

MODULATION OF SALT STRESS EFFECTS ON *VICIA FABA* L. PLANTS GROWN ON A RECLAIMED-SALINE SOIL BY SALICYLIC ACID APPLICATION

Mostafa Mohamed Rady^{1*}, Ragab Salama Taha¹, Wael Morad Semida², Hesham F. Alharby³

¹Department of Botany, Faculty of Agriculture, Fayoum University, 63514 Fayoum, Egypt

²Department of Horticulture, Faculty of Agriculture, Fayoum University, 63514 Fayoum, Egypt

³Department of Biological Sciences, Faculty of Science, King Abdulaziz University, 21589 Jeddah, Saudi Arabia

*Corresponding author. E-mail: mmmr02@fayoum.edu.eg; mrady2050@gmail.com

ABSTRACT

Two field experiments were conducted using two varieties (i.e., Giza 40 and Giza 429) of *Vicia faba* L. to evaluate the effect of 1.0 mM salicylic acid (SA) foliar application on plant tolerance to reclaimed-saline soil conditions. Growth, physiological attributes and yields of water-sprayed plants (controls) grown under reclaimed-saline soil conditions were negatively affected. However, under the adverse soil conditions, SA-treated plants had enhanced growth characteristics, yield components and anatomy of both varieties compared to the controls. Free proline, soluble sugars, chlorophyll fluorescence, relative water content, membrane stability index, and nutrients were also improved significantly in SA-treated plants. Compared to Giza 40, Giza 429 showed better growth and yield, reflecting more salt-tolerance. The SA at 1.0 mM concentration could be recommended to enable plants to mitigate the oxidative damage under the adverse conditions of reclaimed-saline soils.

Key words: Anatomy, chlorophyll fluorescence, dehydration tolerance, Faba bean varieties, salt stress.

INTRODUCTION

Faba beans (*Vicia faba* L.) are popular legume foods and consumed worldwide as an important protein source for human and animal nutrition. Faba bean seeds are rich in carbohydrates, proteins and minerals (Broughton et al., 2003). Although, Saxena et al. (1993) reported that there is a considerable variability in salinity tolerance among legumes, others considered them either sensitive or only moderately tolerant to salinity (Subbarao and Johansen, 1993). Among legumes, *V. faba* plants are proved to be moderately sensitive to salinity (Delgado et al., 1994), exhibiting a reduction in plant growth up to 50% under 6.7 dS m⁻¹ salinity (Mass and Hoffman, 1977). Because of the lack of reliable traits for selection, breeding faba beans for salt tolerance has usually been limited in Egypt. Therefore, it is necessary to determine the differences in resistance mechanisms between genotypes through their growth and yield characteristics (Semida and Rady, 2014a, b).

At recent days, there is a tendency, in Egypt, to expand the cultivated area for many

crops, including faba beans in newly-reclaimed soils, although most of them are affected by salinity. Salinity stress is one of the major abiotic stresses, which decrease plant growth and crop productivity in different regions, particularly in arid and semi-arid regions. Salt stress causes ion toxicity and osmotic stress, producing excess of reactive oxygen species (ROS) in plant cells such as superoxide radicals, hydrogen peroxide, hydroxyl anions, and singlet oxygen that damage lipids, proteins and DNA (Yasar et al., 2006). It also causes ion imbalance and direct ion toxic effects on the metabolic processes by the increased Na⁺ and Cl⁻ levels. These ions adversely affect the morphological, physiological and biochemical responses of plants (Nazar et al., 2011; Guo et al., 2014). In addition, photosynthetic attributes, plant growth and development, yield and antioxidant capacity are affected (Sairam and Tyagi, 2004; Saha et al., 2010; Guo et al., 2014; Yasuta and Kokubun, 2014; Chunthaburee et al., 2015). Several mechanisms are being developed by plants to induce tolerance to overcome the deleterious

effects of salinity. The increased activity of antioxidants in plants is one of these mechanisms. Therefore, foliar application on plants with some antioxidants is considered to contribute for developing the sustainable agriculture under stress conditions.

Salicylic acid (SA) is an antioxidant and is considered a hormone-like substance. Some abiotic stresses, such as drought, chilling, heavy metal toxicity, and salinity can increase the SA content in the plant, playing important roles in enhancing the resistance to these stressed conditions (Yuan and Lin, 2008). SA plays a crucial role in photosynthesis, stomatal conductance and transpiration (Khan et al., 2003). It also reduces Na^+ and Cl^- accumulation in plant tissues, improving the antioxidative protection (Gunes et al., 2007; Xu et al., 2008). Therefore, SA can reduce the harmful effects of salt stress on plants by inducing their salt-tolerance (Manaa et al., 2014; Semida et al., 2014). The protective effects of SA include upregulation of anti-stress processes and the recovery of growth processes after the stress is over (Sharikova et al., 2003).

