10 mL of aqueous 3% sulfosalicylic acid over-night. The extract was centrifuged at 3000*g* for 10 min. Two mL of the supernatant was mixed with 2 mL of fresh acid ninhydrin solution and 2 mL of glacial acetic acid for reaction in a test tube for 1 h at 100 °C. The reaction was terminated in an ice bath, and the mixture was extracted with 4 mL toluene. The extract was vigorously stirred for 20 s using a test tube stirrer. The chromophore-containing toluene was aspirated from the aqueous phase, and its absorbance was measured at 520 nm. Proline content was determined from a standard curve and calculated as mg g$^{-1}$ DW.

### 2.5. Determination of soluble protein content

Soluble protein content of leaves was determined according to Lowry et al. (1951) using Bovine serum albumin as a standard. Leaf samples (0.1 g dry weight) were extracted in 10 mL distilled water for 2 h at 90 °C. The extracts were centrifuged and the supernatants were collected. One mL of extract was added to 5 mL of alkaline reagent (50 mL 2% $Na_2CO_3$ prepared in 0.1 N NaOH and 1 mL 0.5% $CuSO_4{\cdot}5H_2O$ prepared in 1% sodium potassium tartarate) and mixed thoroughly then allowed to stand for 10 min. A total of 0.5 mL of Folin phenol reagent diluted 1:2 (v/v) was then added and mixed immediately. After 30 min, the extinction against appropriate blank was measured at 700 nm. Protein contents were expressed as mg g$^{-1}$ DW.

### 2.6. Determination of soluble carbohydrate content

Soluble carbohydrate content was determined in aqueous solution with anthrone sulfuric acid reagent according to Fales (1951) and Schlegel (1956), using glucose as a standard. To extract water-soluble carbohydrates, a known weight (0.1 g dry weight) of leaf tissue powder was boiled in distilled water in a water bath for 1 h. The extracts were then cooled and filtrated through a centered glass funnel. A total of 0.5 mL of the extract was mixed with 4.5 mL of anthrone reagent (0.2 g anthrone, 8 mL absolute ethyl alcohol, 30 mL distilled water and 100 mL sulfuric acid ($D$ = 1.84). The mixture was then boiled in a water bath for 7 min. After cooling, the developed blue green color was measured at 620 nm against blank. Soluble carbohydrate contents were expressed as mg g$^{-1}$ DW.

### 2.7. Determination of lipid peroxidation

Malondialdehyde (MDA) content was determined as an indication of leaf lipid peroxidation according to Hernández and Almansa (2002). Fresh leaf samples (500 mg) were homogenized in 5 mL of 0.1% trichloroacetic acid (TCA). The homogenate was centrifuged at 15,000*g* for 20 min at 4 °C. One mL aliquot of the supernatant was mixed with 3 mL of 0.5% thiobarbituric acid (TBA) prepared in 20% TCA and incubated at 90 °C for 20 min. After stopping the reaction in an ice bath, samples were centrifuged at 10,000*g* for 5 min. The supernatant absorbance at 532 nm was then measured. After subtracting the non-specific absorbance at 600 nm, MDA concentration was determined using the extinction coefficient 155 mM$^{-1}$ cm$^{-1}$.

### 2.8. Determination of total phenolic compounds

Total phenolics were measured with the Folin–Ciocalteu reagent (Dai et al., 1994). Twenty five μL of the extract was mixed with 110 μL Folin–Ciocalteu reagent, 200 μL 20% sodium carbonate and 1.9 mL distilled water, and placed at 60 °C for 30 min. Optical density was measured with a spectrophotometer at 750 nm. A standard curve was constructed with different concentrations of gallic acid. The results were expressed as μg of gallic acid g$^{-1}$ FW.

### 2.9. Estimation of total antioxidant activity by using DPPH scavenging assay

The free radical-scavenging activity of leaf extract in methanol was measured using the method described by Shimada et al. (1992). Each extract (0.2–10 mg mL$^{-1}$) in methanol (2 mL) was mixed with 2 mL of freshly prepared methanolic solution containing 80 ppm of 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals. The mixture was shaken vigorously and left to stand for 30 min in the dark. The absorbance was then measured at 517 nm. The percentage of DPPH scavenging activity was calculated using the equation below:

$$\text{DPPH scavenging ability} = [1 - (A_i - A_j)/A_c]^{*}100$$

Where, $A_i$ is absorbance of extract + DPPH, $A_j$ is absorbance of extract + methanol, and $A_c$ is absorbance of DPPH + methanol. A lower absorbance indicates a higher scavenging effect.

### 2.10. Determination of $Na^+$ and $K^+$ contents

Fine ground oven dried leaves (0.1 g) were soaked for 12 h in digesting tubes with 10 mL concentrated nitric acid and 3 mL perchloric acid and then digested at 300 °C for 6 h. The extracts were completed to appropriate volume with deionized water. The amount of $K^+$ and $Na^+$ contents was measured using a Flame photometer (Jenway PFP7, UK.).

### 2.11. Statistical analysis

The obtained data were subjected to one-way analysis of variance (ANOVA). Means were compared by least significant differences (LSD) test at $P \leqslant 0.001$, $P \leqslant 0.01$ and $P \leqslant 0.05$ levels. All statistical analyses were carried out using SPSS 9.0 statistical software for Windows. Significant differences at levels of significance are represented by asterisks. Data are given as means values ± standard deviation.

