ISSN 1021-4437, Russian Journal of Plant Physiology, 2018, Vol. 65, No. 1, pp. 104–114. © Pleiades Publishing, Ltd., 2018.

RESEARCH PAPERS

Salicylic Acid (SA) Induced Alterations in Growth, Biochemical Attributes and Antioxidant Enzyme Activity in Faba Bean (*Vicia faba* L.) Seedlings under NaCl Toxicity[1]

P. Ahmad[a, b, *], M. N. Alyemeni[a], M. A. Ahanger[c], D. Egamberdieva[d], L. Wijaya[a], and P. Alam[e]

[a] *Botany and Microbiology Department, College of Science, King Saud University, P. O. Box 2460, Riyadh, 11451 Saudi Arabia*

[b] *Department of Botany, S.P. College, Srinagar, Jammu and Kashmir, 190001 India*

[c] *School of Studies in Botany Jiwaji University, Gwalior, MP-474011 India*

[d] *Institute of Landscape Biogeochemistry, Leibniz Centre for Agricultural Landscape Research, Müncheberg, Germany*

[e] *Biology Department, College of Science and Humanities, Prince Sattam bin Abdulaziz University (PSAU), Alkharj, 11942 Kingdom of Saudi Arabia (KSA)*

**e-mail: parvaizbot@yahoo.com*

Received November 11, 2016

Abstract—In the present study we tried to evaluate the effect of salicylic acid (SA) in alleviating the negative effects of salinity stress. NaCl stress (50 and 100 mM) declines the shoot and root length and maximum decrease was observed at 100 mM concentration of NaCl. Similarly shoot dry weight decreased by 57.14% and root dry weight by 67.24% with 100 mM NaCl stress. The pigments and leaf relative water content (LRWC) were also observed to decline with increase in NaCl concentration. However, supplementation of SA to NaCl stressed seedlings showed enhanced length and dry weight of shoot and root. The pigment and LRWC also increased by the application of SA in the present study. NaCl stress also enhanced proline and glycine betaine (GB) by 3.01 and 2.04 folds, respectively; further enhancement was recorded by the application of SA. Hydrogen peroxide (H_2O_2) and malondialdehyde (MDA) content also showed rise in accumulation, however, seedlings treated with SA and NaCl (100 mM + SA) declines the H_2O_2 accumulation to 1.90 from 2.45 folds and MDA to 1.69 from 2.34 folds over the control. Antioxidants were observed to increase with NaCl concentration and further increase was recorded by the application of SA. Indoleacetic acid (IAA) and indole butyric acid (IBA) decreased by 36.60 and 44.16%, respectively, and ABA increased by 750% with 100 mM NaCl. Addition of SA to NaCl stressed seedlings enhanced the IAA and IBA and decreased the ABA concentration to appreciable level. NaCl is also responsible for the higher accumulation of Na^+ and Na^+/K^+ ratio and decreased uptake of Ca^{2+} and K^+. Supplementation of SA decreased the Na^+ accumulation and enhanced the uptake of Ca^{2+} and K^+ in NaCl stressed seedlings. In conclusion, SA supplementation mitigates the negative effects of NaCl toxicity in faba bean seedlings through the modulation of different osmoprotectants, antioxidants and nutrients uptake.

Keywords: Vicia faba, lipid peroxidation, antioxidants, osmolytes, salicylic acid, salinity stress

DOI: 10.1134/S1021443718010132

INTRODUCTION

Plants are exposed to different environmental pressures, which often lead to decreased plant growth and development and ultimately the yield. NaCl stress is one of the prevalent stress responsible for the pollution of soil and water bodies throughout the globe. Salinity stress is increasing and around 21% of the land is affected and the most affected areas are arid and semi-arid regions of world. Salinity has negative impact on crop yield as it imposes osmotic and ionic stresses that leads to reduced uptake of mineral elements and thus decline the growth and yield. Excess accumulation of Na^+ ions result in reduced leaf relative content and increased electrolyte leakage [1]. Prolonged or high concentrations of NaCl stress leads to secondary stress known as oxidative stress. Oxidative stress is developed by the generation of reactive oxygen species (ROS), and are very much reactive and can damage the biomolecules and other enzymatic activities of the cell. Nature has equipped the plants with different defense systems that include osmoprotectants like proline, glycine betaine, carotenoids and enzymatic antioxidants like superoxide dismutase (SOD), catalase (CAT),

[1] The article is published in the original.

Abbreviations: APX—ascorbate peroxidase; CAT—catalase; DAT—days after treatment; GB—glycine betaine; GR—glutathione reductase; SA—salicylic acid; LRWC—leaf relative water content.

ascorbate peroxidase (APX) and glutathione reductase (GR) [1–3]. Antioxidants neutralize ROS to avoid their negative effects in plant cell. SOD dismutates the oxygen radicals to H_2O_2, and CAT and APX are responsible for the removal of H_2O_2. GR is important enzyme of ascorbate-glutathione system and is responsible for maintaining the reduced glutathione pool [3].

Salicylic acid (SA) is a phytohormone responsible for the regulation of plant growth, photosynthesis and other metabolic processes. Apart from this SA have been reported to enhance the tolerance mechanism to different abiotic stresses [4–6]. Kaya et al. [6] have also reported mitigation of NaCl stress by the application of SA. The positive role of SA under stress might be due to enhanced mineral and water uptake and restoration of photosynthesis, membrane stability etc. [5]. SA is a signaling molecule and can interact with ROS signal pathways thereby can help in regulation of environmental stresses against oxidative stress [7]. SA is also reported to decrease the lipid peroxidation and enhance the activity of antioxidant enzymes in different plants [5].

Faba bean (*Vicia faba* L.) belongs to family Leguminosae and is mainly cultivated for human consumption as it is rich in proteins. Cultivation of faba bean enhances the fertility of the soil as it increases the soil nitrogen compounds. The faba bean is susceptible to salinity stress and attempts are being made to increase the production of this crop especially on wastelands affected with salinity. Thus the present study was conducted to evaluate the role of SA in mitigating the adverse effects of NaCl on growth, physio-biochemical attributes and enzymatic antioxidants in faba bean.

MATERIALS AND METHODS

Plant material and experimental design. Healthy and viable seeds of faba bean (*Vicia faba* L.) were surface sterilized using sodium hypochlorite (0.5%, v/v) for 3 min followed by washing with distilled water and were allowed to germinate on a wet blotting paper. Thereafter, healthy germinated seedlings were transferred to pots filled with acid washed sand and vermicompost in the ratio of 3 : 1. NaCl in varying concentrations (i.e. 0, 50 and 100 mM) dissolved in Hoagland solution was applied to the pots after every alternate day for 60 days under controlled conditions and control plants were given full strength Hoagland solution. SA (10 mL/plant, 1 mM) mixed with surfactant, tween-20 was applied foliarly using a manual sprayer to plants after every alternate day from 10 day up to 60 days after treatment (DAT). Pots were arranged in randomized block design with five replicates. After 60 DAT plants were uprooted carefully and washed with distilled water to remove the dust and different growth and biochemical parameters were analyzed. All chemicals used in the present study were of analytical grade procured from Sigma Aldrich, Merck and Himedia.

