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Full Length Research Paper

Evaluation of salicylic acid (SA) application on growth, osmotic solutes and antioxidant enzyme activities on broad bean seedlings grown under diluted seawater

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Exogenously applied salicylic acid has been shown to be an essential signal molecule involved in both local defense reactions and induction of systemic resistance response of plants after salt stress. Our study was aimed at evaluating the foliar spray with different levels (0.0, 0.5 and 1.0 mM) of salicylic acid (SA) on broad bean (*Vicia faba* L) seedlings grown under diluted seawater on growth and some related physiological responses. Seawater irrigation negatively affected growth parameters, free amino acids and K^+, while the contents of soluble sugars and protein, proline, Cl^-, Na^+, ion leakage, lipid peroxidation and antioxidant enzyme activities were significantly increased. Foliar spray with SA improved all growth parameters and increases the activities of antioxidant enzymes. On the other hand, plants treated with SA had lower Cl^- and Na^+, while K^+ had a reverse pattern. Based on our findings, the effectiveness of SA in inducing seawater stress tolerance depends upon the concentration of SA applied. The inducer effect of SA was greater with 1 than 0.5 mM treatment. This effect includes the stimulation of antioxidant enzyme activities and regulation of osmotic adjustment through accumulation of osmotic solutes and regulation of absorption and distribution of inorganic ions.

Key words: Antioxidant enzyme activities, inorganic ions, proline.

INTRODUCTION

There is increasing consciousness among agricultural scientists and planners for using seawater to irrigate crops (Liu et al., 2003). Soil salinity under the influence of seawater stress irrigation decreases crop yield through increasing osmotic stress on the plant (Azooz and Al-Fredan, 2009). The response of plants to excess salinity is complex and involves changes in morphology, physiology and metabolism (Parida and Das, 2005). At the whole plant level, the effect of stress is usually perceived as a decrease in photosynthesis and growth associated with alteration in carbon and nitrogen metabolism (Mwanamwenge et al., 1999). The maintenance of plant growth and development in saline environments is associated with osmotic adjustment, synthesis of compatible osmolytes and accumulation of Na^+ in the vacuoles (Ramani et al., 2006). Proline, amino acids, soluble protein and carbohydrate compounds, among the most important compatible solutes, play an important role in plant salt tolerance by osmotic adjustment (Munns, 2002; Ashraf and Harris, 2004; Azooz, 2009).

Under salt stress, osmotic adjustment is usually achieved by the uptake of inorganic ions such as Na^+, Cl^- and K^+ from the growth media (Misra and Gupta, 2006). Accumulation of K^+ and limitation of Na^+ and Cl^- in root than in shoot has been considered a physiological trait indicator for salt tolerance in plants (Azooz et al., 2004; Song et al., 2006; Morsy et al., 2007). The ability of plants to limit Na^+ transport to shoots is important for maintenance of high growth rates and protection of

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metabolic processes from Na^+ toxicity (Razmjoo et al., 2008).

High concentrations of salts in seawater result in membrane injury and loss of permeability (Di Baccio et al., 2004). Salt stress induces oxidative damage to plant cells catalyzed by reactive oxygen species (ROS) (Di Baccio et al., 2004; Azevedo-Neto et al., 2006), that are high when plants are exposed to salinity stress (Jaleel et al., 2008a; Ashraf, 2009). Plant cells posses an antioxidant defense system including catalase (CAT), peroxidase (POD), ascorbate peroxidase (APX) and glutathione reductase (GR), which help in detoxifying ROS (Panda and Upadhyay, 2003; Jaleel et al., 2006).

Salicylic acid (SA) is considered as a hormone-like substance, which plays an important role in the regulation of plant growth and development. Improvement or modification of plant growth and development can occur by the direct application of SA to seeds (Arfan et al., 2007; Azooz and Youssef, 2010). Ion uptake and transport (Wang et al., 2006), photosynthetic rate, membrane permeability and transpiration (Khan et al., 2003) could also be affected by SA application. Khodary (2004) found that SA treatment increased the chlorophyll and carotenoid contents in maize plants.

Exogenously applied SA has been shown to be an essential signal molecule involved in both local defense reactions and induction of systemic resistance response of plants after salt stress (Loake and Grant, 2007). It has been shown that SA treatment increased resistance to abiotic stresses of many crop plants (Wang et al., 2006; Noreen and Ashraf, 2008). Interestingly, the majority of SA-regulated abiotic stresses in plants are related to anti-oxidative responses, suggesting that SA as an internal signal molecule that interacts with reactive oxygen species (ROS) signal pathways and could regulate physiological adaptation to some environmental stresses, including oxidation damage (Borsani et al., 2001). Protection of plants from oxidative damage by SA is associated with an increase in antioxidant enzyme activities and a decrease in the level of ROS and lipid peroxidation (Wang et al., 2006; Shi and Zhu, 2008; Sharhrtash et al., 2011).

Broad bean (*Vicia faba* L.) is the most important legume crop and a major source of protein. Cultivation of broad bean leads to the increase of soil N compounds (Hungria and Vargas, 2000). Several researches showed that SA had a positive role in ameliorating the damaging effects of salt stress in many plant species (Arfan et al., 2007; Noreen and Ashraf, 2008; Nazar et al., 2011; Syeed et al., 2011). The effectiveness of SA in inducing salt stress tolerance depends upon the developmental phase of plants (Borsni et al., 2001) or the concentration of SA applied (Noreen and Ashraf, 2008). Thus, the objective of this work was to provide additional information about the evaluation of foliar spray with different levels (0.0, 0.5 and 1.0 mM) of SA on broad bean plants at early vegetative stage grown under diluted seawater.

MATERIALS AND METHODS

Plant cultivation and treatments

Broad bean (*Vicia faba* L.) seeds were surface sterilized in aqueous solution of 0.1% $HgCl_2$ for 1 min, with frequent shaking and then thoroughly washed three times with distilled water. The washed seeds were planted in 2 L plastic pots (5 seeds/pot), lined with polyethylene bags and filled with soil composed of clay and sand (1:1 by volume). The pots were divided into two groups (18 pots/ group). The first group was irrigated with normal water (0% seawater) to serve as control, and the second group was irrigated with 25% seawater (seawater/normal water). Then, the pots were kept in growth chamber maintained at 22/20 ± 2°C day/night (10/14h) temperature cycles and relative humidity 65% ± 5. When the seedlings were at two-leaf stage, the pots of each group were divided into three sub-groups (6 replicates for each sub-group). Foliar spraying with distilled water was applied to the seedlings of the first sub-groups, while the seedlings of the second and third groups were sprayed with 0.5 and 1.0 mM SA, respectively. A constant volume (5 ml/ plant) of the SA solution was sprayed on all pots with a manual sprayer (the soil was covered during spraying). Spraying was conducted two times at intervals of 7 days. The test plants were irrigated daily with water to maintain the seawater level used and left to grow for the period of the experiment (21 days).

Harvesting

Plants were uprooted 21 days after cultivation. The harvested plants were washed, fresh weight (FW) determined and roots and shoots separated. Root and shoot lengths were measured. To determine the dry matter, the fresh materials were dried in an aerated oven (Program oven, MOV-313P, Sanyo, Japan) at 80°C until constant weight. The samples were ground into fine powder and stored in sealed glass containers at room temperature until analysis.

Determination of leaf relative water content, ion leakage and Photosynthetic pigments

Leaf relative water content (LRWC) was determined according to Smart (1974). Ion leakage was measured as electrical conductivity (EC %) according to Yan et al. (1996). Photosynthetic pigments (chlorophyll *a*, chlorophyll *b* and carotenoids) were estimated in 80% acetone extracts using the spectrophotometric method according to Linchtenthaler and Wellburn (1983).

Determination of osmotic solutes

Soluble sugars were extracted from plant tissues and determined using the anthrone sulphuric acid method (Fales, 1951); dried tissue of roots and shoots was extracted with distilled water. One ml of the extract was mixed with 9 ml of anthrone sulphuric acid reagent in a test tube and heated for 7 min at 100°C. The absorbance was read spectrophotometrically (Spectronic Genesys ZPC, Rochester, NY, USA) at 620 nm, against a blank containing only distilled water and anthrone reagent. Soluble protein was determined according to Bradford (1976). Free amino acids were determined according to the method of Lee and Takahashi (1966).

Proline was determined by ninhydrin method according to Bates et al. (1973).

Determination of inorganic ions

The inorganic ions (Na^+ and K^+) were determined by flame photometry (Jenway, PFP-7) according to Williams and Twine (1960). Chloride was analyzed by precipitation as AgCl and titration according to Johnson and Ulrich (1959).