## 3. Results

### 3.1. Estimation of shoot fresh weight and height

The barley shoot fresh weight and height displayed a significant reduction in response to the increasing levels of salt doses and water deficit treatments. For example, under effect of 150 mM NaCl, the shoot fresh weight and height decreased by 30% (Fig. 1) and 36% (Table 1), respectively, compared to those of the control. Leaf chlorosis was observed in plants treated with 150 mM NaCl (Fig. 2). The effect of combination treatments of 150 mM NaCl + 50 μM SA or 10 mM $KNO_3$ improved shoot growth and disappeared leaf chlorosis in comparison to that treated only with 150 mM NaCl (Fig. 1). Water deficit of 50% SWC treated plants displayed 35% reduction in

**Table 1** Effect of salt stress (50, 100 and 150 mM NaCl), water deficit stress (80%, 70% and 50% SWC), 50 μM SA and the combination of 150 mM NaCl + 50 μM SA, 150 mM NaCl + 10 mM $KNO_3$, 50% SWC + 50 μM SA and 50% SWC + 10 mM $KNO_3$ on shoot height (cm) of barley (*Hordeum vulgare* L. cv. Gustoe) relative to the control. Values are means (M) of three replicates ± standard deviation (SD). For a given date, statistically significant of differences compared to the value of control plants was conducted.

| Treatments | Shoot height (cm) | |
|---|---|---|
| | M ± SD | % |
| Control | 23.86 ± 1.58 | 100 |
| 50 μM SA | 24.53 ± 0.51 | 103 |
| 50 mM NaCl | 19.93*** ± 2.22 | 84 |
| 100 mM NaCl | 17.13*** ± 2.20 | 72 |
| 150 mM NaCl | 15.20*** ± 1.76 | 64 |
| 150 mM NaCl + 50 μM SA | 17.10*** ± 1.30 | 72 |
| 150 mM NaCl + 10 mM $KNO_3$ | 17.93*** ± 0.98 | 75 |
| 80% SWC | 21.00* ± 1.09 | 88 |
| 70% SWC | 20.20*** ± 1.02 | 85 |
| 50% SWC | 17.80*** ± 0.79 | 75 |
| 50% SWC + 50 μM SA | 19.76*** ± 2.24 | 83 |
| 50% SWC + 10 mM $KNO_3$ | 21.50* ± 1.64 | 90 |

* Significant at $P \leqslant 0.05$.
** Significant at $P \leqslant 0.01$.
*** Significant at $P \leqslant 0.001$.

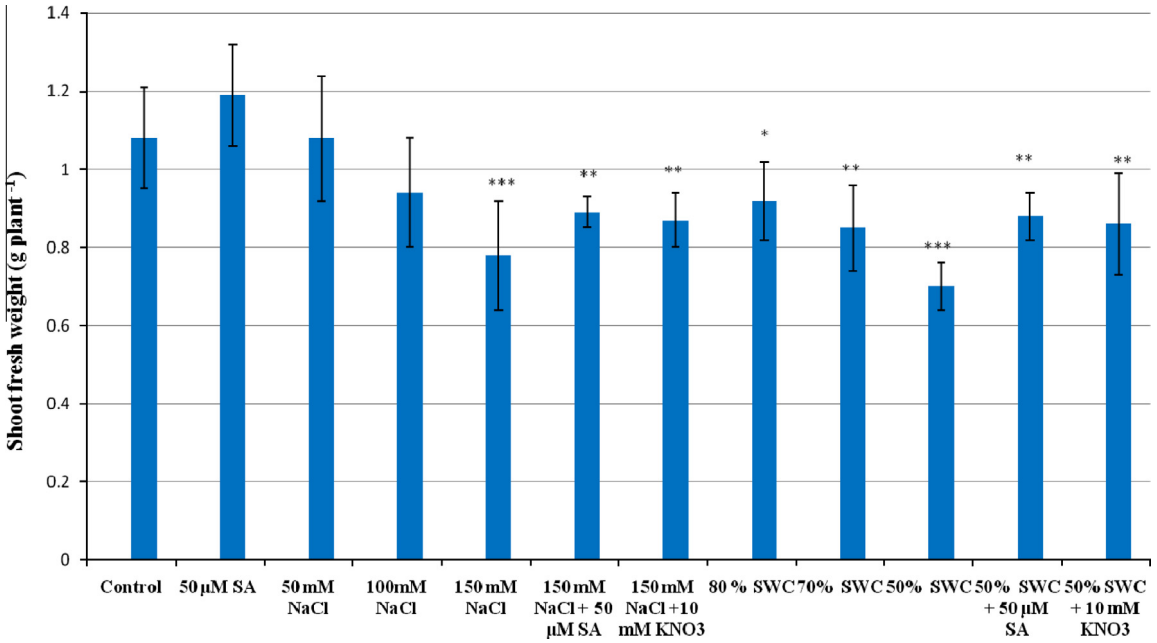


**Figure 1** Effect of salt stress (50, 100 and 150 mM NaCl), water deficit (80%, 70% and 50% SWC), 50 μM SA and the combination of 150 mM NaCl + 50 μM SA, 150 mM NaCl + 10 mM $KNO_3$, 50% SWC + 50 μM SA and 50% SWC + 10 mM $KNO_3$ on shoot fresh weight (g $plant^{-1}$ FW) of barley leaves (*Hordeum vulgare* L. cv. Gustoe). Values are means (M) of three replicates ± standard deviation (SD). For a given date, statistically significant of differences relative to the value of control plants was conducted. *significant at $P \leqslant 0.05$; **significant at $P \leqslant 0.01$; ***significant at $P \leqslant 0.001$.