Despite attempts conducted to identify faba bean cultivars through experiments to be grown in the newly-reclaimed and salt-affected soils, there are no Egyptian cultivars produced by breeders as salt-tolerant that could be recommended for cultivation in such soils. Therefore, the aim of this study was to evaluate the salt-tolerance of two Egyptian faba bean cultivars and their responses to SA under reclaimed saline soil conditions in connection with growth characteristics, physiological attributes, ion accumulation, plant anatomy and yield.

MATERIAL AND METHODS

Plant material, experimental design and treatments

Two field experiments were conducted in two successive seasons (2011/2012 and 2012/2013) at the Experimental Farm of Faculty of Agriculture, Fayoum University, Southeast Fayoum (29°17'N; 30°53'E), Egypt. In both seasons, the daily temperatures averaged $21.2 \pm 2.6^\circ\text{C}$ and $22.1 \pm 2.8^\circ\text{C}$, and the daily relative humidity averaged $58.4 \pm 5.1\%$

and $60 \pm 4.9\%$, respectively. Healthy seeds of two varieties (i.e., Giza 40 and Giza 429) of faba bean (*V. faba* L.) were sown on 22 and 25 October 2011 and 2012, respectively. Seeds were obtained from Horticulture Research Institute, Agricultural Research Centre, Giza, Egypt and were selected for uniformity by choosing those of equal size and of the same colour. The selected seeds were washed with distilled water, sterilized in 1% (v/v) sodium hypochlorite for approx. 2 min, washed thoroughly again with distilled water, and left to dry at room temperature overnight. Uniform, air-dried faba bean seeds were sown in hills spaced 20-25 cm apart, in rows spaced 70 cm apart in 3.0 m \times 3.5 m plots, using an equivalent of 120 kg seed ha^{-1} to generate the recommended planting density. Thinning was done before the first irrigation to remain two plants per hill. During soil preparation and plant growth, the soil was supplemented with the full dose of NPK fertilizer according to the recommendations of the Ministry of Agriculture and Land Reclamation [i.e., 450 kg ha^{-1} calcium superphosphate (15.5% P_2O_5), 250 kg ha^{-1} ammonium sulphate (20.5% N), and 120 kg ha^{-1} potassium sulphate (48% K_2O)]. Irrigation water was added to 100% of the reference crop evapotranspiration (ET_o), values from the Fayoum Meteo Station. Seven irrigations were applied in each season, with total water rates of about 2800 $\text{m}^3 \text{ha}^{-1}$ in each growing season. All other recommended agricultural practices were followed as recommended by the Ministry of Agriculture and Land Reclamation.

One experimental site was chosen for each season in the same location. Soil analyses were carried out. The soil texture was sandy clay loam [sand (% w/v), 49.8 and 50.0; silt (% w/v), 19.7 and 19.9; clay (% w/v), 30.5 and 30.1 in both 2011/2012 and 2012/2013 seasons, respectively]. The other main characteristics of the soil were: pH [at a soil : water (w/v) ratio of 1:2.5], 7.74 and 7.80; E_ce (dS m^{-1} ; soil – paste extract), 8.88 and 8.95; organic matter (% w/v), 1.04 and 1.02; CaCO_3 (% w/v), 6.96 and 6.89; total N (% w/v), 0.072 and 0.068; available P (mg kg^{-1} soil), 8.67 and 8.49; available K (mg kg^{-1} soil), 192 and 187; available Fe (mg kg^{-1}

soil), 6.32 and 5.98; available Mn (mg kg^{-1} soil), 2.32 and 2.27; available Zn (mg kg^{-1} soil), 0.98 and 0.95; and available Cu (mg kg^{-1} soil), 0.48 and 0.52 in both growing seasons, respectively. Based on the above ECe values, soil was classed as being strongly saline according to Dahnke and Whitney (1988). The treatments of both experiments in both seasons were arranged in a randomised complete block design, with two levels of salicylic acid (SA; 0 and 1.0 mM), with three replicate plots. Twenty days after sowing (DAS), faba bean seedlings in each plot were sprayed to run-off with 0 (tap water as a control) and 1.0 mM SA, and then the sprays were repeated at 30 and 40 DAS. The level of SA and the number and timing of sprays were based on a preliminary pot trial using various SA levels (i.e., 0, 0.25, 0.50, 1.0 and 2 mM; data not shown). To ensure optimal penetration into leaf tissues, 0.1% (v/v) Tween-20 was added to the foliar sprays as a surfactant.

Plant growth and yield measurements

Fifty-day-old plants ($n=9$) were carefully removed from each experimental plot and dipped in a bucket of water. Plants were shaken gently to remove all adhering soil particles and the lengths of their shoots were measured using a meter scale. Numbers of leaves and branch plants⁻¹ were counted. The shoots of plants were weighed to record their fresh weights. They were then placed in an oven at 70°C until constant weight and the dry weights were recorded. Using a graph sheet, leaf areas were measured manually, where the squares covered by the leaf were counted. At the end of each experiment (2 and 5 April 2012 and 2013, respectively), all the dry pods on each plant in each experimental plot were collected and counted. The dry faba bean seeds were then extracted from their pods and weighed to calculate the average 100-seed weight, and seed yield per plant and per hectare.