Assays of some antioxidant enzyme activities

Enzyme extraction: The samples were prepared as described by Mukherjee and Choudhuri (1983). A leaf sample (0.5 g) was frozen in liquid nitrogen and ground using a porcelain mortar and pestle. The frozen powder was added to 10 ml of 100 mM phosphate buffer (KH_2PO_4 / K_2HPO_4) pH 7.0, containing 0.1 mM Na_2EDTA and 0.1 g of polyvinylpyrrolidone. The homogenate was filtered through cheesecloth, and then centrifuged at x 15,000 *g* for 10 min at 4°C. The supernatant was recentrifuged at 18,000 x *g* for 10 min, and then the resulted supernatant was collected and stored at 4°C for catalase (CAT), peroxidase (POD),ascorbate peroxidase (APX) and glutationer reductase (GR) assays.

Catalase (EC 1. 11. 1. 6) activity was assayed according to Aebi (1984). The activity of catalase was estimated by the decrease of absorbance at 240 nm for 1 min as a consequence of H_2O_2 consumption (Havir and McHale, 1987). Peroxidase (EC 1. 11. 1. 7) activity was determined according to Maehly and Chance (1954) by the oxidation of guaiacol in the presence of H_2O_2. The increase in absorbance due to formation of tetraguaiacol was recorded at 470 nm (Klapheck et al., 1990). The activity of ascorbate peroxidase (EC 1. 11. 1. 11) was assayed according to (Chen and Asada, 1992), by measuring the decrease in absorbance at 290 nm for 1 min caused by ascorbic acid oxidation. Glutathione reductase (EC 1. 6. 4. 2) activity was measured according to Foyer and Halliwell (1976), from the rate of decrease in the absorbance of NADPH at 340 nm.

Determination of lipid peroxidation

Lipid peroxidation level was measured as the content of malonyldialdehyde (MDA) using the thiobarbioturic method (Zhao et al., 1994), and expressed as nmol of MDA formed using an extinction coefficient of 155 mM^{-1} cm^{-1} as nmol (MDA) g^{-1} fresh weight.

Statistical analysis

All data were analyzed statistically by one-way ANOVA. Values in the figures indicate the mean values ± SD based on independent six determinations (*n* =6). Least significant difference (L.S.D) test was used to assess the differences between treatments; $p \leq 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Growth parameters

Growth performance of broad bean (*Vicia faba* L) under the 0 (control) and 25% seawater irrigation was estimated by measuring the lengths and dry weights of roots and shoots, water content (WC), transpiration rate and leaves relative water content (RWC) (Figures 1A to H).

Treatment with 25% seawater irrigation was found to reduce values of growth parameters as compared with those of controls. These parameters were less affected in shoots than in roots. The reduction in root and shoot dry weight (Figures 1B and E) in plants subjected to seawater stress was 15 and 10% lower than the control, respectively. Foliar applications with salicylic acid (SA) at concentrations of 0.5 or 1.0 mM through the early vegetative stage generally increased the values of such growth parameters under 0 or 25% seawater treatments. This increase in growth parameters was appeared to be larger at the higher concentration of SA (1.0 mM) especially under 25% seawater treatment. These results are in agreement with those of Ates and Tekeli (2007) who showed in their studies that salinity caused a marked reduction in dry weight and WC of roots and shoots in Parsian clover. Similar decreases in growth parameters were found in *Salvodora persica* and *Hibiscus sabdariffa L.* under saline conditions (Dagar et al., 2004; Azooz, 2009). This might be due to the toxic effect of seawater salinity or increased crucial osmotic pressure at which the plant would not be able to take up water. Furthermore, seawater salinity may lead to nutrient balance disorders in the root (Salter et al., 2007). Seawater stress exhibited more reduction in growth parameters of roots than shoots. This may be attributed to the higher water content of shoots than those of roots. Foliar application of SA, generally had a positive effect on the growth parameters and water status of roots, shoots and leaves, whereas transpiration rate was decreased compared to the untreated plants irrigated with 0 or 25% seawater (Figure 1H). However, the addition of 0.5 mM SA had no significant effects (compared to controls) on the root and shoot water contents (WC) of the examined plants under 0 and 25% seawater irrigation (Figures 1C and F, respectively). The positive effect of foliar application with SA on the growth parameters and water status has also been reported under stress conditions (Khodary, 2004; Hussein et al., 2007; Erdal et al., 2011). They studied the growth criteria of maize and wheat plants and found that foliar application of SA at different concentrations stimulated the dry weight of roots and shoots of such plants which is consistent with our results in broad bean plant. Several studies have discussed the effect of salt stress on the growth parameters such as El-Tayeb (2005) who observed in his study that RWC of leaves and WC of shoots and roots in barley plant declined under salt stress. However, the SA treatments induced increases in such parameters. On the other hand, Lu et al. (2002) reported that with increasing salinity, leaf water potential and evaporation rate decrease significantly in the *Suaeda salsa* L. with no changes in leaf RWC. The increase in dry weight of roots and shoots of SA treated plants may be explained by an increased efficiency of water uptake as well as a decrease in transpiration rate.

Photosynthetic pigments

A significant decrease in photosynthetic pigment contents of the broad bean plants under the treatment of seawater irrigation was observed. Exogenous application of SA caused in most cases, an increase in chlorophyll *a*, *b*,

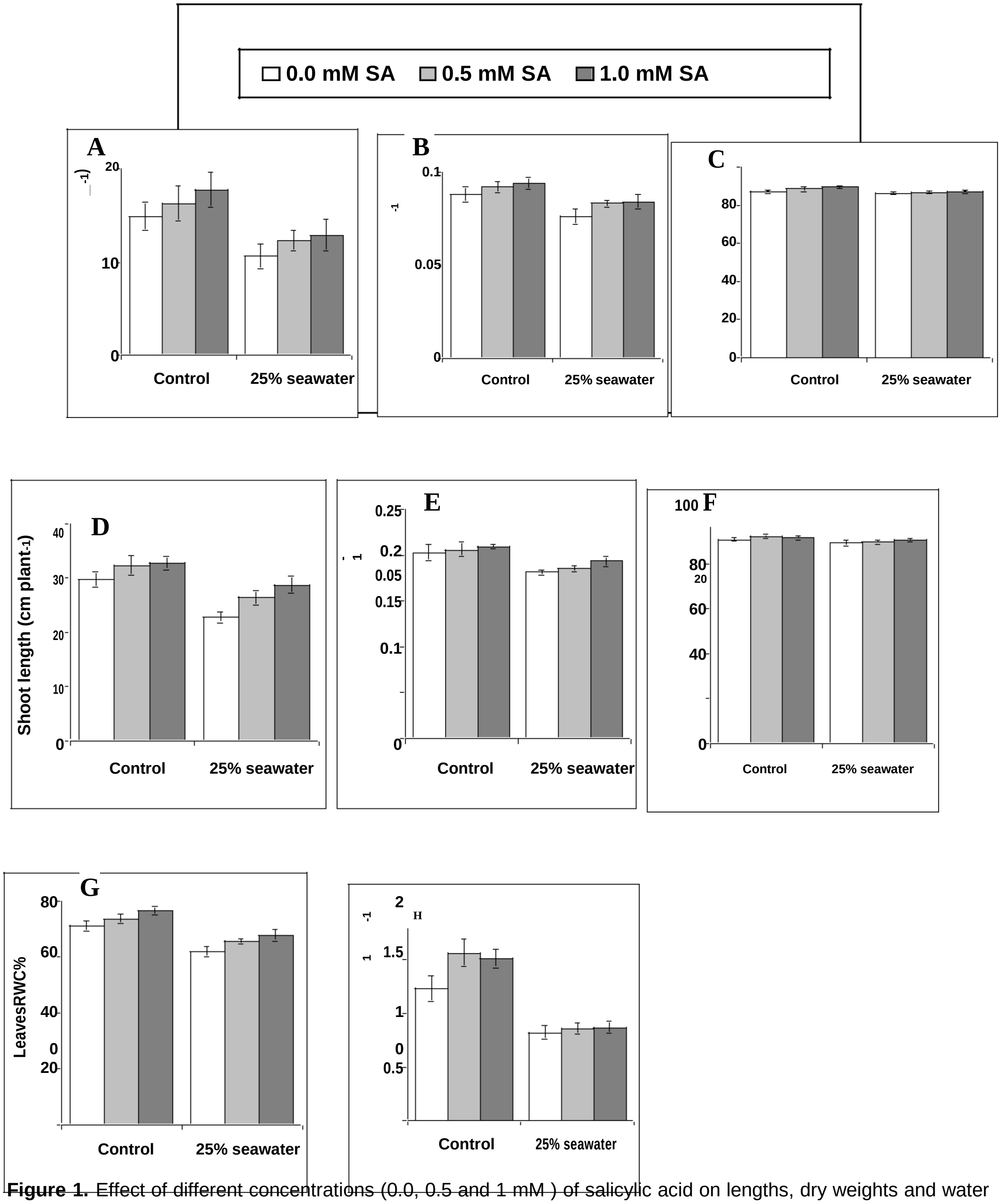

Figure 1. Effect of different concentrations (0.0, 0.5 and 1 mM) of salicylic acid on lengths, dry weights and water content of root (A, B and C) and shoot (D, E and F), leaves relative water content (G) and transpiration (H) of broad bean (*Vicia faba* L.) irrigated with 0.0% (control) or 25% seawater. Vertical bars represent ± SD.