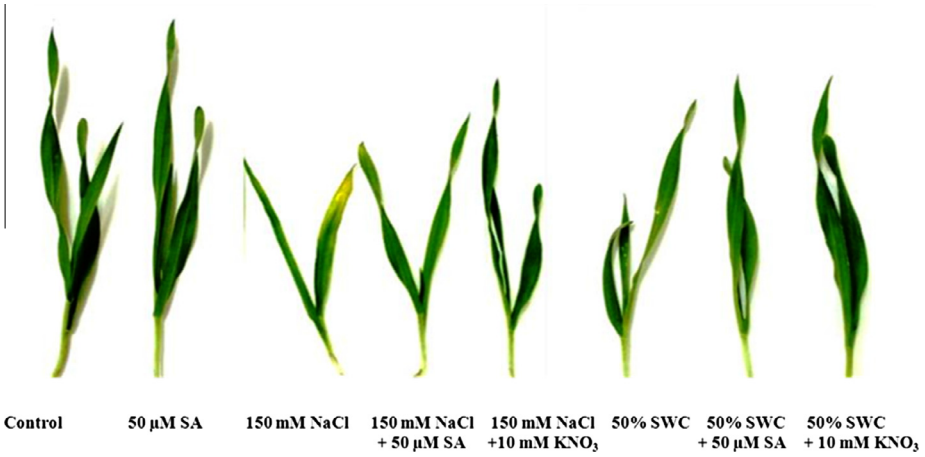


**Figure 2** Effect of 50 µM SA, 150 mM NaCl, 150 mM NaCl + 50 µM SA, 150 NaCl mM + 10 mM $KNO_3$, 50% SWC, 50% SWC + 50 µM SA, and 50% SWC + 10 mM $KNO_3$ on growth and leaf morphology of barley (*Hordeum vulgare* L. cv. Gustoe) relative to the control. Salt stress and water deficit stresses were reduced plant height and a new leaf production. Application of 50 µM SA and $KNO_3$ to 150 mM NaCl or 50% SWC stressed plants enhanced plant height.

the shoot fresh weight (Fig. 1) and 25% in shoot height (Table 1) compared to those of the control plants. Spraying of 50 µM SA or addition of 10 mM $KNO_3$ to water deficit (50% SWC) treated plants improved shoot fresh weight and height compared to that treated only in with water deficit of 50% SWC.

### *3.2. Determination of photosynthetic pigment contents*

In response to salt and water deficit stresses, the photosynthetic pigment contents of barley leaves showed considerable variations in comparison with those of the control plants (Table 2). Spraying of 50 uM SA increased leaf total photosynthetic pigments by 12% in comparison with that of the control plants. Generally, Chl *a*, Chl *b* and carotenoids contents were significantly decreased with increasing salt and water deficit stressed plants. The leaf total pigment content was 2.56 mg $g^{-1}$ FW for the control and 1.10 mg $g^{-1}$ FW for 150 mM NaCl treated plants (43% of the control). In response to the water deficit of 50% SWC, the leaf total pigment content was 1.26 mg $g^{-1}$ FW (49% of the control). Spraying of 50 µM SA to plants treated with 150 mM NaCl increased photosynthetic pigment contents in comparison with those treated only with 150 mM NaCl. The value of total pigment content was 1.57 mg $g^{-1}$ for 150 mM + 50 µM SA treated plants. In response to the combination treatments of 150 mM

**Table 2** Effect of salt stress (50, 100 and 150 mM NaCl), water deficit stress (80%, 70% and 50% SWC), 50 µM SA and the combination of 150 mM NaCl + 50 µM SA, 150 mM NaCl + 10 mM $KNO_3$, 50% SWC + 50 µM SA and 50% SWC + 10 mM $KNO_3$ on pigment contents (mg $g^{-1}$ FW) of barley (*Hordeum vulgare* L. cv. Gustoe) relative to the control. Values are means (M) of three replicates ± standard deviation (SD). For a given date, statistically significant of differences compared to the value of control plants was conducted.

| Treatments | Chl A | Chl B | Carotenoids | A/B ratio | Total | % |
|---|---|---|---|---|---|---|
| | M ± SD | M ± SD | M ± SD | | | |
| Control | 1.52 ± 0.10 | 0.74 ± 0.12 | 0.30 ± 0.03 | 2.05 | 2.56 | 100 |
| 50 µM SA | 1.69 ± 0.34 | 0.97 ± 0.10 | 0.22*** ± 0.02 | 1.74 | 2.88 | 112 |
| 50 mM NaCl | 1.42 ± 0.10 | 0.65 ± 0.12 | 0.22*** ± 0.02 | 2.18 | 2.29 | 89 |
| 100 mM NaCl | 1.10** ± 0.12 | 0.53** ± 0.08 | 0.20*** ± 0.01 | 2.07 | 1.83 | 71 |
| 150 mM NaCl | 0.65*** ± 0.06 | 0.31*** ± .03 | 0.13*** ± 0.03 | 2.09 | 1.10 | 43 |
| 150 mM NaCl + 50 µM SA | 0.93*** ± 0.06 | 0.44*** ± 0.03 | 0.20*** ± 0.02 | 2.11 | 1.57 | 61 |
| 150 mM NaCl + 10 mM $KNO_3$ | 1.11** ± 0.22 | 0.51*** ± 0.08 | 0.24* ± 0.04 | 2.17 | 1.86 | 73 |
| 80% SWC | 1.37 ± 0.14 | 0.69 ± 0.10 | 0.19*** ± 0.02 | 1.98 | 2.25 | 88 |
| 70% SWC | 0.96*** ± 0.06 | 0.48*** ± 0.04 | 0.19*** ± 0.01 | 2.00 | 1.63 | 64 |
| 50% SWC | 0.76*** ± 0.10 | 0.37*** ± 0.04 | 0.13*** ± 0.02 | 2.05 | 1.26 | 49 |
| 50% SWC + 50 µM SA | 1.44 ± 0.19 | 0.68 ± 0.05 | 0.19*** ± 0.02 | 2.11 | 2.31 | 90 |
| 50% SWC + 10 mM $KNO_3$ | 1.26 ± 0.27 | 0.61 ± 0.13 | 0.14*** ± 0.03 | 2.06 | 2.01 | 78 |

* Significant at $P \leqslant 0.05$.
** Significant at $P \leqslant 0.01$.
*** Significant at $P \leqslant 0.001$.