Growth and biomass yield. Length of shoot and root was measured using a manual scale and for dry weight estimation samples were oven dried at 70°C for 48 h and then weighed.

Estimation of photosynthetic pigment content. Method of Hiscox and Israelstam [8] were followed for estimation of photosynthetic pigments. The optical density (OD) was recorded at 480, 510, 645 and 663 nm using spectrophotometer against blank DMSO.

Determination of proline and glycine betaine (GB) content. Method of Bates et al. [9] was followed for estimation of proline content and optical density was recorded at 520 nm against toluene. For estimation of GB method of Grieve and Grattan [10] was employed and absorbance was read at 365 nm. Calculation was done from standard curve of GB.

Determination of leaf relative water content. For determination of leaf relative water content (LRWC) method of Smart and Bingham [11] was adopted and calculation was done according to following formula:

$$\text{LRWC}\ (\%) = [(\text{FW} - \text{DW})/(\text{TW} - \text{DW})] \times 100,$$

where FW—fresh weight; DW—dry weight; TW—turgid weight.

Estimation of hydrogen peroxide (H_2O_2) and lipid peroxidation. Method suggested by Velikova et al. [12] was followed for the estimation of H_2O_2 and concentrations were calculated from the standard curve of H_2O_2. Lipid peroxidation was measured in fresh leaf tissues using the method of Heath and Packer [13] and the formation of malondialdehyde (MDA) was recorded spectrophotometrically at 532 nm and making clarification with non-specific absorption at 600 nm.

Determination of electrolyte leakage. Electrolyte leakage in fresh leaves was determined following Dionisio-Sese and Tobita's [14] method and was calculated by the following formula:

$$\text{Electrolyte leakage}\ (\%) = [(EC_1 - EC_0)/(EC_2 - EC_0)] \times 100,$$

where EC_0—electrical conductivity was observed at 0 time point; EC_1—electrical conductivity after contents heated at 60°C; EC_2—electrical conductivity after contents heated at 100°C.

Antioxidant enzymes assay. Antioxidant enzymes were extracted from fresh leaf sample (500 mg) in Tris-HCl (100 mM, pH 7.5) containing dithiotrol (5 mM), $MgCl_2$ (10 mM), EDTA (1.0 mM), magnesium acetate (5 mM), PVP (1.5%), PMSF (1.0 mM) and 1 μg/mL aproptinin in a pre-chilled pestle and mortar. The homogenate was subjected to centrifugation at 10000 rpm for 15 minutes at 4°C and the supernatant was used as enzyme source. However, the extraction buffer for APX contained 2 mM ascorbate in addition of the above ingredients. Soluble protein was estimated employing method of Bradford [15].

For carrying the assay of superoxide dismutase (SOD, EC 1.15.1.1) the ability of enzyme to inhibit the photochemical reduction of NBT was observed at

560 nm in accordance with the method of VanRossum et al. [16]. Catalase (CAT, EC 1.11.1.6) activity was estimated by monitoring the change in absorbance at 240 nm [17]. For ascorbate peroxidase (APX, EC1.11.1.11) assay oxidation of ascorbate was measured as decrease in absorbance at 290 nm [18]. Foyer and Halliwell's [19] method was adopted for assaying the glutathione reductase (GR, EC 1.6.4.2) activity and change in optical density was recorded at 340 nm for 3 minutes. The activities of all the enzymes were expressed as U/mg protein.

Estimation of phytohormones. Phytohormones including indoleacetic acid (IAA), indolebutyric acid (IBA) and abscisic acid (ABA) were extracted by the method of Kusaba et al. [20] and endogenous concentrations in the sample were determined by subjecting the extract to HPLC.

Determination of ion accumulation. Na^+, K^+ and Ca^{2+}were estimated in oven dried leaf samples using flame photometer after digesting the samples in acid following Ahanger and Agarwal [3].

Statistical analysis. Data presented is mean of five replicates with ±SD calculated and statistical analyses was performed using one-way analysis of variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT). *P*-values (≤0.05) were considered as significant.

RESULTS

Salicylic Acid Enhanced Growth and Biomass Yield

NaCl stress decreased the growth and biomass yield and the results are presented in Fig. 1. Shoot and root length decreased by 54.58 and 66.98%, respectively, with high concentration (100 mM) of NaCl stress. Exogenous application of SA showed less decrease of 33.94% in shoot length and 30.56% in root length with 100 mM NaCl + SA over the control plants.

Shoot and root dry weight also decline with NaCl stress and maximum decrease of 57.14 and 67.24%, respectively, was observed with 100 mM NaCl stress compared to control. Supplementation of SA to NaCl stressed plants restored the dry weight with both stress level (Fig. 1).

Salicylic Acid Restores Pigment Content

Plants exposed to salinity stress showed decreased pigment content in the present study (Fig. 2). The maximum decrease was observed to be 49.50, 58.85, 51.85 and 54.76% in chlorophyll *a*, chlorophyll *b*, total chlorophylls and carotenoids, respectively, with 100 mM NaCl stress relative to control plants. SA supplementation to NaCl stressed plants restored the pigment content and showed less decrease of 6.93% in chlorophyll *a*, 5.88% in chlorophyll *b*, 6.66% in total chlorophylls and 19.04% in carotenoids with 100 mM + SA over the control plants.

Salicylic Acid Boosts Proline, Glycine Betaine and Leaf Relative Water Content

In the present study, 50 mM NaCl stress induced proline and GB content by 3.01 and 2.04 fold, respectively. Further increase of 4.98 fold in proline content and 3.20 fold in GB was recorded with 100 mM NaCl stress relative to control plants. Foliar spray of SA boosted the proline accumulation by 6.05 folds and GB by 3.46 folds with 100 mM + SA as compared to control plants (Figs. 3a and 3b).

LRWC was decreased by 65.82% with 50 mM and 44.72% with 100 mM NaCl stress. However, application of SA recorded increase of 14.30 and 17.49% with 50 mM + SA and 100 mM + SA, respectively, over the untreated plants (Fig. 3c).

Salicylic Acid Declines Accumulation of H_2O_2, MDA Content and Electrolyte Leakage

Salinity stress increased the accumulation of H_2O_2 and MDA content in the present study (Figs. 4a and 4b). Maximum accumulation of 2.45 and 2.84 folds in H_2O_2 and MDA, respectively, was reported with 100 mM NaCl stress. Supplementation of SA decreased the accumulation to 1.90 fold in H_2O_2 and 1.69 fold in MDA content with 100 mM + SA over control plants.