carotenoids and total pigment contents under non-saline and 25% saline conditions, compared with the control (Figures 2A to D). Application of foliar SA at 1.0 mM was found to be more effective in increasing such fractions of pigments either under 0 (control) or 25% seawater application. Results obtained in this study are in agreement with those of Dagar et al. (2004) for *Salvadora persica* and Jaleel et al. (2008a) for *Withania somnifera*. The reduction in leaf pigment contents under salt stress has been attributed to destruction of chlorophyll pigments and the instability of the pigment complex (Jaleel et al., 2008a). It may be also attributed to the interference of salt ions with the *de novo* synthesis of proteins, the structural component of chlorophyll, rather than the breakdown of chlorophyll (El-Tayeb, 2005). Zhou et al. (1999) reported that photosynthetic pigments increased in corn with SA application. Moreover, Khan et al. (2003) showed that SA increased photosynthetic rate in corn

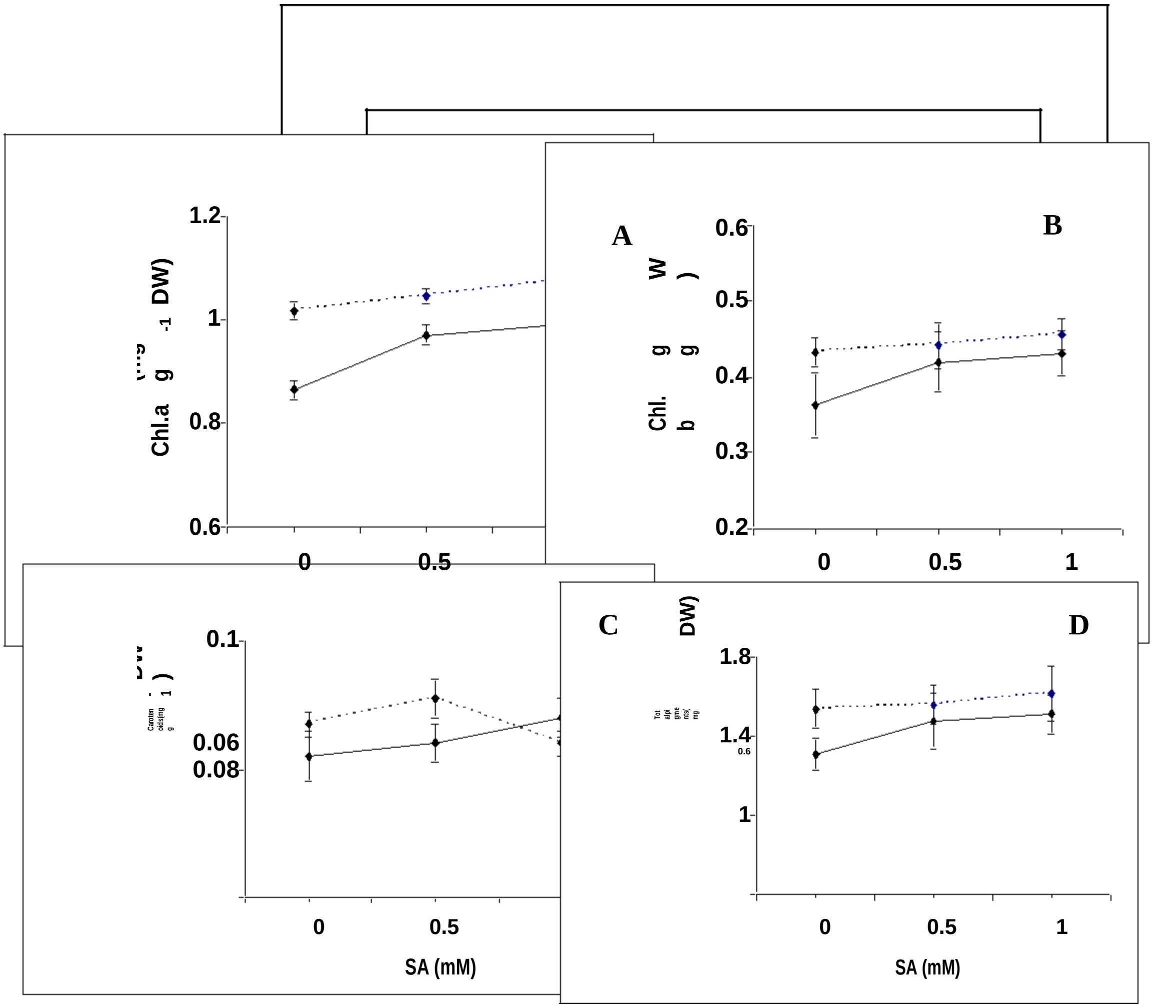

Figure 2. Effect of different concentrations (0.0, 0.5 and 1 mM) of salicylic acid on chlorophyll a (A), chlorophyll b (B), carotenoids (C) and total photosynthetic pigments (D), in leaves of broad bean (*Vicia faba* L.) irrigated with 0.0% (control) or 25% seawater. Vertical bars represent ± SD.

and soybean.

Osmotic solutes

Osmotic solutes (soluble sugars, soluble proteins, free amino acids and proline contents) of *Vicia faba* L. plants grown under seawater treatment and at different application of SA (Figures 3 A to H) showed that, soluble sugars (Figures 3A and B) and proline (Figures 3G and H) contents in roots and shoots respectively, of the examined plants under 25% seawater condition, were significantly increased as compared with the control plants. Furthermore, it was found that foliar application of SA on the tested plants significantly enhanced the contents of soluble sugars and proline in roots and shoots of these plants. The 25% seawater treatments supplemented with the 1.0 mM SA to the plants, have recorded the highest levels of soluble sugars, while the opposite trend was recorded in proline content in roots and shoots of broad bean plants.

Regarding soluble proteins content (Figures 3C and D); the present data revealed that soluble proteins were mostly unaffected in roots, while they were increased in shoots under treatment of 25% seawater irrigation. The accumulation of soluble proteins under such condition was more prominent in shoots (about 2-fold) rather than those attained in roots. Application of SA stimulated the biosynthesis of soluble proteins, especially at 0.5 mM SA, under 25% seawater treatment.

The content of free amino acids (Figures 3E and F) had a reverse pattern to those observed in each of soluble sugars and proline contents. In relation to the free amino acids in roots and shoots of *Vicia faba* plants, there was a decrease in the free amino acids values under the treat-ment of 25% seawater (about 13 and 33%, respectively) lower than those recorded at non-saline condition. Application of the tested concentrations of SA, enhanced the free amino acids content for the examined organs in the broad bean plants under non-saline and saline conditions. It is noticed in the present study that the concentration of the different estimated fraction from the organic solutes was higher in shoots than those in roots at different concentrations of SA and under saline or non-saline conditions.