Measurement of proline and total soluble sugar concentrations

Using the rapid colorimetric method outlined by Bates et al. (1973), proline concentration (in $\mu\text{g } 100 \text{ g}^{-1}$ DW of leaf) was

measured. The leaf sample was extracted from 0.5 g DW of leaf tissue by grinding in 10 ml of 3% (v/v) sulfosalicylic acid. The mixture was then centrifuged at $10,000 \times g$ for 10 min. In a test tube, 2 ml of the supernatant followed by 2 ml of freshly prepared acid-ninhydrin solution was placed. The tubes were incubated in a water bath at 90°C for 30 min and the reaction was terminated in an ice-bath. Each reaction mixture was extracted with 5 ml of toluene and was then vortex-mixed for 15 s. The tubes were allowed to stand for at least 20 min in the dark at room temperature to separate the toluene and aqueous phases. The toluene phase was collected carefully into a test tube and its absorbance was read at 520 nm. Proline concentrations were determined from a standard curve prepared using analytical grade proline. Total soluble sugar (T.S. sugar) concentrations were extracted and determined according to the method described by Irigoyen et al. (1992). A dried leaf sample (0.2 g) was homogenized in 5 ml of 96% (v/v) ethanol and washed with 5 ml 70% (v/v) ethanol. The extract was centrifuged at $3500 \times g$ for 10 min and the supernatant was stored at 4°C prior to measurement. T.S. sugar concentrations were determined by reacting 0.1 ml of the ethanolic extract with 3 ml of freshly-prepared anthrone reagent [150 mg anthrone plus 100 ml of 72% (v/v) sulphuric acid] by placing it in a boiling water bath for 10 min. After cooling, the absorbance of the mixtures was recorded at 625 nm using a Bausch and Lomb-2000 Spectronic Spectrophotometer.

Measurements of chlorophyll fluorescence

Using a portable fluorometer (Handy PEA, Hansatech Instruments Ltd, Kings Lynn, UK), chlorophyll fluorescence was measured on two different sunny days. On each day, three leaves (at the same age) were randomly chosen from different three plants in each experimental plot of each treatment ($n=9$). Fluorescence measurements were conducted by calculating the maximum quantum yield of PS II Fv/Fm using the following equation (Maxwell and Johnson, 2000):

$$Fv/Fm = (Fm - F0)/Fm.$$

Performance index of photosynthesis based on the equal absorption (PIABS) was calculated as reported by Clark et al. (2000).

Determination of membrane stability index and relative water content

Using duplicate 0.2 g samples of fully-expanded leaf tissue, membrane stability indices (MSI) were estimated in 9 samples for each treatment (Rady, 2011). A sample of each duplicate was placed in a test tube containing 10 ml of double-distilled water and heated at 40°C in a water bath for 30 min, and the electrical conductivity (C1) of the solution was then recorded using a conductivity bridge. The second sample was boiled at 100°C for 10 min, and the conductivity was also measured (C2). The MSI was calculated using the following formula:

$$\text{MSI (\%)} = [1 - (C1 \div C2)] \times 100$$

Excluding the midrib, fresh (2=9) were used to determine the relative water contents (RWC). The discs were weighed (fresh mass; FM) and immediately floated on double-distilled water in Petri dishes at 25°C for 24 h, in the dark, to saturate them with water. Any adhering water was blotted dry and the turgid mass (TM) was measured. The dry mass (DM) was recorded after dehydrating the discs at 70°C for 48 h. The RWC was then calculated using the formula of Hayat et al. (2007) as follows:

$$\text{RWC (\%)} = [(FM - DM) \div (TM - DM)] \times 100$$

Determination of leaf nitrogen (N), phosphorus (P), potassium (K), calcium (Ca) and sodium (Na) concentrations

Using the Orange-G dye method, leaf N concentrations (in mg g⁻¹ DW) were determined (Hafez and Mikkelsen, 1981). The dye solution was prepared by dissolving 1.0 g of 96% (w/w) assay-dye in 1 L of distilled water, with 21.0 g citric acid which acted as a buffer to maintain the correct pH, and 2.5 ml 10% (v/v) thymol in 10% (v/v) ethanol as an inhibitor of microbial growth. A sample of dried leaf tissue (0.2 g) was placed in a centrifuge tube and 20 ml of the dye reagent solution was added. The contents of each tube were shaken for 15 min, and then filtered

using Whatman No. 1 filter paper. The solution was diluted 100-fold with distilled water and its absorbance was measured at 482 nm. N concentrations were calculated using the formulae:

$$\text{N (\%)} = 0.39 + 0.954 \times \text{Dye absorbed (g/100 g)}$$

and

$$\text{Dye absorbed (g/100 g)} = (a - b/a) (cfv/w) \times 100$$

where: a was the absorbance of the dye reagent solution at 482 nm without plant material (the blank), b was the absorbance of the dye reagent solution at 482 nm with plant material, c was the concentration of the dye reagent (1.0 g l⁻¹ distilled water), f was the purity factor of the dye reagent (96%), v was the volume of the dye reagent solution used per sample (20 ml), and w was the weight of ground dry material (0.2 g).