NaCl + 10 mM $KNO_3$ treated plants, the leaf photosynthetic pigment contents (Chl *a*, Chl *b* and carotenoids) significantly increased in comparison with those treated only with 150 mM NaCl. Application of 50 µM SA or 10 mM $KNO_3$ to plants treated with 50% SWC caused greater increase in pigment contents than those of the 50% SWC treated plants. The Chl *a*/chl *b* ratio increased with increasing salt and water deficit stresses as well as with the combination treatments.

### 3.3. Determination of proline content

Proline content in barley leaves was markedly increased in salt and water deficit stressed plants in comparison with that of the control plants (Fig. 3). This increase in proline content was concomitant with increasing salt doses and water deficit level treated plants. On this, the proline content was 1.60 mg $g^{-1}$ DW for the control and 2.78 mg $g^{-1}$ DW for 150 mM NaCl treated plant. Spraying of 50 µM SA or addition of 10 mM $KNO_3$ to plants treated with 150 mM NaCl decreased proline content in comparison with that treated only with 150 mM NaCl. The proline content (Fig. 3) was 5.40 mg $g^{-1}$ DW for 50% SWC-treated plants (337% of the control). Spraying of 50 µM SA to barley leaves or addition of 10 mM $KNO_3$ to soil grown plants subjected to water deficit (50% SWC) decreased proline content relative to that of 50% SWC treated plants. On the other side, plants sprayed with 50 µM SA resulted in 16% increase in the proline content relative to that of the control plants.

### 3.4. Determination of soluble protein and carbohydrate contents

The soluble protein of barley leaves was slightly changed in response to salt and water deficit stresses as well as with the combination treatments (Table 3). The soluble protein content was 45.26 mg $g^{-1}$ DW for the control plants and 49.58 mg $g^{-1}$ DW for 150 mM NaCl treated plants. With effect of 50% SWC treated plants, the soluble protein content was 49.32 mg $g^{-1}$ DW (Table 3). Application of SA or $KNO_3$ to plants treated with 150 mM NaCl or water deficit (50% SWC) did not affect soluble protein content.

In case of carbohydrate, spraying of 50 µM SA to the control plants increased leaf soluble carbohydrate content by 5%. With salt stress, the soluble carbohydrate content of barley leaves was significantly increased with increasing salt doses (Table 3). The soluble carbohydrate content of leaves was 70.41 mg $g^{-1}$ DW for the control plants and 94.84 mg $g^{-1}$ DW for 150 mM NaCl treated plants (Table 3). Spraying of 50 µM SA or addition of 10 mM $KNO_3$ to plants treated with 150 mM NaCl decreased leaf soluble carbohydrate content in comparison with that of 150 mM NaCl only treated plants. Increasing levels of water deficit stress significantly increased leaf soluble carbohydrate content (Table 3). On the percentage basis calculation, the soluble carbohydrate content of barley leaves subjected to water deficit of 50% SWC increased by 32% relative to that of the control plants. Spraying of 50 µM SA or 10 mM $KNO_3$ to barley plants treated with 50% SWC decreased soluble carbohydrate in comparison with that treated only with 50% SWC.

### 3.5. Determination of lipid peroxidation

The oxidative damage of lipids was evaluated by measuring the changes in malondialdehyde (MDA) content. Lipid peroxidation content was determined to assess the membrane damage after salt and drought stress as well as the combination stress treatments. Spraying of 50 µM SA decreased MDA by 11%