It is well known that organic solutes play a major role in

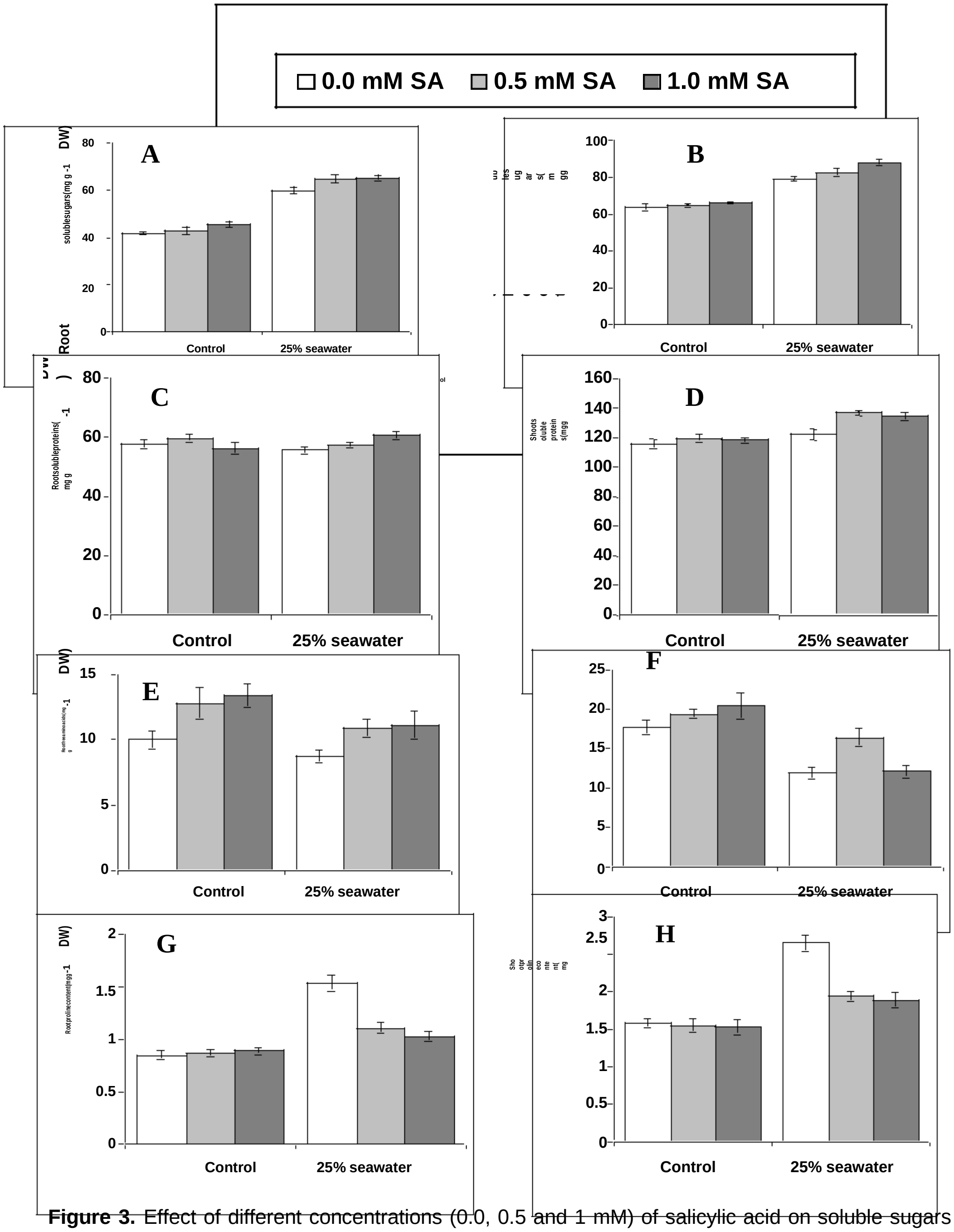

Figure 3. Effect of different concentrations (0.0, 0.5 and 1 mM) of salicylic acid on soluble sugars (A and B), soluble proteins (C and D), free amino acids (E and F) and proline (G and H), in root and shoot respectively of broad bean (*Vicia faba* L.) irrigated with 0.0% (control) or 25% seawater. Vertical bars represent ± SD.

the mitigation of salt stress (Azooz et al., 2004; Misra and Gupta, 2005; Silva et al., 2008). Accumulation of these compatible solutes reduces osmotic potential in the cytoplasm and contributes to maintaining water homeostasis among several cellular compartments (Sairan and Tyagi, 2004). Among all organic solutes, soluble sugars (Ashraf and Harris, 2004) and proline (Azooz 2004; Jaleel et al., 2007a) represent the most osmotically active organic solutes. Higher proline accumulation can be appreciated as a further important factor of adaptation to salinity as reported in number of species (Ashraf and Harris, 2004; Hameed and Ashraf, 2008). In our study, the proline content of seawater stressed plants increased significantly, suggesting an excellent mechanism of plants to decrease the osmotic potential. These results support the hypothesis that proline accumulation is a part of physiological response of the plant to intense stress (Ain-Lhout et al., 2001). The accumulation of proline may be thought an increase in its synthesis constantly with inhibition of its catabolism (Yoshiba et al., 1997; Jaleel et al., 2007b). Our results revealed that seawater salinity significantly decreased the free amino acids content in shoots and roots. This indicates that the accumulation of proline seemed to be at the expense of reduction in the other free amino acids under seawater stress.

A strong correlation between sugar accumulation and salt tolerance has been widely reported (Hameed and Ashraf, 2008). Accordingly, soluble sugars and proteins contents in shoots and roots were much higher than those of free amino acids. Our results suggest a greater participation of soluble sugars and proteins than free amino acids in maintaining water relations in both roots and shoots. Proteins may be increased (Hameed and Ashraf, 2008) or decreased (Jaleel et al., 2008b) under salt stress. However, Bacheller and Romeo (1992) in *Spartina alternifolia* reported no change in soluble proteins under salt stress. This confirms our results which revealed that soluble proteins were increased in shoots, while mostly unaffected in roots under seawater stress.

Foliar application of SA concentrations, significantly increased the contents of soluble sugars, proteins as well as free amino acids, while the proline content was decreased in both roots and shoots as compared with untreated plants either under seawater or non-seawater (control) conditions, suggesting an ameliorative function of SA on seawater toxicity. The accumulation of these osmolytes seems to be correlated with greater tolerance against seawater stress when stressed plants treated with SA. The stimulation effect of SA on the biosynthesis of soluble sugars and proteins was associated to an increase in photosynthetic pigments and consequently the photosynthetic apparatus (Yildirim et al., 2008). The reduction of proline as a result of SA treatments was associated with an increase in soluble proteins. This indicates that foliar spray with SA could stimulate the incorporation of proline into proteins leading to increase of broad bean growth. It can be notable that 1.0 mM SA accumulated lower soluble proteins than 0.5 mM SA in plant roots and shoots. These results imply that effect of SA on these biochemical events is not a general effect of SA and the effectiveness of SA in inducing salt stress tolerance depends upon the concentration of SA applied as reported by Noreen and Ashraf (2008).

Inorganic ions

Concerning the inorganic ions (Figures 4A to H), the results indicated that, Cl^- and Na^+ contents were significantly increased with 25% seawater (Figures 4A to D), whereas the opposite trends was observed for K^+ and K^+/Na^+ ratio (Figures 4E-H) in both roots and shoots. The contents of Cl^- and Na^+ were higher in roots than shoots. SA application decreased the amounts of Cl^- and Na^+ under non-saline or seawater treatments for both plant organs. The reduction was more prominent in root than in shoot, and in plants subjected to seawater stress than those of control. The lowest values of Cl^- (60 and 78%) and Na^+ (74 and 85%) were attained at 1.0 mM SA concentration in roots and shoots, respectively under 25% seawater condition compared with the corresponding SA-untreated plants. The treatments of SA did not significantly influence the K^+ concentration in roots of the plants. However, K^+ concentrations in shoots were highly significant under the application of 1.0 mM SA in both non-saline (30%) and saline (43%) conditions, over the corresponding plants untreated with SA. Foliar spray with SA decreased markedly Na^+ and increased K^+ content leading to consequently increased of K^+/Na^+ ratio in the two organs of control and seawater-stressed plants. K^+/Na^+ ratio were higher in roots and shoots of seawater-stressed plants than plants irrigated with normal water (control).

Seawater salinity enhances the content of Na^+ and Cl^- as reported by Gunes et al. (2007). Soil salinity affects plant growth and development by way of osmotic stress and injurious effects of toxic Na^+ and Cl^- ions caused by the excess of these ions (Sairan and Tyagi, 2004). Therefore, a low rate of Na^+ and Cl^- transport to shoots is one of the important adaptive components of plant to salt stress (Munns, 2002). This explains why the injuries effects of seawater on growth parameters were lower in shoots than in roots of broad bean plants. Our results showed that shoots had the highest K^+/Na^+ than roots in accordance with results obtained by others (Ates and Tekeli, 2007; Lopez et al., 2008). Ashraf and Orooj (2006) reported that the maintenance of higher K^+/Na^+ ratios in shoots of *Trachyspermum ammi* L. could be an important component of its salt tolerance. Gharsa et al. (2008) concluded that the better tolerance of plant to salt stress was primarily due to better K^+ assimilation, resulting in higher K^+/Na^+ ratios. High K^+/Na^+ selectivity in plants under saline conditions is considered as one of the important selection criteria for salt tolerance (Ashraf and Harris, 2004). SA succeeded in reducing the levels of Cl^- and Na^+ and increasing the content of K^+ and K^+/Na^+ ratio as reported by Gunes et al. (2007) and Syeed et al. (2011). This means that foliar spray with SA reduced the toxicity of Cl^- and Na^+ which reflected the positive effect of SA on growth and development of broad bean plants when subjected to seawater stress. Thus, the restricted uptake of Cl^- and Na^+ as a result of SA treatments was increased and consequently maintenance of high K^+/Na^+ ratio in roots and shoots. This indicates that SA could play important role in selectivity of these ions under saline or non-saline conditions. The positive effect of SA on K^+ uptake and inhibitory effect on Na^+ and Cl^- could be responsible for managing of seawater stress on broad bean plant.