The molybdenum-reduced molybdophosphoric blue colour method (Jackson, 1967), in sulphuric acid (with reduction to exclude arsenate), was used to determine P concentrations (in mg g⁻¹ DW). Sulphomolybdic acid (molybdenum blue), diluted sulphomolybdic acid, and 8% (w/v) sodium bisulphite-H₂SO₄ solution were used as reagents.

Leaf K⁺ and Na⁺ ion concentrations (in mg g⁻¹ DW) were assessed using a Perkin-Elmer Model 52-A Flame Photometer (Glenbrook, Stamford, CT, USA; Page et al., 1982). Leaf Ca²⁺ concentrations were determined using a Perkin-Elmer Model 3300 Atomic Absorption Spectrophotometer (Chapman and Pratt, 1961).

Anatomical study

Leaf and stem samples were collected at 50 DAS. Leaf samples were taken from the middle of the fifth leaf from apex, and the fifth internodes were taken as the stem samples. Samples were killed and fixed in FAA solution (50 ml 95% ethyl alcohol + 10 ml formalin + 5 ml glacial acetic acid + 35 ml distilled water) for 48 h. Samples were then washed in 50% ethyl alcohol, dehydrated and cleared in tertiary butyl alcohol series, embedded in paraffin wax of 54-56°C m.p. Using a rotary microtome, cross sections, 20 μ thick, were cut and adhesived by Haupt's adhesive, and then

stained with the crystal violet-erythrosine combination (Sass, 1961). The sections were cleared in carbol xylene and mounted in Canada balsam, and were then observed and documented using an upright light microscope (AxioPlan, Zeiss, Jena, Germany). Measurements were done, using a micrometer eyepiece and an average of five readings were calculated.

Statistical analysis

All data were subjected to analysis of variance (ANOVA) for a randomised complete block design, after testing for homogeneity of error variances according to the procedure outlined by Gomez and Gomez (1984). Significant differences between treatments were compared at $p \leq 0.05$ by Fisher's least-significant difference test.

RESULTS

Growth characteristics of *Vicia faba* L. varieties

Foliar spray of 1.0 mM salicylic acid (SA) to both varieties of faba bean (i.e., Giza 40 and Giza 429) significantly increased all tested growth traits (i.e., shoot length, leaf number, leaf area, branch number, shoot fresh weight and shoot dry weight) compared to control plants sprayed with tap water (Table 1). The increases in the above growth characteristics were 80.1, 46.3, 122.5, 43.5, 95.6 and 98.2%, respectively for Giza 40, and by 63.2, 43.8, 120.0, 60.9, 77.0 and 94.0%, respectively for Giza 429 compared to their controls. For varieties, there were significant increases in growth characteristics of Giza 429 compared to those of Giza 40.

Table 1. Effect of exogenous treatment of salicylic acid (SA; mM) on growth characteristics of two varieties of *Vicia faba* L. plants grown in reclaimed-saline calcareous soil

Treatment		Shoot length (cm)	Number of leaves plant ⁻¹	Leaf area plant ⁻¹ (dm ²)	Number of branches plant ⁻¹	Shoot fresh weight (g)	Shoot dry weight (g)
Variety	SA						
Giza 40	0	30.7 ± 1.2d [#]	12.1 ± 0.5d	7.1 ± 0.3c	2.3 ± 0.1c	47.2 ± 2.3d	5.6 ± 0.3d
	1	55.3 ± 1.9b	17.7 ± 0.8b	15.8 ± 0.5b	3.3 ± 0.2b	92.3 ± 4.4b	11.1 ± 0.7b
Giza 429	0	36.7 ± 1.4c	13.7 ± 0.5c	8.0 ± 0.3c	2.3 ± 0.1c	56.4 ± 2.6c	6.7 ± 0.4c
	1	59.9 ± 1.9a	19.7 ± 0.9a	17.6 ± 0.6a	3.7 ± 0.2a	99.8 ± 5.1a	13.0 ± 0.9a

[#]Values are means ± SE (n=9). Mean values in each column followed by a different lower-case-letter are significantly different by Fisher's least-significant difference test (LSD) at $p \leq 0.05$ (Measurements were made in 50-day-old plants).

Physiological attributes of *Vicia faba* L. varieties

Exogenous application of 1.0 mM SA significantly increased chlorophyll fluorescence

(i.e., F_v/F_m and PI) MSI and RWC, and the concentrations of free proline and total soluble sugars in both faba bean cultivars compared to their controls (Table 2).