Electrolyte leakage increased by 30.15 and 62.11% with 50 mM and 100 mM NaCl stress. However, NaCl treated plants supplied with SA showed less decrease of 15.77% with 50 mM + SA and 45.21% with 100 mM + SA relative to control (Fig. 4c).

Salicylic Acid Enhanced Activities of Antioxidant Enzymes

Salinity stress increased the activity of SOD, CAT, APX and GR by 35.62, 48.13, 37.91 and 31.57%, respectively, with 50 mM NaCl stress. The NaCl concentration of 100 mM showed further enhancement of 68.86% in SOD, 75.52% in CAT, 89.59% in APX and 42.74% in GR relative to control. Exogenous application of SA further boosted the activity of SOD by 79.49%, CAT by 112%, APX by 105% and GR by 75.59% with 100 mM + SA over the control plants (Figs. 5a and 5b).

Salicylic Acid Enhanced Phytohormones

Salinity stress decreased the IAA by 36.60% and IBA by 44.16% with 100 mM NaCl stress. Plants treated with NaCl in combination with SA showed less decrease of 21.42 and 30.21% in IAA and IBA, respectively, with 100 mM + SA over control plants. ABA enhanced with NaCl stress to 512% with 50 mM and 750% with 100 mM NaCl stress. However, application of SA maintains the ABA content to 277 and 507% with 50 mM + SA and 100 mM + SA, respectively, relative to control plants (Figs. 6a–c).

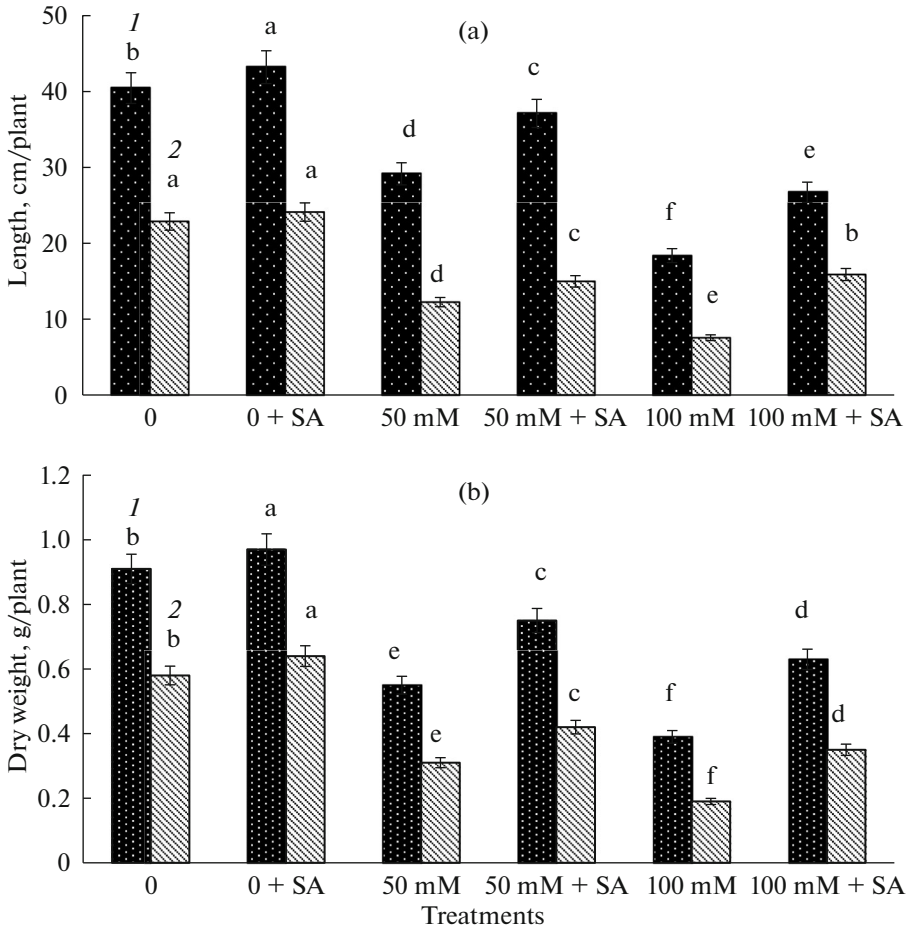

Fig. 1. Salicylic acid application enhanced shoot and root length (a) and shoot and root dry weight (b) in faba bean seedlings under 0, 50 and 100 mM NaCl treatments. *1*—Shoots; *2*—roots. 0—control; SA—salicylic acid. Data presented are the means ± SE (n = 5). Different letters indicate significant difference at $P \leq 0.05$.

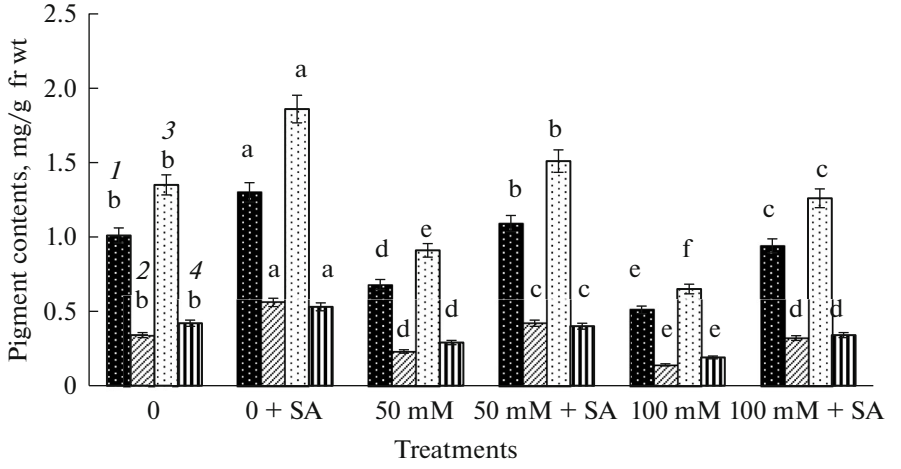

Fig. 2. Salicylic acid boosts the pigment content in faba bean seedlings under 0, 50 and 100 mM NaCl stress. *1*—Chlorophyll *a*; *2*—chlorophyll *b*; *3*—total chlorophyll, *4*—carotenoids. 0—control; SA—salicylic acid. Data presented are the means ± SE (n = 5). Different letters indicate significant difference at $P \leq 0.05$.