Antioxidant enzyme activities

Compared with the control, the 25% seawater treatment significantly increased the activity of different evaluated antioxidant enzyme activities (CAT, POD, APX and GR)

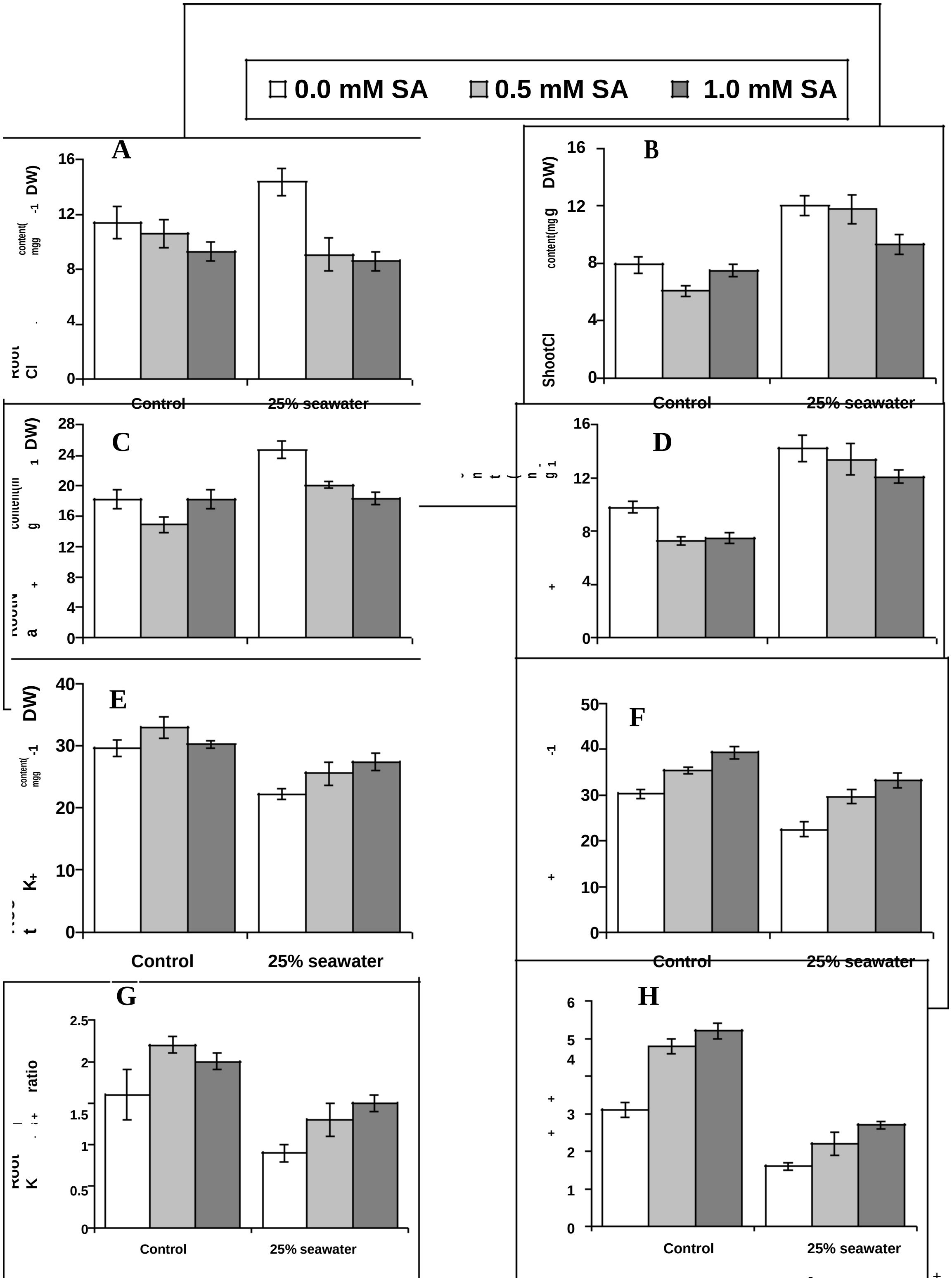

Figure 4. Effect of different concentrations (0.0, 0.5 and 1 mM) of salicylic acid on contents of Cl$^-$ (A and B), Na$^+$ (C and D), K$^+$ (E and F), and K$^+$/Na$^+$ (G and H), of root and shoot respectively of broad bean (*Vicia faba* L.) irrigated with 0.0% (control) or 25% seawater. Vertical bars represent ± SD.

measured in leaves of seawater-treated *Vicia faba* L. (Figures 5A to D). CAT activity of seawater-stressed plants increased in plants treated with seawater alone and in plants with seawater and applied with SA.

However, CAT activity (Figure 5A) was more obvious under the treatment of seawater applied to the plant leaves with 0.5 mM SA. POD activity of seawater-stressed leaves of the examined plants, increased under

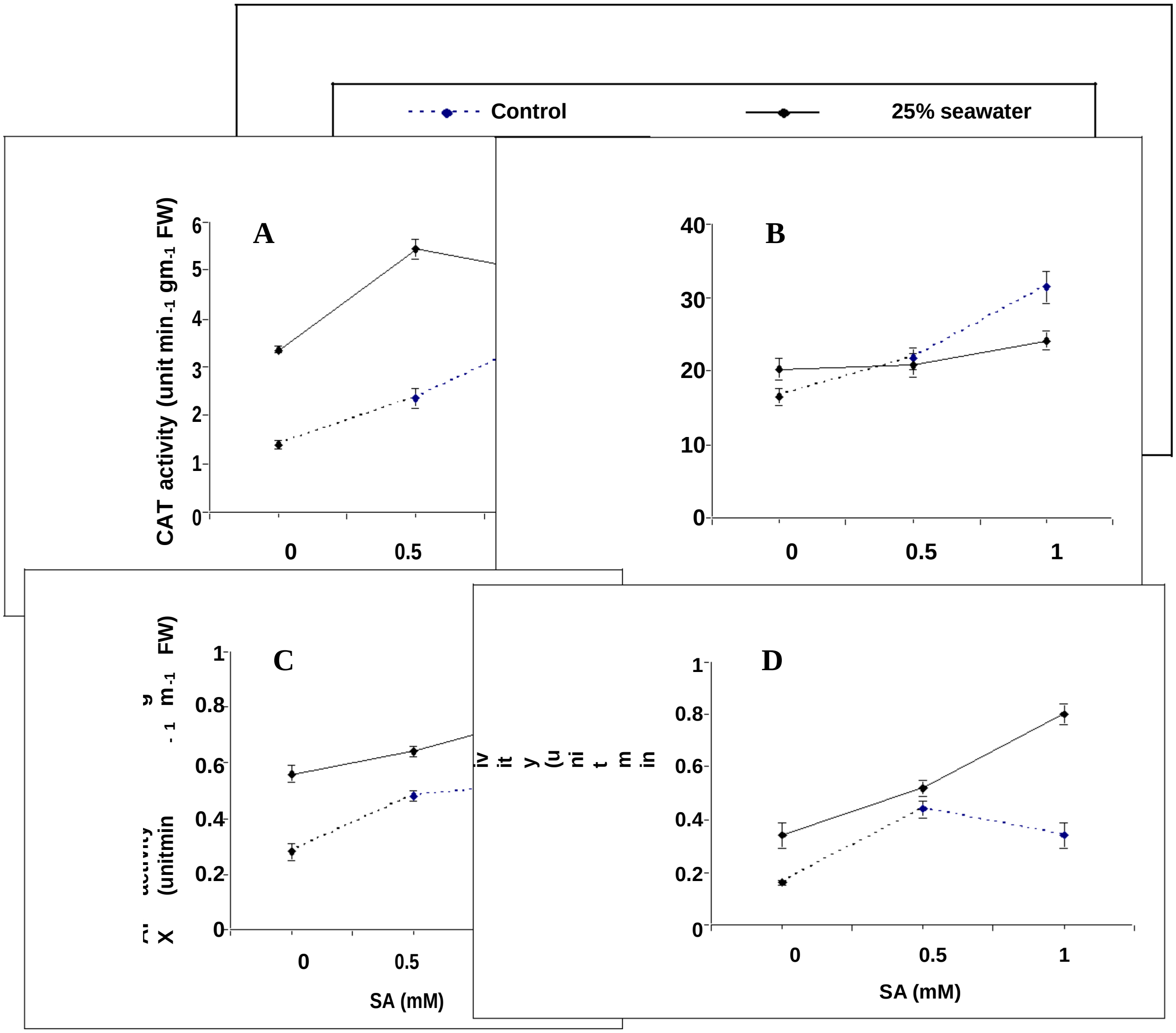

Figure 5. Effect of different concentrations (0.0, 0.5 and 1 mM) of salicylic acid on catalase (CAT) (A), peroxidase (POD) (B), ascorbate peroxidase (APX) (C) and glutathione reductase (GR) (D) in leaves of broad bean (*Vicia faba* L.) irrigated with 0.0% (control) or 25% seawater. Vertical bars represent ± SD.