Table 2. Effect of exogenous treatment of salicylic acid (SA; mM) on physiological attributes of two varieties of *Vicia faba* L. plants grown in reclaimed-saline calcareous soil

Treatment		Free proline (µg g ⁻¹ DW)	Total soluble Sugars (mg g ⁻¹ DW)	F_v/F_m	PI	MSI (%)	RWC (%)
Variety	SA						
Giza 40	0	101.6 ± 5.3d [#]	3.28 ± 0.12d	0.76 ± 0.02b	3.23 ± 0.14c	63.6 ± 3.1c	42.1 ± 2.1c
	1	172.4 ± 8.6b	6.70 ± 0.22b	0.84 ± 0.03a	4.56 ± 0.18b	76.2 ± 4.4b	57.0 ± 2.4b
Giza 429	0	116.7 ± 5.8c	4.27 ± 0.15c	0.78 ± 0.02b	3.41 ± 0.15c	65.0 ± 3.5c	43.0 ± 2.8c
	1	189.8 ± 9.7a	7.66 ± 0.24a	0.85 ± 0.04a	4.96 ± 0.21a	83.3 ± 4.5a	62.6 ± 3.4a

[#]Values are means ± SE (n=9). Mean values in each column followed by a different lower-case-letter are significantly different by Fisher's least-significant difference test (LSD) at $p \leq 0.05$ (Measurements were made in 50-day-old plants).

F_v/F_m = uses to estimate the potential efficiency of PSII by taking dark adapted measurements; PI = photosynthetic performance index; MSI = membrane stability index; RWC = relative water content.

These physiological parameters in SA-applied plants exceeded those in their controls by 69.7 and 62.6%, 104.3 and 79.4%, 10.5 and 9.0, 41.2 and 45.5, 19.8 and 28.2, and 35.4 and 45.6% for Giza 40 and Giza 429, respectively. Results also indicated that, Giza 429 was better than Giza 40 in tolerating the adverse effects of reclaimed saline soil conditions.

Nutrient status of *V. faba* L. varieties

Plants of both faba bean varieties sprayed with 1.0 mM SA had significantly increased concentrations of N, P, K and Ca, while showed declined concentrations of Na⁺

ions compared to water-sprayed controls (Table 3). The relations between useful nutrients (K and Ca) and harmful ions (Na⁺) were higher in SA-applied plants than controls (Table 4). The 1.0 mM SA application increased the concentrations of N, P, K and Ca in both Giza 40 and Giza 429 varieties by 32.4 and 34.9%, 38.8 and 38.5%, 40.2 and 41.9%, and 48.0 and 49.2%, respectively, and reduced Na⁺ ion concentrations by 41.1 and 40.7%, respectively compared to controls (tap water spraying). Results also showed a superiority of Giza 429 in restoring N, P, K and Ca nutrients and excluding Na⁺ ions compared to Giza 40.

Table 3. Effect of exogenous treatment of salicylic acid (SA; mM) on nutrient status of two varieties of *Vicia faba* L. plants grown in reclaimed-saline calcareous soil

Treatment		N (mg g ⁻¹ DW)	P (mg g ⁻¹ DW)	K (mg g ⁻¹ DW)	Ca (mg g ⁻¹ DW)	Na (mg g ⁻¹ DW)
Variety	SA					
Giza 40	0	26.2 ± 0.6b [#]	2.27 ± 0.06d	23.4 ± 0.6d	6.15 ± 0.07b	8.24 ± 0.11a
	1	34.7 ± 0.9a	3.15 ± 0.09b	32.8 ± 0.8b	9.10 ± 0.09a	4.85 ± 0.06c
Giza 429	0	27.2 ± 0.7b	2.57 ± 0.07c	25.8 ± 0.6c	6.30 ± 0.06b	7.42 ± 0.10b
	1	36.7 ± 0.9a	3.56 ± 0.10a	36.6 ± 0.9a	9.40 ± 0.08a	4.40 ± 0.07d

[#]Values are means ± SE (n=9). Mean values in each column followed by a different lower-case-letter are significantly different by Fisher's least-significant difference test (LSD) at p≤0.05 (Measurements were made in 50-day-old plants).

Table 4. Effect of exogenous treatment of salicylic acid (SA; mM) on relations of Ca and/or K with Na of two varieties of *Vicia faba* L. plants grown in reclaimed-saline calcareous soil

Treatment		K:Na ratio	Ca:Na ratio	K + Ca:Na
Variety	SA			
Giza 40	0	2.84 ± 0.14d [#]	0.75 ± 0.03c	3.59 ± 0.17d
	1	6.76 ± 0.33b	1.88 ± 0.08b	8.64 ± 0.41b
Giza 429	0	3.48 ± 0.18c	0.85 ± 0.04c	4.33 ± 0.22c
	1	8.32 ± 0.42a	2.14 ± 0.11a	10.45 ± 0.53a

[#]Values are means ± SE (n=9). Mean values in each column followed by a different lower-case-letter are significantly different by Fisher's least-significant difference test (LSD) at p≤0.05 (Measurements were made in 50-day-old plants).

Yield and its components of *V. faba* L. varieties

Exogenous application of 0.1 mM SA significantly increased the number of dry pod

plant⁻¹, average 100-seed weight, dry seed yield plant⁻¹ and dry seed yield hectare⁻¹ compared to the controls (Table 5).