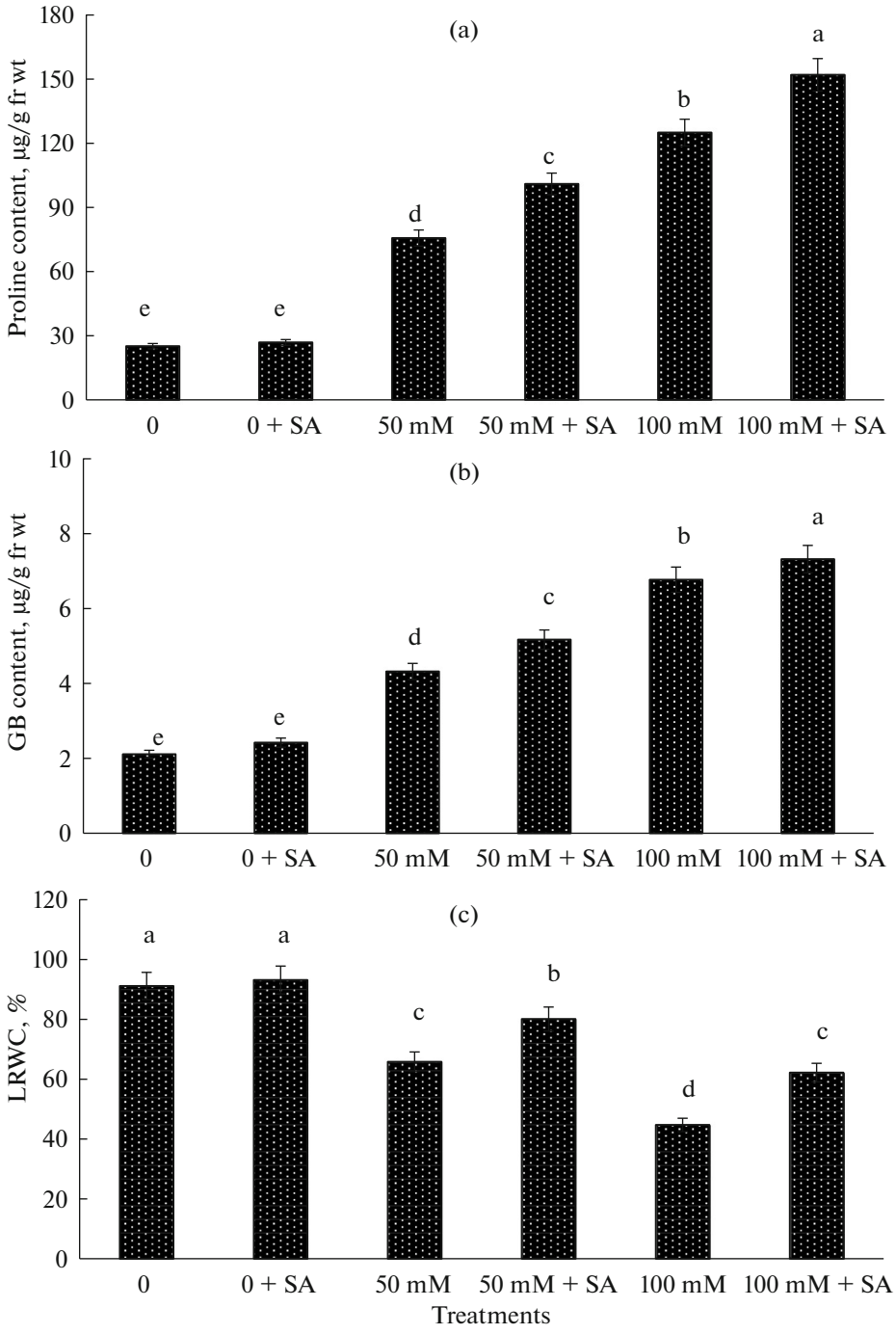

Fig. 3. Effect of different concentrations of NaCl (0, 50 and 100 mM) and salicylic acid on proline (a), glycine betaine (b) and LRWC (c) in faba bean seedlings. 0—Control; SA—salicylic acid; LRWC—leaf relative water content. Data presented are the means ± SE ($n = 5$). Different letters indicate significant difference at $P \leq 0.05$.

Salicylic Acid Restores Ion Accumulation

Na and Na/K ratio enhanced with NaCl concentration and maximum increase of 215 and 669%, respectively, was reported with 100 mM NaCl treatment. Supplementation of SA decreased the accumulation of Na^+ ions by 153% and Na^+/K^+ ratio by 252% relative to control. K^+ and Ca^{2+} decreased by 23.17 and 42.33%, respectively, with 50 mM NaCl stress. Further decrease of 58.39% in K^+ and 60.58% in Ca^{2+} was recorded with 100 mM NaCl stress over control plants. However, application of SA to NaCl treated plants restored the K^+ and Ca^{2+} ions and less decrease of 26.99 and 35.52% was observed in K^+ and Ca^{2+}, respectively, with 100 mM + SA relative to control plants (Fig. 7).