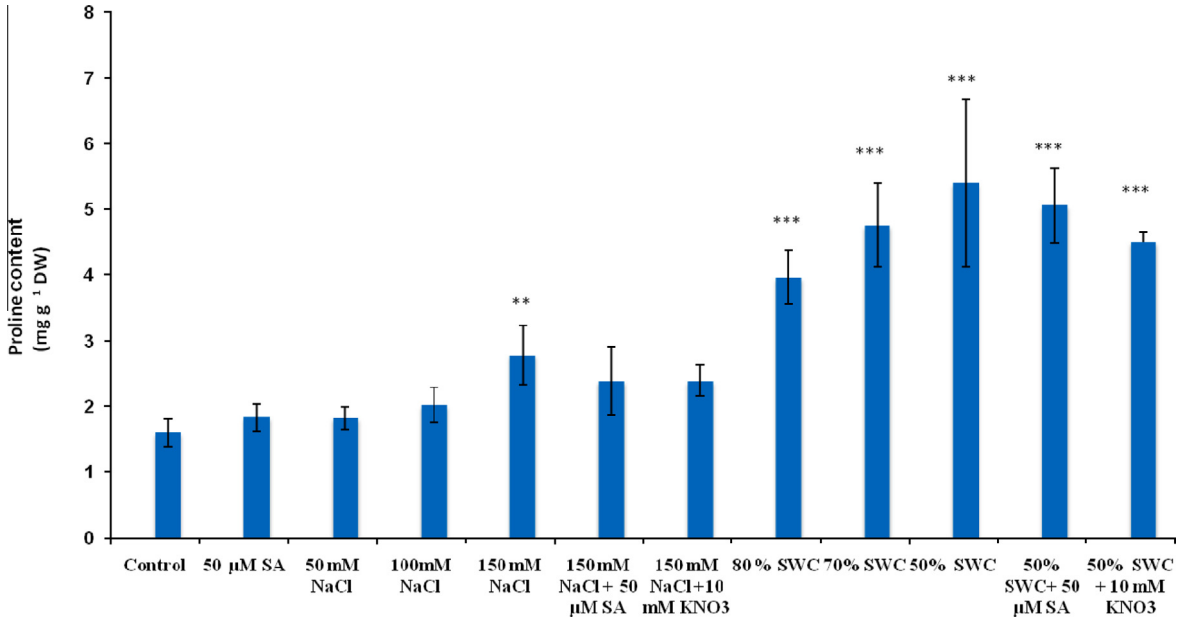


**Figure 3** Effect of salt stress (50, 100 and 150 mM NaCl), water deficit stress (80, 70 and 50% SWC), 50 lM SA and the combination of 150 mM NaCl + 50 µM SA, 150 mM NaCl + 10 mM $KNO_3$, 50% SWC + 50 µM SA and 50% SWC + 10 mM $KNO_3$, on proline content (mg $g^{-1}$ DW) of barley leaves (*Hordeum vulgare* L. cv. Gustoe) relative to the control. Values are means (M) of three replicates ± standard deviation (SD). For a given date, statistically significant of differences relative to the value of control plants was conducted. $^{*}$significant at $P \leqslant 0.05$; $^{**}$significant at $P \leqslant 0.01$; $^{***}$significant at $P \leqslant 0.001$.

**Table 3** Effect of salt stress (50, 100 and 150 mM NaCl), water deficit stress (80%, 70% and 50% SWC), 50 μM SA and the combination of 150 mM NaCl + 50 μM SA, 150 mM NaCl + 10 mM $KNO_3$, 50% SWC + 50 μM SA and 50% SWC + 10 mM $KNO_3$ on soluble protein and carbohydrate contents (mg $g^{-1}$ DW) of barley leaves (*Hordeum vulgare* L. cv. Gustoe) relative to the control. Values are means (M) of three replicates ± standard deviation (SD). For a given date, statistically significant of differences compared to the value of control plants was conducted.

| Treatments | Soluble protein mg $g^{-1}$ DW | | Soluble carbohydrate mg $g^{-1}$ DW | |
|---|---|---|---|---|
| | M ± SD | % | M ± SD | % |
| Control | 45.26 ± 4.03 | 100 | 70.41 ± 8.17 | 100 |
| 50 μM SA | 48.58 ± 3.98 | 107 | 74.15 ± 8.78 | 105 |
| 50 mM NaCl | 44.40 ± 6.50 | 98 | 78.53 ± 7.09 | 111 |
| 100 mM NaCl | 48.09 ± 7.20 | 106 | 87.01** ± 5.00 | 123 |
| 150 mM NaCl | 49.58 ± 6.04 | 109 | 94.84*** ± 8.44 | 135 |
| 150 mM NaCl + 50 μM SA | 41.21 ± 2.62 | 91 | 90.42** ± 3.77 | 128 |
| 150 mM NaCl + 10 mM $KNO_3$ | 45.74 ± 4.03 | 101 | 88.18** ± 10.82 | 125 |
| 80% SWC | 45.78 ± 4.75 | 100 | 73.04 ± 4.73 | 104 |
| 70% SWC | 49.78 ± 5.37 | 110 | 88.06** ± 6.59 | 125 |
| 50% SWC | 49.32 ± 4.84 | 109 | 92.82*** ± 8.64 | 132 |
| 50% SWC + 50 μM SA | 46.12 ± 1.17 | 102 | 81.35 ± 7.35 | 115 |
| 50% SWC + 10 mM $KNO_3$ | 46.00 ± 2.88 | 102 | 82.11 ± 4.90 | 117 |

* Significant at $P \leqslant 0.05$.
** Significant at $P \leqslant 0.01$.
*** Significant at $P \leqslant 0.001$.

**Table 4** Effect of salt stress (50, 100 and 150 mM NaCl), water deficit stress (80%, 70% and 50% SWC), 50 μM SA and the combination of 150 mM NaCl + 50 μM SA, 150 mM NaCl + 10 mM $KNO_3$, 50% SWC + 50 μM SA and 50% SWC + 10 mM $KNO_3$ on MDA content (μg $g^{-1}$ FW) of barley leaves (*Hordeum vulgare* L. cv. Gustoe) relative to the control. Values are means (M) of three replicates ± standard deviation (SD). For a given date, statistically significant of differences compared to the value of control plants was conducted.

| Treatments | MDA (μg $g^{-1}$ FW) | |
|---|---|---|
| | M ± SD | % |
| Control | 12.17 ± 1.58 | 100 |
| 50 μM SA | 10.81 ± 0.51 | 89 |
| 50 mM NaCl | 13.67 ± 2.22 | 112 |
| 100 mM NaCl | 14.89* ± 2.20 | 122 |
| 150 mM NaCl | 17.02*** ± 1.76 | 140 |
| 150 mM NaCl + 50 μM SA | 15.53* ± 1.30 | 127 |
| 150 mM NaCl + 10 mM $KNO_3$ | 13.54 ± 0.98 | 111 |
| 80% SWC | 14.40 ± 1.09 | 118 |
| 70% SWC | 14.95* ± 1.02 | 123 |
| 50% SWC | 19.28*** ± 0.79 | 158 |
| 50% SWC + 50 μM SA | 16.37** ± 2.24 | 134 |
| 50% SWC + 10 mM $KNO_3$ | 14.62 ± 1.64 | 120 |

* Significant at $P \leqslant 0.05$.
** Significant at $P \leqslant 0.01$.
*** Significant at $P \leqslant 0.001$.

in compassion to the untreated barley plant decreased (Table 4). With increasing salt doses and water deficit stress, the MDA content significantly increased. The values of the MDA content were 12.17 for the control plant, 17.02 for 150 mM NaCl treated plant and 19.28 μg $g^{-1}$ FW for 50% SWC treated plants. On the basis of percentage, the increase in MDA content was 40% and 58% for plants treated with 150 mM NaCl and 50% SWC, respectively, relative to that of the control plants. Spraying of 50 μM SA to 150 mM NaCl or 50% SWC treated plants displayed reduction in MDA content in comparison with those of 150 mM NaCl or 50% SWC treated plants. In case of addition of 10 mM $KNO_3$ to 150 mM NaCl or 50% SWC treated plants, the reduction in MDA content was more obvious in comparison with those of plants treated only with 150 mM NaCl or 50% SWC (Table 4).