Table 5. Effect of exogenous treatment of salicylic acid (SA; mM) on the yield and its components of two varieties of *Vicia faba* L. grown in reclaimed-saline calcareous soil

Treatment		No. of dry pod plant ⁻¹	Average 100-seed weight	Dry seed yield plant ⁻¹ (g)	Dry seed yield hectare ⁻¹ (ton)
Variety	SA				
Giza 40	0	13.3 ± 0.6d [#]	53.8 ± 2.5c	36.5 ± 2.1d	1.68 ± 0.12d
	1	22.7 ± 1.3b	73.7 ± 3.1b	54.0 ± 2.9b	2.55 ± 0.15b
Giza 429	0	15.8 ± 0.7c	56.8 ± 2.6c	41.1 ± 2.4c	1.92 ± 0.14c
	1	25.4 ± 1.7a	81.2 ± 3.3a	60.3 ± 3.2a	2.82 ± 0.16a

[#]Values are means ± SE (n=9). Mean values in each column followed by a different lower-case-letter are significantly different by Fisher's least-significant difference test (LSD) at p≤0.05 (Measurements were made in 50-day-old plants).

SA-applied plants of both Giza 40 and Giza 429 exceeded the controls (water-sprayed plants) by 70.7 and 60.8%, 37.0 and 43.0%, 47.9 and 46.7%, and 51.8 and 46.9% for the aforementioned yield and its components, respectively. Giza 429 was found to produce more yield and its components than Giza 40 due to its higher tolerability to saline conditions.

Stem and leaf anatomy of *V. faba* L. varieties

Plants of both faba bean varieties treated with 1.0 mM SA had clear improved stem and leaf anatomy compared to those of tap

water-sprayed controls (Tables 6 and 7). SA application increased stem length, stem width, cortex thickness, stem pith diameter, vessels diameter, leaf blade thickness, midvein thickness, vascular bundle length, vascular bundle width and number of leaf xylem vessels in both Giza 40 and Giza 429 varieties by 28.0 and 47.0%, 26.8 and 36.4%, 50.0 and 88.0%, 15.7 and 25.7%, 43.3 and 31.6%, 21.4 and 25.6, 14.3 and 34.2, 11.1 and 25.0, 50.0 and 40.0, and 60.0 and 71.4%, respectively compared to the controls (tap water spraying). Results also indicated that Giza 429 had better leaf and stem anatomy than Giza 40.

Table 6. Effect of exogenous treatment of salicylic acid (SA; mM) on the stem anatomy of two varieties of *Vicia faba* L. grown in reclaimed-saline calcareous soil

Treatment		Dimensions of stem (μ)		Cortex thickness (μ)	Pith diameter (μ)	Vessels diameter (μ)
Variety	SA	length	width			
Giza 40	0	3125 \pm 106c [#]	3175 \pm 111c	40 \pm 2d	713 \pm 24c	30 \pm 1d
	1	4000 \pm 128b	4025 \pm 133b	60 \pm 3b	825 \pm 28b	43 \pm 2b
Giza 429	0	2925 \pm 101c	3300 \pm 120c	50 \pm 3c	700 \pm 22c	38 \pm 1c
	1	4300 \pm 148a	4500 \pm 154a	94 \pm 4a	880 \pm 35a	50 \pm 2a

[#]Values are means \pm SE (n=9). Mean values in each column followed by a different lower-case-letter are significantly different by Fisher's least-significant difference test (LSD) at $p \leq 0.05$ (Measurements were made in 50-day-old plants).

Table 7. Effect of exogenous treatment of salicylic acid (SA; mM) on the leaf anatomy of two varieties of *Vicia faba* L. grown in reclaimed-saline calcareous soil

Treatment		Leaf blade thickness (μ)	Midvein thickness (μ)	Vascular bundle length (μ)	Vascular bundle width (μ)	No. of xylem vessels
Variety	SA					
Giza 40	0	420 \pm 16c [#]	700 \pm 27c	180 \pm 7c	200 \pm 9d	15 \pm 1d
	1	504 \pm 21b	800 \pm 33b	200 \pm 8b	300 \pm 13b	24 \pm 1b
Giza 429	0	430 \pm 17c	730 \pm 30c	200 \pm 8b	250 \pm 11c	21 \pm 1c
	1	540 \pm 24a	980 \pm 41a	250 \pm 9a	350 \pm 15a	36 \pm 2a

[#]Values are means \pm SE (n=9). Mean values in each column followed by a different lower-case-letter are significantly different by Fisher's least-significant difference test (LSD) at $p \leq 0.05$ (Measurements were made in 50-day-old plants).