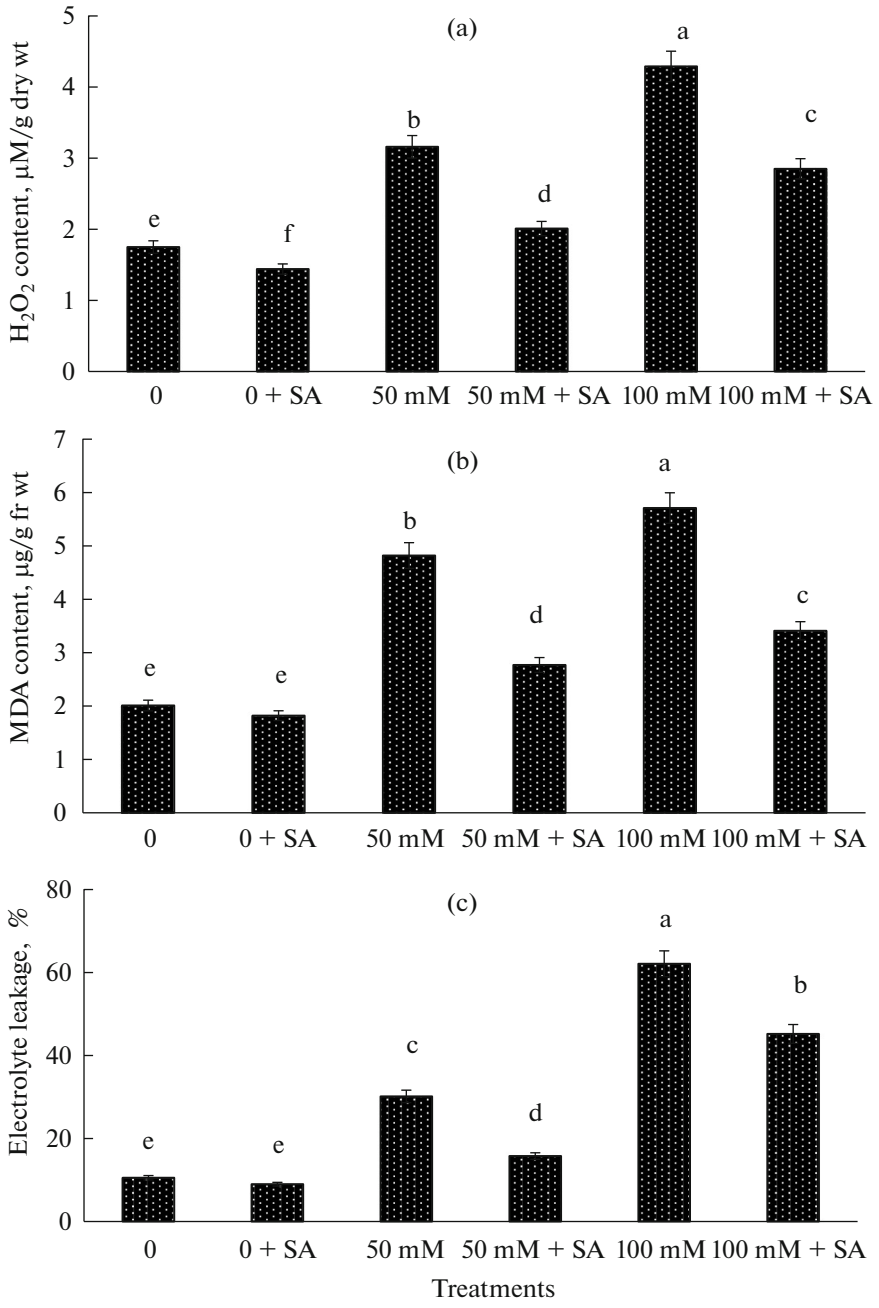

Fig. 4. Decrease in accumulation of H_2O_2 (a), MDA content (b) and electrolyte leakage (c) by the application of salicylic acid to 0, 50 and 100 mM NaCl treated faba bean seedlings. Data presented are the means ± SE ($n = 5$). Different letters indicate significant difference at $P \leq 0.05$.

DISCUSSION

The NaCl concentration declined the growth in terms of shoot and root length and biomass yield in the present study. The decreasing growth and biomass yield is also reported by other workers like Ahmad et al. [21] in mustard, Ahmad et al. [2] in chickpea and Ashraf et al. [22] in wheat. Inhibition in cell division and cell elongation induced by NaCl stress is the main reason of decreasing growth and biomass yield [1, 2]. Other reasons of reduced growth might be reduced uptake of mineral nutrients, production of reactive oxygen species, inhibition in cytoplasmic enzyme activity, turgor loss and hormonal imbalance [22]. Exogenous application of SA enhanced the length of root and shoot in NaCl stressed plants. The dry weight of shoot and root also enhanced in NaCl stressed plants supplemented with SA and the results corroborates Karlidag et al. [23] in *Fragaria ananassa* and Li et al. [24] in *Torreya grandis*. The ameliorating effect

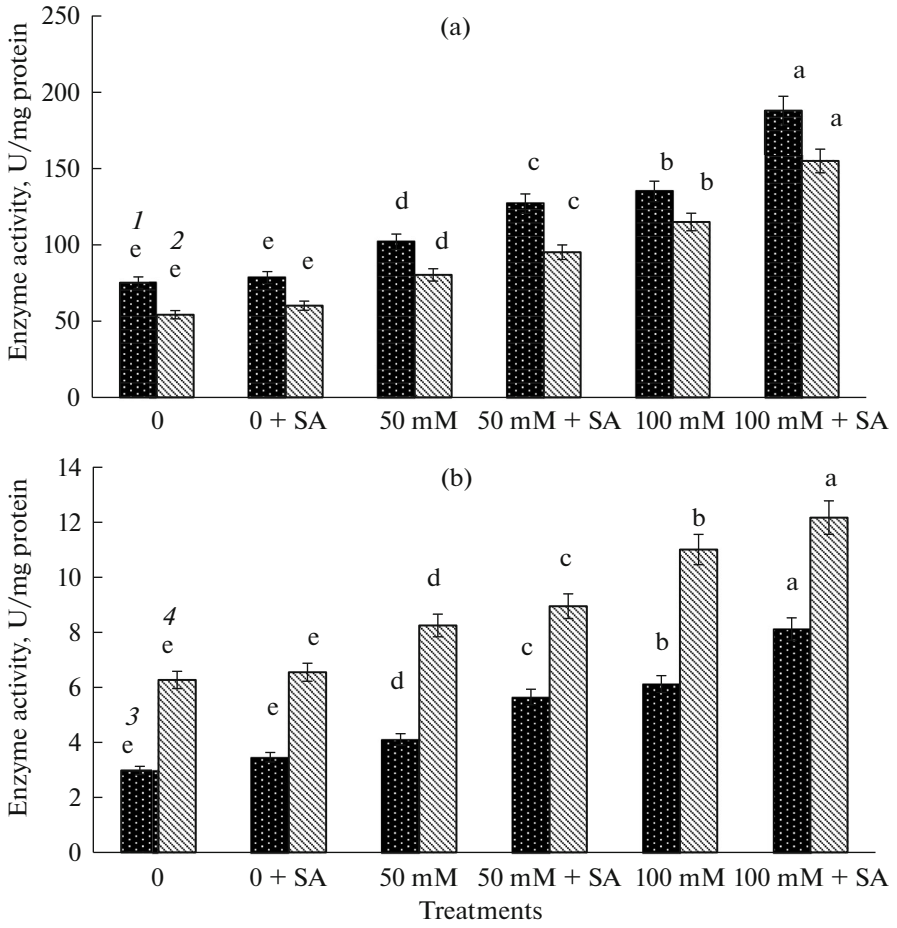

Fig. 5. Activities of superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX) and glutathione reductase (GR) in 0, 50 and 100 mM NaCl treated faba bean seedlings by application of salicylic acid: a—activity of SOD (*1*) and CAT (*2*); b—activity of APX (*3*) and GR (*4*). 0—Control; SA—salicylic acid. Data presented are the means ± SE ($n = 5$). Different letters indicate significant difference at $P \leq 0.05$.

of SA may be due to its role in enhanced CO_2 assimilation rate, enhanced rate of photosynthesis and uptake of essential elements in NaCl stressed plants [25].

Decreased pigment content due to NaCl in the present study coincides with the reports of Ahmad et al. [1, 21] in mustard and Ashraf et al. [22] in wheat. NaCl stress reduced the pigment system may be due to the destruction in chlorophyll pigments, increased activity of chlorophyllase, abnormality in pigment protein complex and inhibition of mineral uptake especially Mg^{2+} [20, 22]. SA enhanced the chlorophyll and carotenoid content in NaCl stressed plants is also reported in different plants [4, 29, 23]. The positive role of SA in maintaining the chlorophyll content may be due to enhanced uptake of Ca, Mg, Fe etc. SA also enhanced the rate of photosynthetic electron transport, maintains higher Rubisco activation, boosts PSII efficiency [4]. Foliar application of SA improved the activity of PSII, net photosynthesis, transpiration rate and enzymatic antioxidants in cotton seedlings under NaCl stress [4].