the application of 1.0 mM SA However, the highest value of POD activity was recorded in the 0.0 seawater treatment at 1.0 mM SA (Figure 5B). The activity of APX and GR in the leaves of *Vicia faba* L. plants was also induced by the treatment of 25% seawater (Figures 5C and D). The activity of both enzymes increased significantly in response to the different application of SA, reached the maximum activity of APX under 1.0 mM SA, whereas the highest activity of GR was recorded at the 0.5 mM SA application. There are reports that salt stress increases the content of H_2O_2 and peroxidation of lipid membrane leading to disrupter of its permeability or induce oxidative damage in plant tissues (Demiral and Turkan, 2005; Jaleel et al., 2008a). Membrane injury under salt stress is related to increased production of high toxic reactive oxygen species (ROS) (Hernández et al., 2000; Khan and Panda, 2008). The higher activity of antioxidant enzymes either in plants irrigated with seawater or in combination with foliar spray with SA is a good implication of plant ability to cope with ROS produced as a result of seawater stress as reported by others in many plant species (Di Baccio et al., 2004; Parida and Das, 2005; Athar et al., 2008; Azooz, 2009). They suggested that salt tolerance could be induced by enhancing antioxidant capacity of plants. Hydrogen peroxide produced during salt stress can easily permeate membranes and can be removed by catalase or peroxidase (Meloni et al., 2003; Khan and Panda, 2008). Bor et al. (2003) reported that increased activity of GR closely related with salt tolerance capacity in sugar beet plant. The enhancement effect of SA on growth parameters could be related to enhanced activity of antioxidant enzyme activities that protect the plants from the oxidative damage (Noreen and Ashraf, 2008; Nazar et al., 2011). Thus, the increases observed in growth parameters of broad bean plants irrigated with seawater when treated with SA may be attributed to the inductive responses of antioxidant enzyme activities, and the protective role of membranes

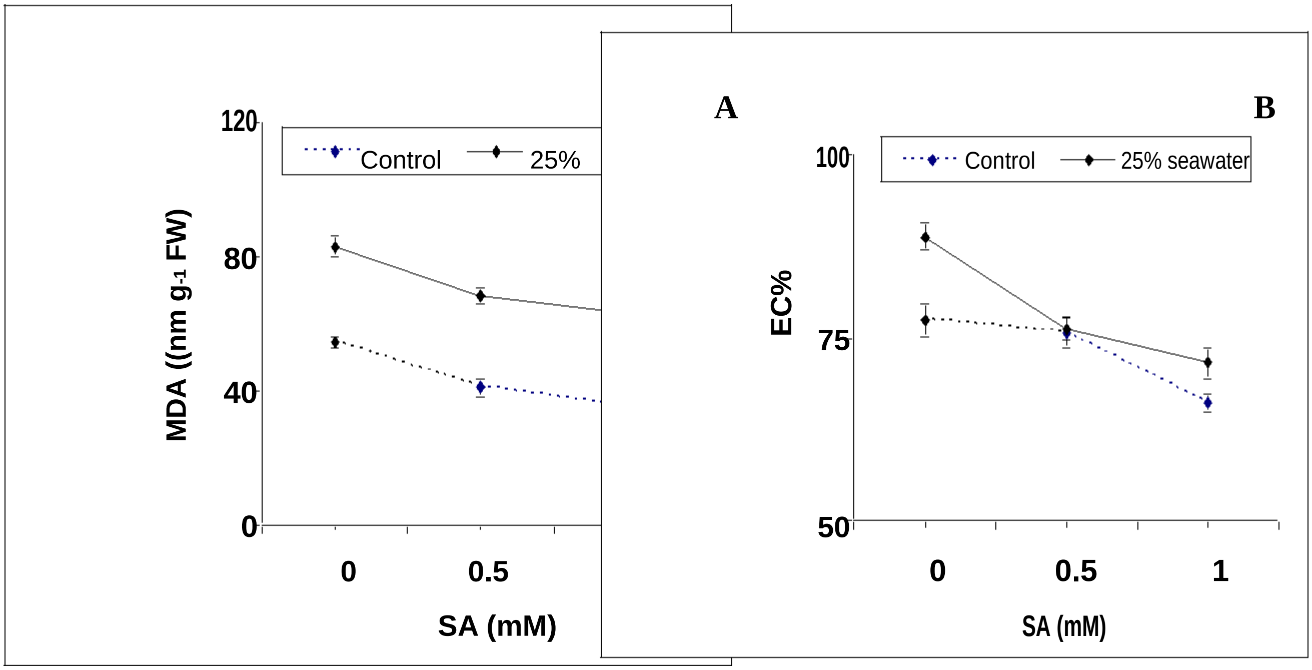

Figure 6. Effect of different concentrations (0.0, 0.5 and 1 mM) of salicylic acid on MDA (A) and electrical conductivity (B) in leaves of broad bean (*Vicia faba* L.) irrigated with 0.0% (control) or 25% seawater. Vertical bars represent ± SD.

that increase the tolerance of plant damage (Gunes et al., 2007). The variations observed in response of antioxidant enzyme activities to the concentrations of SA indicated that, these responses depend upon the concentration of SA applied.

Lipid peroxidation and ion leakage

Lipid peroxidation which measured as MDA and ion leakage which measured as electrical conductivity (EC %) showed that, MDA and EC% levels (Figures 6A and B) significantly increased (about 52 and 15%, respectively) in response to 25% seawater treatment over those of control. However, the applications of SA were effective in decreasing both MDA and EC % in plants under 0 or 25% seawater treatments. The stimulation effect of seawater on the value of EC% and MDA may be attributed to injury of plasma membrane caused by reactive oxygen species which could induce lipid peroxidation (Yin et al., 2008). Reduction of MDA levels and EC % in response to SA application might be related to induction of antioxidant responses that protect the plants from oxidative damage (Afzal et al., 2006; Sharhrtash et al., 2011). The improvement of growth of seawater stressed plants as a result of SA treatments may be related to maintenance of the membrane integrity as well as to the retardation of lipid peroxidation and reduction of membranes injury.

Conclusion

From the above mentioned results, it could be concluded that, seawater stress had inhibitory effects on the growth parameters of broad bean (*Vicia faba* L.) plants. While, osmotic solutes, antioxidant enzyme activities, lipid peroxidation in the different organs of *Vicia faba* L. plants were increased. Foliar spray with different concentration of SA solution could contribute to a reduction of the injurious effect of seawater stress on growth and some related physiological and antioxidant responses of broad bean (*Vicia faba* L.) plants. The protective role of SA mainly includes the stimulation of antioxidant enzyme activities, reducing the level of lipid peroxidatin and ion leakage, regulation of osmotic adjustment through the accumulation of osmotic solutes (soluble sugars, soluble proteins and free amino acids), reducing of proline and regulation the absorption and distribution of inorganic ions (Cl^-, Na^+ and K^+) in seawater-stressed plants. The inorganic contents, osmotic solutes and antioxidant enzymes could be have a specific role in the adjustment of the plant organs toward seawater stress. The effectiveness of SA in inducing seawater stress tolerance depends upon the concentration of SA applied.

REFERENCES

Aebi H (1984). Catalase *in vitro*. Meth. Enzymol., 105: 121-126.

Afzal I, Basara MA, Farooq M, Nawaz A (2006). Alleviation of salinity stress in spring wheat by hormonal priming with ABA, salicylic acid and ascorbic acid. Int. J. Agric. Biol., 8: 23-28.