DISCUSSION

The environmental conditions in the Middle Eastern countries are characterized by some problems, including soil salinity. There are many reasons that cause soil salinity, such as low rainfall, high evaporation rate, poor irrigation water and its management, and accumulation of salts in the top layer of the soil due to over-irrigation, proximity to the sea and the capillarity rise of salts from underground water into the root zone (Rady et

al., 2013). In the present study, the decreased plant growth (Table 1) and productivity (Table 5) under the adverse conditions of the newly-reclaimed saline soil could be attributed to the osmotic effect that resulted from salt stress. These conditions caused increase of abscisic acid (a growth inhibitor), reduction in indole-3-acetic acid (IAA) and gibberellins (growth promoters) and disrupted the water balance of stressed plants. These salinity-induced problems lead to stomatal closure, ionic imbalance, reduction in

photosynthesis, increase in ROS production in chloroplasts, and accumulation of toxic ions, which inhibit plant growth (Rady et al., 2013; Semida and Rady, 2014a; Semida et al., 2014). Results of this study also show that, salt stress negatively affected the efficiency of photosynthesis, stability of cell membranes, relative water content (Table 2), plant nutrient status (Table 3) and plant anatomy (Tables 6 and 7). One of the solutions to overcome these adverse effects of soil salinity and to improve growth and productivity of crop plants is the exogenous application of antioxidants (Manaa et al., 2014; Semida et al., 2014; Klein et al., 2015), including salicylic acid (SA).

Plants develop some complex mechanisms to induce their tolerance to alleviate salt toxicity to reduce soil water potential (Munns and Tester, 2008), protect ion homeostasis, support osmotic adjustments, control stress damage and repair growth regulation (Zhu, 2002). But, that is not enough to obtain a satisfactory crop growth and economic yields under increased soil salinity. Therefore, the exogenous application of antioxidants such as SA supported faba bean plants to overcome soil salinity ($EC_e = 8.88 - 8.95 \text{ dS m}^{-1}$) and was significantly effective in accelerating the restoration of growth processes, increasing growth characteristics (Table 1). The mitigating effect of SA has been reported in different crop plants grown under abiotic stress conditions (Manaa et al., 2014; Semida et al., 2015). This alleviating effect of SA has been attributed to the enhancing role of SA in nutrient uptake (Glass, 1974), water relations (Semida and Rady, 2014b), photosynthetic capacity and plant growth (Popova et al., 2009), as well as in antioxidant defence system (Semida and Rady, 2014b). Exogenous application of SA reduced the adverse effects of soil salinity and increased the growth characteristics of saline-stressed faba bean plants, because SA acts as an iron-chelating molecule which can directly scavenge hydroxyl (OH^\bullet) radicals (Dinis et al., 1994), improving the antioxidant defence system (Idrees et al., 2011) that enables plants to overcome the negative effects of soil salinity. Semida and Rady (2014b) reported that exogenous application of SA was very

effective in reducing adverse effects of salt stress on *Phaseolus vulgaris* seedlings and increased seedling growth that resulted in the improved antioxidant capacity. SA can be considered an important part of the plant defence system, maintaining the integrity and normal function of the photosynthetic apparatus, confirmed by the significant increase in the Fv/Fm and performance index (PI) by the application of SA under salinity stress (Table 2). The reduction in the concentration of Na^+ ions and the increased concentration of K^+ ions in salt-stressed faba bean plants due to the application of 1.0 mM SA may cause maintenance of photosynthetic efficiency measured as Fv/Fm and PI. The accumulation of proline and soluble sugars in salt-stressed faba bean plants in response to SA application (Table 2), may be attributed to the crucial roles played by these two parameters. Proline is a compatible osmolyte with an ability to scavenge free radicals (Matysik et al., 2002). The accumulation of proline is an important physiological index for the response of plants to salt stress (Shi and Yin, 1993). It might have protected cell membranes against ion toxicity and salt-induced oxidative stress, increasing cellular growth (Banu et al., 2009). It was also reported that, the potential mechanism by which proline overcomes free radical damages is the physical quenching of singlet oxygen ($^1\text{O}_2$) and chemical reactions with hydroxyl radicals (Alia et al., 2001), improving water availability and nutritional status, stabilizing the photosynthetic reactions (Abdelhamid et al., 2013) and increasing salt tolerance of faba bean plants. Depending on these possible mechanisms by which SA protects plant against salinity stress, our results suggested that the increase of proline accumulation by SA application in saline-stressed faba bean plants may be, at least in part, responsible for the alleviated photosynthetic efficiency, membrane stability index, relative water content and nutritional status (Tables 2, 3 and 4). In addition, soluble sugars are a major category of organic compatible solutes that play a key role in alleviating salinity stress either by osmotic adjustment or by conferring some desiccation resistance to plant cells

(Pérez-López et al., 2010). The exogenous SA application overcomes the negative effects of salinity stress along with the accumulation of proline and soluble sugars in plant cells, which consequently, could promote the entire plant growth as noted in our results (Tables 1 and 2).