Improved LRWC is considered as the one of the adaptations to stress [3]. Decreased LRWC under NaCl stress is also reported by Ahmad et al. [21] in *B. juncea* and Karlidag et al. [23] in strawberry plants. NaCl stress induced the osmotic and ionic stress by hampering the uptake of water and nutrients [2, 5] which in turn decreased the LRWC. Foliar application of SA enhanced the leaf diffusive resistance and lower transpiration rates [22] and that could be one of the reasons of enhanced LRWC under stress.

Proline accumulation was reported to increase with increasing concentrations of NaCl in present study. Similar reports have been described for other plants like *B. juncea* [1] and *Vigna radiate* [4]. Salt tolerant cultivars have been reported to accumulate more proline content than susceptible cultivars indicating the role of proline in stress tolerance [21]. Proline is involved in osmoregulation and helps in stabilizing membranes and maintains protein structures under stress [1, 3, 21]. Proline is also reported to have antioxidant property that quenches the ROS and reduces

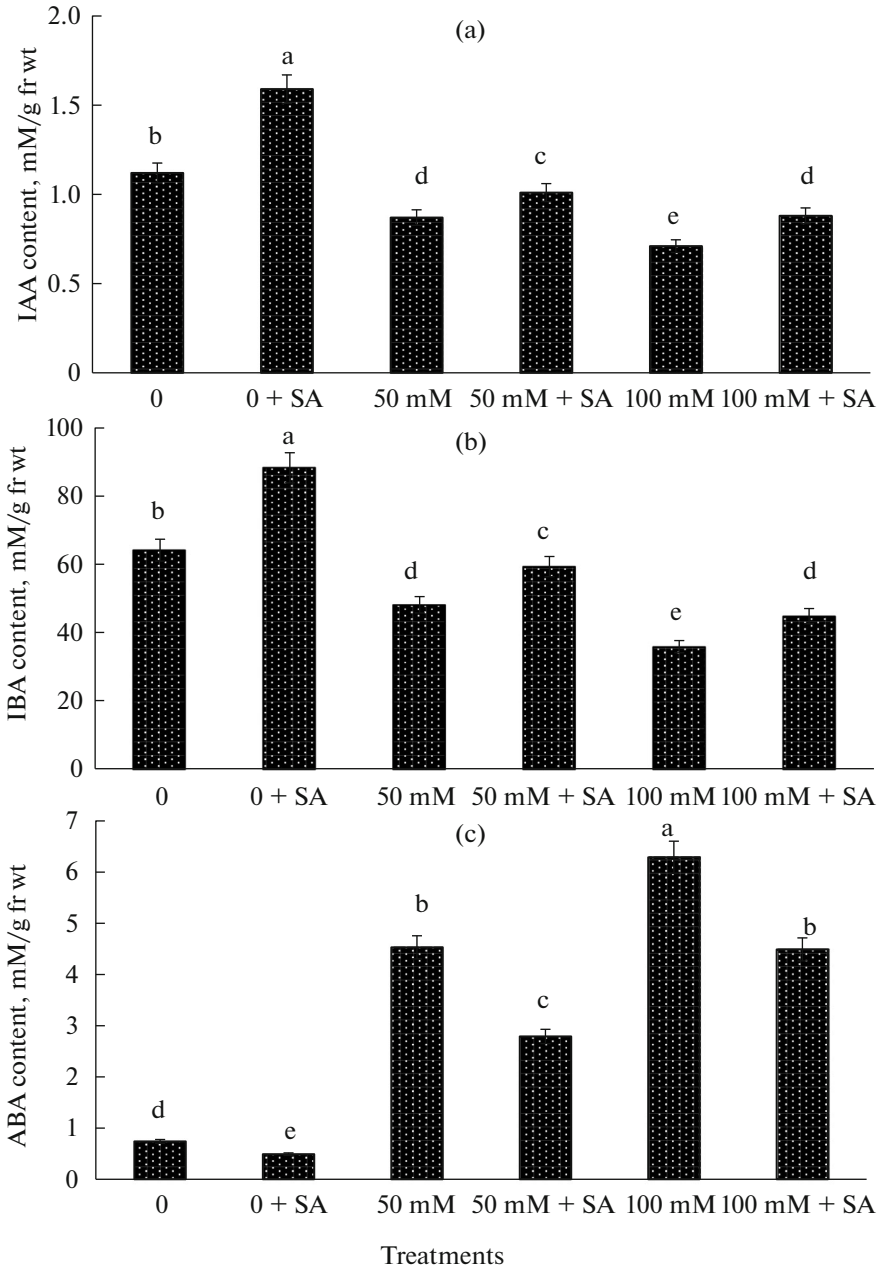

Fig. 6. Effect of different concentrations of NaCl (0, 50 and 100 mM) and salicylic acid on phytohormones content in faba bean seedlings: a—indoleacetic acid (IAA); b –indolebutyric acid (IBA); c—abscisic acid (ABA). 0—Control; SA—salicylic acid. Data presented are the means ± SE ($n = 5$). Different letters indicate significant difference at $P \leq 0.05$.

photo-damage of thylakoid membranes [4] and acts as energy storage and boosts N_2 fixation in plants. GB is also involved in cell osmoregulation and is also reported to inhibit accumulation of ROS, shields photosynthetic machinery and activates genes related to stress and protects the protein structures from the negative effects of abiotic stress [4]. Supplementation of SA enhanced the LRWC in the present study and the results corroborates with the findings of Karlidag et al. [23] in *Fragaria* x *ananassa*. Supplementation of SA enhanced the proline and GB content in NaCl stresses plants and the results coincides Li et al. [24] in *Torreya grandis* and Khan et al. [4] in *Vigna radiate*, respectively. GB provides protection under NaCl stress by boosting the antioxidant defense machinery. The main role of GB is osmoprotection and stabilization of quaternary structure against NaCl stress [4].

Salt stress increased the H_2O_2, MDA and electrolyte leakage in the present study corroborates with the findings of Ahmad et al. [21]. Ashraf et al. [22] also reported increased H_2O_2 content in wheat under NaCl