### 3.6. Determination of total phenolic compounds

Spraying of 50 μM SA to untreated plants increased phenolic compounds of barley leaves by 7% relative to that of the control plants. All treatments used in the experiment showed an increase in phenolic compounds relative to the control plants (Table 5). With increasing salt or water deficit stresses, the total phenolic compounds increased. Phenolic contents of control were 14.73, 20.10 for 150 mM NaCl treated plants (136% of the control), and 23.26 μg $g^{-1}$ FW for water deficit 50% SWC treated plants (158% of the control). Application of SA or $KNO_3$ to plants treated with 150 mM NaCl did not affect phenolic compound contents.

### 3.7. Estimation of total antioxidant activity by using DPPH scavenging assay

Table 5 shows the effect of various treatments on the total antioxidant activity (TAA) of barley leaf extract by using DPPH free radical scavenging assay. Most treatments induced formation of antioxidants and then improved the antioxidant status of leaves (Table 5). Spraying of 50 μM SA to the untreated plants increased antioxidant activity of leaf extracts (36.19%) in comparison to that of the control (32.44%)

**Table 5** Effect of salt stress (50, 100 and 150 mM NaCl), water deficit stress (80%, 70% and 50% SWC), 50 μM SA and the combination of 150 mM NaCl + 50 μM SA, 150 mM NaCl + 10 mM $KNO_3$, 50% SWC + 50 μM SA and 50% SWC + 10 mM $KNO_3$ on total phenolic content (μg $g^{-1}$ FW) and DPPH assay (%) of barley leaves (*Hordeum vulgare* L. cv. Gustoe) relative to the control. Values are means (M) of three replicates ± standard deviation (SD). For a given date, statistically significant of differences compared to the value of control plants was conducted.

| Treatments | Phenolic compounds (μg $g^{-1}$ FW) | | DPPH assay (%) |
|---|---|---|---|
| | M ± SD | % | M ± SD |
| Control | 14.73 ± 2.25 | 100 | 32.44 ± 2.08 |
| 50 μM SA | 15.76 ± 1.10 | 107 | 36.19 ± 3.27 |
| 50 mM NaCl | 16.48 ± 0.90 | 112 | 39.43 ± 5.39 |
| 100 mM NaCl | 17.73* ± 1.50 | 120 | 55.47*** ± 4.51 |
| 150 mM NaCl | 20.10*** ± 1.82 | 136 | 62.15*** ± 3.96 |
| 150 mM NaCl + 50 μM SA | 19.48*** ± 1.20 | 132 | 56.68*** ± 4.59 |
| 150 mM NaCl + 10 mM $KNO_3$ | 20.33*** ± 2.44 | 138 | 58.16*** ± 3.40 |
| 80% SWC | 16.20 ± 1.83 | 110 | 42.32* ± 3.33 |
| 70% SWC | 19.61*** ± 1.42 | 133 | 52.34*** ± 3.40 |
| 50% SWC | 23.26*** ± .642 | 158 | 60.73*** ± 2.95 |
| 50% SWC + 50 μM SA | 22.60*** ± 1.21 | 153 | 58.20*** ± 5.52 |
| 50% SWC + 10 mM $KNO_3$ | 19.80*** ± 1.31 | 134 | 52.06*** ± 8.44 |

* Significant at $P \leqslant 0.05$.
** Significant at $P \leqslant 0.01$.
*** Significant at $P \leqslant 0.001$.

**Table 6** Effect of salt stress (50, 100 and 150 mM NaCl), water deficit stress (80%, 70% and 50% SWC), 50 μM SA and the combination of 150 mM NaCl + 50 μM SA, 150 mM NaCl + 10 mM $KNO_3$, 50% SWC + 50 μM SA and 50% SWC + 10 mM $KNO_3$ on sodium and potassium contents of barley leaves (*Hordeum vulgare* L. cv. Gustoe) relative to the control. Values are means (M) of three replicates ± standard deviation (SD). For a given date, statistically significant of differences compared to the value of control plants was conducted.