Ain-Lhout F, Zunzunegui FA, Diaz Barradas MC, Tirado R, Clavijio A, Garcia NF (2001). Comparison of proline accumulation in two Mediterranean shrubs subjected to natural and experimental water deficit. Plant Soil, 230: 175–183.

Arfan M, Athar HR, Ashraf M (2007). Does exogenous application of salicylic acid through the rooting medium modulate growth and photosynthetic capacity in two differently adapted spring wheat cultivars under salt stress? J. Plant Physiol., 164: 685–694.

Ashraf M (2009). Biotechnological approach of improving plant salt tolerance using antioxidants markers. Biotech. Advan., 27: 84-93.

Ashraf M, Orooj A (2006). Salt stress effects on growth, ion accumulation and seed oil concentration in an arid zone traditional medicinal plant ajwain (*Trachyspermum ammi* L. Sprague). J. Arid. Environ., 64: 209–220.

Ashraf M, Harris PJC (2004). Potential biochemical indicators of salinity tolerance in plants. Plant Sci., 166: 3–16.

Ates E, Tekeli AS (2007). Salinity tolerance of Persian clover (*Trifolium resupinatum* var. majus boiss) lines at germination and seedling stage. World. J Agric. Sci., 3: 71-79.

Athar H, Khan A, Ashraf M (2008). Exogenously applied ascorbic acid alleviates salt-induced oxidative stress in wheat. Environ. Exp. Bot., 63:224-231.

Azevedo-Neto AD, Prisco JT, Enéas-Filho J, Abreu CEB, Gomes-Filho E (2006). Effect of salt stress on antioxidative enzymes and lipid peroxidation in leaves and roots of salt-tolerant and salt-sensitive maize genotypes. J. Environ. Exp. Bot., 56: 87-94.

Azooz MM (2004). Proteins, sugars and ion leakage as a selection criterion for the salt tolerance of three sorghum cultivars at seedling stage grown under NaCl and nicotinamide. Int. J. Agric. Biol., 6 : 27-35.

Azooz MM (2009). Foilar application with riboflavin (Vitamin B2) enhancing the resistance of *Hibiscus sabdariffa* L. (Deep red sepals variety) to salinity stress. J. Biol. Sci., 9: 109-118.

Azooz MM, Al-Fredan MA (2009). The inductive role of vitamin C and its mode of application on growth, water status, antioxidant enzyme activities and protein patterns of *Vicia faba* L cv. Hassawi grown under seawater irrigation. Amer. J. Plant Physiol., 4: 38-51.

Azooz MM, Youssef MM (2010). Evaluation of heat shock and salicylic acid treatments as inducers of drought stress tolerance in Hassawi wheat. Amer. J. Plant Physiol., 5: 56 – 70.

Azooz MM, Shaddad MA, Abdel-Latef AA (2004). Leaf growth and K$^+$/Na$^+$ ratio as an indication of the salt tolerance of three sorghum cultivars grown under salinity stress and IAA treatment. Acta. Agron. Hung., 52: 287-296.

Bacheller JD, Romeo JT (1992). Biotic and abiotic stress effects on nitrogen chemistry in the salt marsh cordgrass *Spartina alterniflora* (Poaceae) Chemoecol., 3: 74-80.

Bates LS, Waldren RP, Tear LD (1973). Rapid determination of free proline for water-stress studies. Plant Soil, 39: 205-207.

Bor M, Ozdemir F, Türkan I (2003) The effect of salt stress on lipid peroxidation and antioxidants in leaves of sugar beet *Beta vulgaris* L. and wild beet *Beta maritime* L. Plant Sci., 164: 77-84.

Borsani O, Valpuesta V, Botella MA (2001). Evidence for a role of salicylic acid in the oxidative damage generated by NaCl and osmotic stress in Arabidopsis seedlings. Plant Physiol., 126:1024–1030.

Bradford MM (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein–dye binding. Anal. Biochem., 72: 248–254.

Chen G, Asada K (1992). Inactivation of ascorbate peroxidase by thoils requires hydrogen peroxide. Plant Cell Physiol., 33:117-123.

Dagar JC, Bhagwan H, Kumar Y (2004). Effect on growth performance and biochemical contents of *Salvadora persica* when irrigated with water of different salinity. Indian J. Plant Physiol., 9: 234-238.

Demiral T, Turkan I (2005). Comparative lipid peroxidation, antioxidant defense systems and proline content in roots of two rice cultivars differing in salt tolerance. Environ. Exp. Bot., 53: 247-257.

DiBaccio D, Navaria-Izzo F, Izzo R (2004). Seawater irrigation: antioxidant defense responses in leaves and roots of a sunflower (*Helianthus annus* L.) ecotype. J. Plant Physiol., 161: 1359-1366.

El-Tayeb MA (2005). Response of barley grains to the interactive effect of salinity and salicylic acid. Plant Growth Regul., 45: 215–224.

Erdal S, Aydın M, Genisel M, Taspınar MS, Dumlupinar R, Kaya O, Gorcek Z (2011). Effects of salicylic acid on wheat salt sensitivity. Afr. J. Biotech., 10 (30):5713-5718.

Fales FW (1951). The assimilation and degradation of carbohydrate by Yeast cells. J. Biol. Chem., 193: 113-124.

Foyer CH, Halliwell B (1976). The presence of glutathione and glutathione reductase in chloroplasts: A proposed role in ascorbic acid metabolism. Planta 133: 21-25.

Gharsa MA, Parre E, Debez A, Bordenave M, Richard L, Leport L, Bouchereau A, Savouré A, Abdelly C (2008). Comparative salt tolerance analysis between Arabidopsis thaliana and *Thellungiella halophila*, with special emphasis on K$^+$/Na$^+$ selectivity and proline accumulation. J. Plant Physiol., 165:588-599.

Gunes A, Anal A, Alpaslan M, Eraslan F, Bagci EG, Cick N (2007). Salicylic acid induced changes on some physiological parameters symptomatic for oxidative stress and mineral nutrition in maize (*Zea mays* L.) grown under salinity. J. Plant Physiol., 164:728-736.

Hameed M, Ashraf M (2008). Physiological and biochemical adaptations of *Cynodon dactylon* (L.) Pers. from the salt range (Pakistan) to salinity stress. Flora 203: 683-694.

Havir EA, McHale NA (1987). Biochemical and developmental characterization of multiple forms of catalase in tobacco leaves. Plant Physiol., 84: 450-455.

Hernández JA, Jiménez A, Mullineaux PM, Sevilla F (2000). Tolerance of pea (*Pisum sativum* L.) to long-term salt stress is associated with induction of antioxidant defenses. Plant Cell Environ., 23: 853–862.

Hungria M, Vargas MAT (2000). Environmental factors affecting nitrogen fixation in grain legumes in the topics, with an emphasis on Brazil. Field Crops Res., 65:151-164.

Hussein MM, Balbaa LK, Gaballah MS (2007). Salicylic acid and salinity effect on growth of maize plants. Res. J. Agric. Biol. Sci., 3: 321-328.

Jaleel CA, Gopi R, Alagu Lakshmanan GM, Panneerselvam R (2006). Triadimefon induced changes in the antioxidant metabolism and ajmalicine production in *Catharanthus roseus* (L.) G. Don. Plant Sci., 171: 271–276.

Jaleel CA, Gopi R, Sankar B, Manivannan P, Kishorekumar A, Sridharan R, Panneerselvam R (2007a). Studies on germination, seedling vigour, lipid peroxidation and proline metabolism in *Catharanthus roseus* seedlings under salt stress. South Afr. J. Bot., 73:190–195.

Jaleel CA, Manivannan P, Kishorekumar A, Sankar B, Panneerselvam R (2007b). Calcium chloride effects on salinity induced oxidative stress, proline metabolism and indole alkaloid accumulation in *Catharanthus roseus*. Comptes Rendus Biol., 330 (9): 674-683.

Jaleel CA, Lakshmanan GM, Gomathinayagam M, Panneerselvam R (2008a). Triadimefon induced salt stress tolerance in *Withania somnifera* and its relationship to antioxidant defense system. South Afric. J. Bot., 74 :126-132.

Jaleel CA, Kishorekumar P, Manivannan A, Sankar B, Gomathinayagam M, Panneerselvam R (2008b). Salt stress mitigation by calcium chloride in *Phyllanthus amarus*. Acta. Bot. Croat., 67: 53-62.

Johnson CM, Ulrich A (1959). Analytical methods for use in plant analysis. 1st ed. Clifornia Agriculture Experiment Station Bulletin, CA USA.