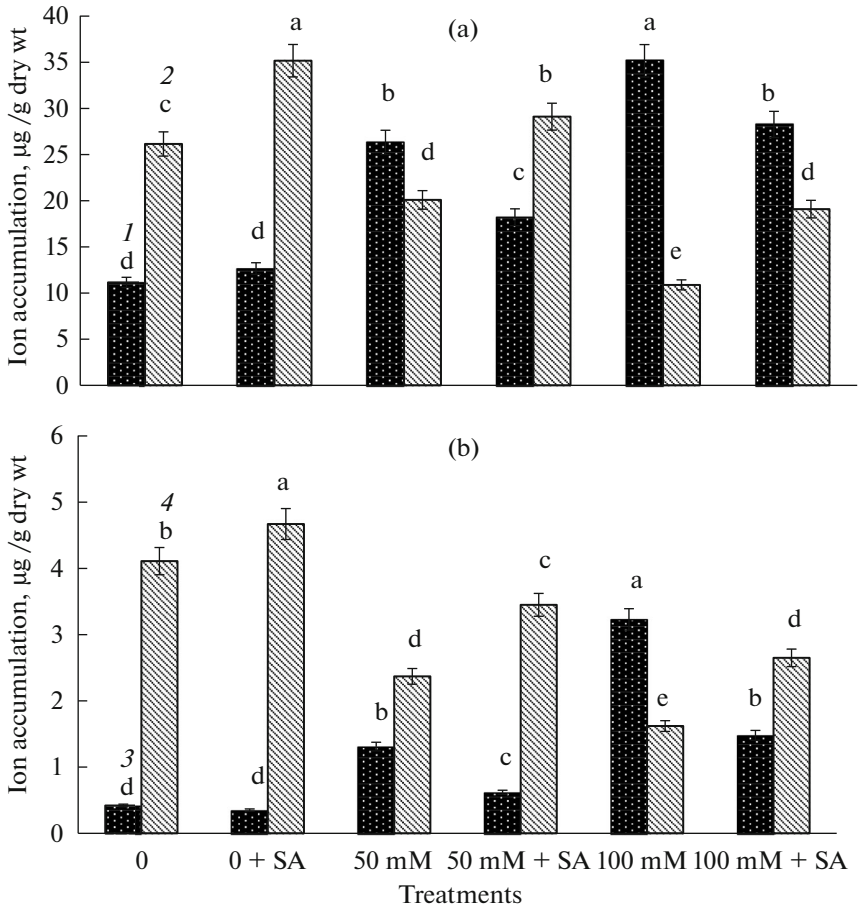

Fig. 7. Application of salicylic acid maintains the uptake of Na^+ and K^+ (a) and Na^+/K^+ ratio and Ca^{2+} (b) in faba bean seedlings under 0, 50 and 100 mM NaCl stress. *1*—Na^+; *2*—K^+; *3*—Na^+/K^+ ratio; *4*—Ca^{2+}. 0—control; SA—salicylic acid. Data presented are the means ± SE ($n = 5$). Different letters indicate significant difference at $P \le 0.05$.

stress reflecting in increased MDA content. ROS directly attacks the biomolecules and polyunsaturated fatty acids (PUFAs) linoleic (18:2) and lenolenic (18:3) and forms complex mixture of lipid hydroperoxides and decreases the permeability of the membranes. Electrolyte leakage under NaCl stress was also reported in other plants like strawberry Karlidag et al. [23] and *B. juncea* [20]. Protection from membrane disintegration is an important criterion for salinity tolerance mechanism and SA treatment has been shown to decrease the H_2O_2 levels.

NaCl stress induces antioxidants has also been reported by many authors on different plants [1, 4, 20, 21]. SA enhanced the antioxidant activity and has been also reported by Li et al. [24] in *Torreya grandis* under NaCl stress. SA mitigates the salinity stress due to enhanced activity of APX and proline [24]. External supply of SA mitigated NaCl stress in *S. lycopersicum* by expression of GST gene family [26]. Enhanced expression of antioxidants genes like *GPX1*, *GPX2*, *GST1*, *GST2* and *ASA-GSH* pathway due to SA application restores the membrane integrity with concomitant increase in photosynthetic pigments [24] and may be due to the reduced generation of ROS.

Phytohormones are very important constituents for imparting tolerance to the plants against abiotic stresses. Similar to our results Sakhabutdinova et al. [27] also reported the decrease in IAA under stress and NaCl decreased the IAA and IBA in *S. lycopersicum* has been reported by Hashem et al. [28]. External supplementation of IAA has been reported to improve the NaCl tolerance in wheat [29]. Priming of wheat seeds with IBA showed enhanced tolerance against NaCl stress [30]. Actual mechanisms underlying SA induce enhancement in the endogenous levels of IAA and IBA with subsequent decline in ABA is not known yet. However, SA being an important signaling molecule crosstalk with other hormones including IAA and IBA so can induce their synthesis.

NaCl stress accumulated Na^+ and Na^+/K^+ ratio and decreased K^+ and Ca^{2+} uptake in the present study corroborated with Hashem et al. [28] in *S. lycopersicum*. Similar results were obtained in mustard by Ahmad et al. [1]. Na^+ and K^+ shares similar physico-

chemical structure due to which competition exists between the two ions for the uptake [3]. Ahmad et al. [1] have reported that Na^+ uptake is increased with increasing concentration of NaCl, which eventually decreased the uptake of K^+ and Ca^{2+}. Decreased K^+ and Ca^{2+} uptake in presence of NaCl stress is also reported by Karlidag et al. [23] in strawberry. Uptake of minerals is one of the main adaptations to confer stress tolerance [1, 3]. SA enhanced the uptake of essential nutrients like K^+, Ca^{2+} and the results corroborates the findings of Szepesi et al. [25] in tomato. The enhanced uptake of mineral nutrients might be due to SA-induced H^+-ATPase activity. The positive correlation of SA and mineral nutrient uptake suggested the ameliorating role of SA against salinity stress.

To conclude, salinity stress affects growth, biomass yield and pigment system and other physiological attributes in the present study. However, plants have some mechanism to deal with this type of environmental pressure. The activity of antioxidants got enhanced with NaCl exposure. Application of SA to NaCl treated seedlings further enhanced the proline, GB and activities of antioxidant enzymes. SA also enhanced the uptake of nutrients, which were hampered by under NaCl stress. Thus application of SA to enhance the growth and crop yield can be a sustainable approach to deal with salinity affected soils and can bring these wastelands under cultivation. A possible involvement of exogenously applied SA in regulating the signaling events for better NaCl tolerance in *Vicia faba* can be proposed and future research is needed to reach a conclusion.

ACKNOWLEDGMENT

The authors would like to extend their sincere appreciation to the Deanship of Scientific Research at King Saud University for its funding this Research group (project no. RGP-199).