| Treatments | $Na^+$ contents (mg $g^{-1}$ DW) | | $K^+$ contents (mg $g^{-1}$ DW) | | $Na^+/K^+$ ratio |
|---|---|---|---|---|---|
| | M ± SD | % | M ± SD | % | |
| Control | 11.08 ± 0.75 | 100 | 35.81 ± 2.92 | 100 | 0.309 |
| 50 μM SA | 11.67 ± 0.56 | 105 | 32.08 ± 1.37 | 89 | 0.363 |
| 50 mM NaCl | 14.83** ± 0.76 | 134 | 30.91 ± 5.21 | 86 | 0.479 |
| 100 mM NaCl | 17.02*** ± 1.00 | 154 | 27.73** ± 2.59 | 77 | 0.613 |
| 150 mM NaCl | 19.75*** ± 0.49 | 178 | 19.75*** ± 3.13 | 55 | 1.000 |
| 150 mM NaCl + 50 μM SA | 18.17*** ± 1.67 | 164 | 20.33*** ± 3.16 | 57 | 0.897 |
| 150 mM NaCl + 10 mM $KNO_3$ | 15.83*** ± 1.94 | 143 | 37.28 ± 2.00 | 104 | 0.424 |
| 80% SWC | 12.46 ± 1.76 | 112 | 32.80 ± 2.22 | 91 | 0.379 |
| 70% SWC | 12.50 ± 2.53 | 113 | 35.41 ± 2.40 | 99 | 0.353 |
| 50% SWC | 13.66* ± 0.57 | 123 | 28.15** ± 3.22 | 78 | 0.485 |
| 50% SWC + 50 μM SA | 13.36* ± 0.87 | 120 | 33.75 ± 4.25 | 94 | 0.395 |
| 50% SWC + 10 mM $KNO_3$ | 12.53 ± 0.50 | 113 | 40.16 ± 2.74 | 112 | 0.312 |

* Significant at $P \leqslant 0.05$.
** Significant at $P \leqslant 0.01$.
*** Significant at $P \leqslant 0.001$.

indicating an increase in the amounts of antioxidant compounds. With increasing salt and water deficit stresses, the total antioxidant activity was significantly increased. For example, the 150 mM NaCl or water deficit of 50% SWC treated plants caused the highest increase in TAA (62.15% and 60.73%, respectively) of leaves relative to that of the control which captured only 32.44% (Table 5). Spraying of 50 μM SA or addition of 10 mM $KNO_3$ to plants treated with 150 mM NaCl or 50% SWC displayed reductions in antioxidant values relative to those of salt or water deficit stressed plants (Table 5).

### 3.8. Determination of sodium and potassium contents

Increasing salt stress increased $Na^+$ content of barley leaves. $Na^+$ content was 11.08 mg $g^{-1}$ DW for the control and 19.75 mg $g^{-1}$ DW for 150 mM NaCl treated plants (Table 6). In contrast, $K^+$ content of leaves was decreased with increasing NaCl doses. This resulted in increased $Na^+/K^+$ ratios salt and water deficit treated plants. Application of 50 μM SA or 10 mM $KNO_3$ to plants treated with 150 mM NaCl resulted in reduction of $Na^+$ content compared to that of plants treated with 150 mM NaCl. However, the $Na^+$ content was still

higher than that of the untreated plants. Also, water deficit stressed plants increased $Na^+$ and decreased $K^+$ contents (Table 6). Spraying of 50 μM SA or 10 mM $KNO_3$ to the water deficit (50% SWC) treated plants decreased $Na^+$ and increased $K^+$ content of leaves (Table 6). The $Na^+/K^+$ ratios in 150 mM NaCl or 50% SWC water deficit stressed plants decreased due to application of 50 μM SA or 10 mM $KNO_3$.

## 4. Discussion

The environmental condition in Middle East countries is characterized by drought and soil salinity resulting from scarcity of rain which influences negative growth and productivity of crop plants. Barley is an important crop cultivated in Saudi Arabia. One of the approaches to improve growth and productivity of crop plants under water deficit and soil salinity is the hormonal and fertilizer treatments which are capable to withstand unfavorable environmental conditions. To resist or avoid stress conditions, plants have evolved complex mechanisms to counter NaCl toxicity and low water potential in soil caused by salinity (Munns and Tester, 2008) or drought (Vaseva et al., 2012). Salt stress induced inhibition on plant growth could be attributed to specific ion toxicity, disturbance in homeostasis of $Na^+$ and $Cl^-$ ions, stomatal closure, and the increased production of ROS in chloroplasts (Meneguzzo et al., 1999; Steduto et al., 2000; Gunes et al., 2007; Daneshmand et al., 2010). In the present study, salt and water deficit stress treatments decreased shoot fresh weight, plant height, photosynthetic pigments and potassium content while increased the contents of proline, soluble carbohydrate, MDA, total phenolic compounds, antioxidant activity and sodium. It was observed that foliar application of SA or addition of $KNO_3$ increased the plant growth, photosynthetic pigment contents, potassium content and decreased sodium content in stressed barley plants. Foliar application of SA reduced the damaging effect of salinity on plant growth and accelerated the restoration of growth processes and reversed the effects of salinity (Idrees et al., 2011). Hussain et al. (2008) also found that exogenous SA application was very effective in reducing adverse affects of drought stress in sunflower. Exogenous application of potassium salts alleviated the adverse effects of salt stress in winter wheat (Zheng et al., 2008), and improved the growth, enhanced photosynthetic capacity, water use efficiency, leaf turgor and relative water content of stressed sunflower (Akram et al., 2009). The protective role of SA in membrane integrity and regulation of ion uptake has also been reported (El-Tayeb, 2005; Erasalan et al., 2007; Gunes et al., 2007).

In the present study, saline and water deficit stresses significantly led to a remarkable decrease in the chl *a*, *b*, and carotenoid contents (Table 2). Exogenous spraying of SA or addition of $KNO_3$ successfully ameliorated leaf chlorophyll and carotenoid contents of plant grown in salt and water stresses. Very severe drought conditions result in limited photosynthesis due to a decline in Rubisco activity and reduced gas exchange (Bota et al., 2004). Similarly, Idrees et al. (2010) reported that SA protected photosynthesis and enhanced Rubisco activity in water stress treated wheat. Application of SA or $KNO_3$ to salt and water stress treated barley plants enhanced the contents of photosynthetic pigments. This enhancement in pigments was greater in water stress treated plant relative to salt treated plants. The decrease $Na^+$ and increase $K^+$ in stressed barley plants in response to application of SA or $KNO_3$ may be caused maintenance of photosynthesis.