Khan MH, Panda SK (2008). Alterations in root lipid peroxidation and antioxidative responses in two rice cultivars under NaCl-salinity stress. Acta Physiol. Plant, 30: 81-89.

Khan W, Prithiviraj B, Smith DL (2003). Photosynthetic response of corn and soybean to foliar application of salicylates. J. Plant Physiol., 160: 485-492.

Khodary SEA (2004). Effect of salicylic acid on the growth, photosynthesis and carbohydrate metabolism in salt-stressed maize plants. Int. J. Agric. Biol., 6:5-8.

Klapheck S, Zimmer I, Cosse H (1990). Scavenging of hydrogen peroxide in the endosperm of *Ricinus communis* by ascorbate peroxidase. Plant Cell Physiol., 31: 1005-1013

Lee YP, Takanashi T (1966). An improved colorimetric determination of amino acids with the use of ninhydrin. Anal. Biochem., 14: 71-77.

Linchtenthaler HK, Wellburn AR (1983). Determination of total carotenoids and chlorophyll a and b of leaf extract in different solvents. Biochem. Soc.Trans., 11: 591-592.

Liu ZP, Liu L, Chen MD, Deng LQ, Zhao GM, Tang QZ, Xia TX (2003). Study on the irrigation systems in agriculture by seawater. J. Nat. Resour., 18: 423–429.

Loake G, Grant M (2007). Salicylic acid in plant defense–the players and protagonists. Curr. Opi. Plant Biol., 10: 466– 472.

López M, Tejera NA, Lluch C (2008). Differential strategies of the model legumes *Lotus japonicus* and *Medicago truncatula* in the adaptation to salt stress: Photosynthetic and nutritional responses. Am. J. Plant Physiol., 3: 121-130.

Lu C, Qiu N, Lu O, Wang B, Kuang T (2002). Does salt stress lead to increased susceptibility of photosystem II to photoinhibition and changes in photosynthetic pigment composition in halophyte *Suaeda salsa* grown outdoors? Plant Sci., 163: 1063-1068.

Maehly AC, Chance B (1954). The assay of catalase and peroxidase. Meth. Anal. Biochem. (D. Glick, ed.). 1:357-424.

Meloni DA, Oliva MA, Martinez CA, Cambraia J (2003). Photosynthesis and activity of superoxide dismutase, peroxidase and glutathione reductase in cotton under salt stress. Environ. Exp. Bot., 49: 69–76.

Misra N, Gupta AK (2005). Effect of salt stress on proline metabolism in two high yielding genotypes of greengram. Plant Sci., 169: 331–339.

Misra N, Gupta AK (2006). Effect of salinity and different nitrogen sources on 12the activity of antioxidant enzymes and indole alkaloid content in *Catharanthus roseus* seedlings. J. Plant. Physiol., 163: 11–18.

Morsy MR, Jouve L, Hausman J, Hoffmann L, Stewart JMcD (2007). Alteration of oxidative and carbohydrate metabolism under abiotic stress in two rice (*Oryza sativa* L.) genotypes contrasting in chilling tolerance. J. Plant Physiol., 164: 157-167.

Mukherjee SP, Choudhuri MA (1983). Implications of water stress-induced changes in the levels of endogenous ascorbic acid and hydrogen peroxide in Vigna seedlings. Plant Physiol., 58: 166-170.

Munns R (2002). Comparative physiology of salt and water stress. Plant Cell Environ., 28: 239-250.

Mwanamwenge J, Loss SP, Siddique KH, Cocks PS (1999). Effect of water stress during floral initiation, flowering and podding on the growth and yield of faba bean (*Vicia faba* L.). Eur. J. Agron., 11: 1-11.

Nazar R, Iqbal N, Syeed S, Khan NA (2011). Salicylic acid alleviates decreases in photosynthesis under salt stress by enhancing nitrogen and sulfur assimilation and antioxidant metabolism differentially in two mungbean cultivars. J. Plant Physiol., 168:807-815.

Noreen S, Ashraf M (2008). Alleviation of adverse effects of salt stress on sunflower (*Helianthus Annus* L.) by exogenous application of salicylic acid: Growth and photosynthesis. Pak. J. Bot., 40 (4): 1657-1663.

Panda SK, Upadhyay RK (2003). Salt stress injury induces oxidative alteration and antioxidative defense in the roots of *Lemna minor*. Biol. Plant, 48:249–253.

Parida AK, Das AB (2005). Salt tolerance and salinity effects on plants: a review Ecotox. Environ. Safety, 60: 324–349.

Ramani B, Reeck T, Debez A, Stelzer R, Huchzermeyer B, Schmidt A, Papenbrock J (2006). *Aster tripolium* L. and *Sesuvium portulacastrum* L.: two halophytes, two strategies to survive in saline habitats. Plant Physiol. Biochem., 44: 395 - 408.

Razmjoo K, Heydarizadeh P, Sabzalian MR (2008). Effect of salinity and drought stresses on growth parameters and essential oil content of *Matricaria chamomile*. Int. J. Agric. Biol., 10: 451- 454.

Sairan RK, Tyagi A (2004). Physiology and molecular biology of salinity stress tolerance in plants. Curr. Sci., 86: 407–421.

Salter J, Morris K, Bailey PC, Boon PI (2007). Interactive effects of salinity and water depth on the growth of *Melaleuca ericifolia* Sm. (Swamp paperbark) seedlings. Aquatic Bot., 86: 213-222.

Sharhrtash M, Mohsenzadeh S, Mohabatkar H (2011). Salicylic acid alleviates paraquat oxidative damage in maize seedling. Asian J. Exp. Biol. Sci., 2: 377-382.

Shi Q, Zhu Z (2008). Effects of exogenous salicylic acid on manganese toxicity, element contents and antioxidative system in cucumber. Environ. Exp. Bot., 63:317-326.

*S*ilva EC, Nogueira RJMC, Araújo FP, Melo NF, Azevedo Neto AD (2008). Physiological responses to salt stress in young umbu plants. Environ. Exp. Bot., 63: 147-157.

Smart RE (1974). Rapid estimation of relative water content. Plant Physiol., 53:258-260.

Song J, Feng G, Tian CY, Zhang FS (2006). Osmotic adjustment traits of *Suaeda physophora*, *Haloxylon ammodendron* and *Haloxylon persicum* in field or controlled conditions. Plant Sci., 170: 113–119.

Syeed S, Anjum NA, Nazar R, Iqbal N, Masood A, Khan NA ((2011). Salicylic acid-mediated changes in photosynthesis, nutrients content and antioxidant metabolism in two mustard (*Brassica juncea* L.) cultivars differing in salt tolerance. Acta Physiol. Plant 33(3): 877-886.

Wang LJ, Chen SJ, Kong WF, Li SH, Archibold DD (2006). Salicylic acid pretreatment alleviates chilling injury and affects the antioxidant system and heat shock proteins of peaches during cold storage. Postharvest. Biol. Technol., 41:244–25.

Williams, Twine (1960). Flame photometric method for sodium, potassium and calcium. – In: Peach, K. and M.V. Tracey, (eds.) Modern Methods of Plant Analysis. Vol. V. pp 3-5, Springer-Verlag., Berlin.

Yan B, Dai Q, Liu X, Huang S, Wang Z (1996). Flooding induced membrane damage, lipid oxidation and activated oxygen generation in corn leaves. Plant Soil 179: 261-268.

Yildirim B, Yaser F, Ozpay T, Ozpay DT, Turkozu D, Terziodlu O, Tamkoc A (2008). Variations in response to salt stress among field pea genotypes (*Pisum sativum* sp. arvense L.). J. Anim. Veter. Advan., 7: 907-910.

Yin H, Chen Q, Yi M (2008). Effect of short-term heat stress on oxidative damage and responses of antioxidant system in *Lilium longiflorum*. Plant Growth Regul., 54: 45-54.

Yoshiba Y, Kiyouse T, Nakashima K, Yamaguchi-Shinozaki K, Shinozaki K (1997). Regulation of levels of proline as an osmolyte in plants under water stress, Plant Cell Physiol., 38:1095–1102.

Zhao SJ, Xu CC, Zhou Q, Meng QW (1994). Improvements of the method for measurement of malondialdehyde in plant tissue. Plant Physiol. Commun., 30: 207–210.

Zhou XM, MacKenzie AF, Madramootoo CA, Smith DL (1999). Effects of stem-injected plant growth regulator, with or without sucrose, on grain production, biomass and photosynthetic activity of field-grown corn plants. J. Agron. Crop Sci., 183:103-110.