REFERENCES

1. Ahmad, P., Hashem, A., Abd-Allah, E.F., Alqarawi, A.A., John, R., and Egamberdieva, D., Role of *Trichoderma harzianum* in mitigating NaCl stress in Indian mustard (*Brassica juncea* L.) through antioxidative defense system, *Front. Plant Sci.*, 2015, vol. 6, p. 868.
2. Ahmad, P., Abdel Latef, A.A., Hashem, A., Abd-Allah, E.F., Gucel, S., and Tran, L.S.P., Nitric oxide mitigates salt stress by regulating levels of osmolytes and antioxidant enzymes in chickpea, *Front. Plant Sci.*, 2016, vol. 7, p. 347.
3. Ahanger, M.A. and Agarwal, R.M., Potassium up-regulates antioxidant metabolism and alleviates growth inhibition under water and osmotic stress in wheat (*Triticum aestivum* L.), *Protoplasma*, 2017, vol. 254, no. 4, pp. 1471–1486. doi 10.1007/s00709-016-1037-0
4. Khan, M.I.R., Asgher, M., and Khan, N.A., Alleviation of salt-induced photosynthesis and growth inhibition by salicylic acid involves glycine betaine and ethylene in mung bean (*Vigna radiata* L.), *Plant Physiol. Biochem.*, 2014, vol. 80, pp. 67–74.
5. Ahmad, P., Nabi, G., and Ashraf, M., Cadmium-induced oxidative damage in mustard [*Brassica juncea* (L.) Czern. & Coss.] plants can be alleviated by salicylic acid, *S. Afr. J. Bot.*, 2011, vol. 77, pp. 36–44.
6. Kaya, C., Kirnak, H., Higgs, D., and Saltali, K., Supplementary calcium enhances plant growth and fruit yield in strawberry cultivars grown at high salinity, *Sci. Hortic.*, 2002, vol. 93, pp. 65–74.
7. Borsani, O., Valpuesta, V., and Botella, M.A., Evidence for a role of salicylic acid in the oxidative damage generated by NaCl and osmotic stress in Arabidopsis seedlings, *Plant Physiol.*, 2001, vol. 126, pp. 1024–1030.
8. Hiscox, J.T. and Israelstam, G., A method for the extraction of chlorophyll from leaf tissue without maceration, *Can. J. Bot.*, 1979, vol. 57, pp. 1332–1334.
9. Bates, L., Waldren, R., and Teare, I., Rapid determination of free proline for water-stress studies, *Plant Soil*, 1973, vol. 39, pp. 205–207.
10. Grieve, C.M. and Grattan, S.R., Rapid assay for determination of water soluble quaternary ammonium compounds, *Plant Soil*, 1983, vol. 70, pp. 303–307.
11. Smart, R.E. and Bingham, G.E., Rapid estimates of relative water content, *Plant Physiol.*, 1974, vol. 53, pp. 258–260.
12. Velikova, V., Yordanov, I., and Edreva, A., Oxidative stress and some antioxidant systems in acid rain-treated bean plants: protective role of exogenous polyamines, *Plant Sci.*, 2000, vol. 151, pp. 59–66.
13. Heath, R.L. and Packer, L., Photoperoxidation in isolated chloroplasts. I. Kinetics and stoichiometry of fatty acid peroxidation, *Arch. Biochem. Biophys.*, 1968, vol. 125, pp. 189–198.
14. Dionisio-Sese, M.L. and Tobita, S., Antioxidant responses of rice seedlings to salinity stress, *Plant Sci.*, 1998, vol. 135, pp. 1–9.
15. Bradford, M.M., A rapid and sensitive method for the quantitation of microgram quantities of protein using the principle of protein–dye binding, *Anal. Biochem.*, 1976, vol. 72, pp. 248–259.
16. Van Rossum, M.V., Alberda, M. and van der Plas, L.H., Role of oxidative damage in tulip bulb scale micropropagation, *Plant Sci.*, 1997, vol. 130, pp. 207–216.
17. Luck, H., Catalases, in *Methods of Enzymatic Analysis*, Bergmeyer, H.U., Ed., New York: Academic, 1963, pp. 885–894.
18. Nakano, Y. and Asada, K., Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts, *Plant Cell Physiol.*, 1981, vol. 22, pp. 867–880.
19. Foyer, C.H. and Halliwell, B., The presence of glutathione and glutathione reductase in chloroplasts: a proposed role in ascorbic acid metabolism, *Planta*, 1976, vol. 133, pp. 21–25.
20. Kusaba, S., Kano-Murakami, Y., Matsuoka, M., Tamaoki, M., Sakamoto, T., Yamaguchi, I., and Fukumoto, M., Alteration of hormone levels in transgenic tobacco plants over-expressing a rice homeobox gene *OSH1, Plant Physiol.*, 1998, vol. 116, pp. 471–476.

21. Ahmad, P., Hakeem, K.R., Kumar, A., Ashraf, M., and Akram, N.A., Salt induced changes in photosynthetic activity and oxidative defense system of three cultivars of mustard (*Brassica juncea* L.), *Afr. J. Biotechnol.*, 2012, vol. 11, pp. 2694–2703.

22. Ashraf, M.A., Ashraf, M., and Ali, Q., Response of two genetically diverse wheat cultivars to salt stress at different growth stages: leaf lipid peroxidation and phenolic contents, *Pak. J. Bot.*, 2010, vol. 42, pp. 559–566.

23. Karlidag, H., Yildirim, E., and Turan, M., Salicylic acid ameliorates the adverse effect of salt stress on strawberry, *Sci. Agric.*, 2009, vol. 66, pp. 180–187.

24. Li, T., Hu, Y., Du, X., Tang, H., Shen, C., and Wu, J., Salicylic acid alleviates the adverse effects of salt stress in *Torreya grandis* cv. *merrillii* seedlings by activating photosynthesis and enhancing antioxidant systems, *PLoS One*, 2014, vol. 9: e109492.

25. Szepesi, A., Csiszar, J., Bajkan, S., Gemes, K., Horvath, F., Erdei, L., Deer, A.K., Simon, M.L., and Tari, I., Role of salicylic acid pre-treatment on the acclimation of tomato plants to salt and osmotic stress, *Acta Biol. Szeged.*, 2005, vol. 49, pp.123–125.

26. Csiszar, J., Horvath, E., Vary, Z., Galle, A., Bela, K., and Brunner, S., Glutathione transferase super gene family in tomato: salt stress-regulated expression of representative genes from distinct GST classes in plants primed with salicylic acid, *Plant Physiol. Biochem.*, 2014, vol. 78, pp. 15–26.

27. Sakhabutdinova, A.R., Fatkhutdinova, D.R., Bezrukova, M.V., and Shakirova, F.M., Salicylic acid prevents the damaging action of stress factors on wheat plants, *Bulg. J. Plant Physiol.*, 2003, special issue, pp. 314–319.

28. Hashem, A., Abd-Allah, E.F., Alqarawi, A.A., Al Huqail, A.A., Egamberdieva, D., and Wirth, S., Alleviation of cadmium stress in *Solanum lycopersicum* L. by arbuscular mycorrhizal fungi via induction of acquired systemic tolerance, *Saudi J. Biol. Sci.*, 2015, vol. 23, pp. 272–281.

29. Egamberdieva, D., Alleviation of salt stress by plant growth regulators and IAA producing bacteria in wheat, *Acta Physiol. Plant.*, 2009, vol. 31, pp. 861–864.

30. Ghodrat, V., Rousta, M.J., and Tadaion, M.S., Effect of priming with indole-butyric acid (IBA) on germination and growth of wheat under saline conditions, *Int. J. Agric. Crop Sci.*, 2012, vol. 4, pp. 289–292.