ROS act as a signal molecule and plants initiate antioxidant mechanism for protection against ROS (Khan et al., 2003). In the present study, maximum oxidative stress (as content of MDA and total antioxidant activity) was observed in barley under both stresses. The application of SA or $KNO_3$ decreased MDA and total antioxidant activity under both stresses. Proline accumulation in response to abiotic stresses is widely reported, and may play a role in plant stress adaptation within the cell (Fayez, 2000; Ashraf and Foolad, 2007; Shahbaz et al., 2011; Sperdouli and Moustakas, 2012). Salt stress and water deficit resulted in a significant accumulation of proline in barley leaves (Fig. 3). Depending on these possible mechanisms by which proline protects plant against abiotic stress, our results suggested that the increase of proline in stressed barley plants may be at least partially responsible for the alleviated lipid peroxidation and photosynthetic pigments. Of the several biochemical indices of water deficit injury, proline accumulation and decline in protein synthesis in plants have been reported (Irigoyen et al., 1992). However, in the present study, soluble protein content of stressed barley plants as well as with the combination treatments, to some extent, was unaffected (Table 3).

Salt and water deficit stresses caused a significant increase in soluble carbohydrate content of barley leaves. With increasing salt doses, the rate of increase in soluble carbohydrate content was increased, indicating a role of soluble carbohydrate in the osmotic adjustment. The accumulation of sugars in plants under stress conditions might be involved in the osmotic adjustment was reported (Cheeseman, 1988; Pérez-López et al., 2010). Interestingly, application of SA and $KNO_3$ to water stressed plants (50% SWC) decreased soluble carbohydrate in comparison to plants subjected only to water deficit of 50% SWC. This decrease in soluble carbohydrate was concomitant with decrease $Na^+$ and increase $K^+$ contents.

The salt stress and water deficit caused lipid peroxidation (measured as MDA) in barley leaves. The level of MDA is often used as an indicator of oxidative damage due to enhanced generation of ROS (Mittler, 2002; Miller et al., 2010). The increase in membrane damage (lipid peroxidation) with increasing water stress levels has been also reported in wheat (Ezzat-Ollah et al., 2007; Hameed et al., 2011). The application of $KNO_3$ to plants treated with 150 mM NaCl or water deficit (50% SWC) maintained the value of MDA content around that of the untreated plants, suggesting a role for $K^+$ in enhancing antioxidant system under stress conditions.

Under salt and water deficit stresses, the content of phenolic compounds was increased in barley leaves (Table 5). Our results are in conformity with previous studies in cucumber (Tiwari et al., 2010), wheat (Keles and Oncel, 2004) and potato (Daneshmand et al., 2010). Application of SA and $KNO_3$ to salt or water stressed plants displayed reductions in phenolic compounds. Phenolic compounds can act as antioxidant to scavenge ROS in plants under stresses (Solecka, 1997). The results showed improved antioxidant status indicating formation of antioxidants in all treatments. Salt and water stresses significantly increased the TAA of barley leaves, which increased with increasing the stress. Application of SA and $KNO_3$ to salt or water deficit stressed plants decreased the TAA of barley leaves, suggesting a decrease in oxidative stress which probably resulted from the decrease in $Na^+$ and this may enhance the

stress tolerance of barley. In previous studies, it was suggested that SA acts as an antioxidant (Erasalan et al., 2007). In the present study, SA or $KNO_3$ displayed reduction in the TAA in stressed barley plants, suggesting their ability for acting as indirect antioxidants by reducing $Na^+$ entry into the cell. Antioxidant activity mainly depends on the dissociation of hydrogen radical from phenolic compounds to form a stable compound with DPPH radical (Viljanen, 2005).

Salinity stress caused a significant increase in $Na^+$ content and a considerable decrease in $K^+$ content, resulting in a significant increase in the $Na^+/K^+$ ratio (Table 6). The $Na^+$ content was increased in barley leaves with increasing NaCl doses and water deficit. In contrast, the $K^+$ content decreased with increasing NaCl doses. According to Blumwald et al. (2000), the decrease in $K^+$ concentration due to NaCl may be attributed to a high external $Na^+$ concentration. Wakeel et al. (2011) suggested that the $Na^+$ toxicity affects plant growth, increased $Na^+/K^+$ ratio and thus displacement of $K^+$ by $Na^+$ in the plant cell affects the activity of plasma membrane (PM) $H^+$-ATPase. Addition of $K^+$ to NaCl and water deficit stressed plants reduced the $Na^+$ and increased $K^+$ content within leaves. $Na^+/K^+$ ratio increased with increasing salt doses. The elevation of $KNO_3$ concentration in the saline nutrient solution has been proven to be effective in increasing $K^+/Na^+$ ratio in barley leaves.

## 5. Conclusions

In conclusion, the results of this study signify the role of SA and $K^+$ in regulating the salt and water stress response of barley, and suggest that SA or $KNO_3$ acts as a potential growth enhancer to improve plant growth, photosynthetic pigment content and reduce $Na^+$ uptake. SA and $KNO_3$ provoked reduction in oxidative stress in plants subjected to salt and water deficit stresses. In the present study we concluded that soluble carbohydrate plays an important role in the osmotic adjustment in stressed plants. SA and $KNO_3$ can help reduce the adverse effects of salt and drought and may increase the barley growth, enhance antioxidant activity and $K^+$ content in stressed plants and thus protecting membrane against oxidative stress. This protective effect led to a decrease in the ratio of $Na^+/k^+$ in barley leaves, which is a critical determinant under salt and water deficit stresses. These results indicate that barley could be possibly cultivated in moderate saline and drought stressed soils due to its capacity for osmotic adjustment.

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