Rafique et al. (2011) reported positive effects of SA in alleviating the leakage of the chloroplast contents in saline-stressed plants. Our findings confirmed this result and showed increased MSI and RWC values in saline-stressed faba bean plants applied with SA (Table 2). The protective role of SA in membrane integrity and regulation of ion uptake was also reported by Erasalan et al. (2007) and Gunes et al. (2007). Results of this study exhibited an improvement in Na^+ ion uptake under soil salinity that was accompanied by corresponding reductions in the concentrations of K^+ and Ca^{2+} , showing an apparent antagonism between K^+ and/or Ca^{2+} and Na^+ (Table 3). Salts in the soil also antagonized N and P elements, reducing their concentrations in plants. However, SA application reversed the status of these ions, increasing the concentrations of K^+ and Ca^{2+} , reducing Na^+ concentration and increasing ratios of K^+/Na^+ and $\text{Ca}^{2+}/\text{Na}^+$ (Table 4), in addition to the increase in the concentrations of N and P that positively reflected in plant growth and yield. This improvement in nutrient concentrations may be attributed to the role of antioxidants, including SA, in increasing osmo-tolerance and/or regulating various processes such as absorption of nutrients from soil solution and enhancing membrane permeability (Table 2). The antagonistic relations between Na^+ and Ca^{2+} and/or K^+ may be taken as an indication of the role played by antioxidants such as SA in modifying K^+/Na^+ and $\text{Ca}^{2+}/\text{Na}^+$ selectivity under salt stress. All improved parameters (i.e., growth traits, plant water relations, concentrations of nutrient elements and their relations with Na^+ ion, and final yields) in response to the foliar application of SA were accompanied with the improved anatomy of faba bean stem and leaf (Tables 6 and 7). Agami (2013) reported a negative effect of

salinity on anatomy of maize plants, but exogenous SA application improved plant anatomy, and that confirms our results. This enhanced stem and leaf anatomy by SA application allowed improved translocation of the absorbed nutrients into healthy cells to be used in different metabolic processes, positively reflecting in vigorous growth and satisfactory yield under the adverse conditions of the tested newly-reclaimed saline soil.

Data of the present study also show that, the variety Giza 429 exhibited better growth, water relations, nutritional status, pod and seed yields and anatomy than the variety Giza 40, concluding that Giza 429 was more salt-tolerant compared to Giza 40.

CONCLUSIONS

SA is a plant hormone-like substance and is considered one of the antioxidants that can play different roles in plant metabolism. It can also play important roles in alleviation of a wide range of biotic and abiotic stresses. Exogenous application of SA enabled faba bean plants to tolerate soil salinity at different levels, by restoration of their vigorous growth, recovery of plant water relations maintenance of the photosynthetic efficiency and rehabilitation of the nutritional status due to the improvement in stem and leaf anatomy that positively reflected in the final yields. Therefore, this study recommends using SA at the level of 1 mM for the faba bean variety Giza 429 when grown under the newly-reclaimed saline soils.

REFERENCES

- Abdelhamid, M.T., Rady, M.M., Osman, A.Sh., Abdalla, M.A., 2013. *Exogenous application of proline alleviates salt-induced oxidative stress in Phaseolus vulgaris L. plants*. Journal of Horticultural Science and Biotechnology, 88: 439-446.
- Agami, R.A., 2013. *Alleviating the adverse effects of NaCl stress in maize seedlings by pretreating seeds with salicylic acid and 24-epibrassinolide*. South African Journal of Botany, 88: 171-177.
- Alia, J.M., Mohanty, P., Matsyik, J., 2001. *Effect of proline on the production of singlet oxygen*. Amino Acids, 21: 195-200.

- Banu, M.N., Hoque, M.A., Watanabe-Sugimoto, M., Matsuoka, K., Nakamura, Y., Shimoishi, Y., Murata, Y. 2009. *Proline and glycinebetaine induce antioxidant defense gene expression and suppress cell death in cultured tobacco cells under salt stress*. Journal of Plant Physiology, 166: 146-156.
- Bates, L.S., Waldeen, R.P., Teare, I.D., 1973. *Rapid determination of free proline for water stress studies*. Plant and Soil, 39: 205-207.
- Broughton, W.J., Hernandez, G., Blair, M., Beebe, S., Gepts, P., Vanderleyden, J. 2003. *Beans (Phaseolus spp.) – model food legumes*. Plant and Soil, 252: 55-128.
- Chapman, H.D., Pratt, P.F., 1961. *Methods of Analysis for Soil, Plants and Water*. pp. 56-63, University of California, Division of Agricultural Science, Berkeley, CA, USA.
- Chunthaburee, S., Sanitchon, J., Pattanagul, W., Theerakulpisut, P., 2015. *Effects of salt stress after late booting stage on yield and antioxidant capacity in pigmented rice grains and alleviation of the salt-induced yield reduction by exogenous spermidine*. Plant Production Science, 18(1): 32-42.
- Clark, A.J., Landolt, W., Bucher, J.B., Strasser, R.J., 2000. *Beech (Fagus sylvatica) response to ozone exposure assessed with a chlorophyll a fluorescence performance index*. Environmental Pollution, 109: 501-507.
- Dahnke, W.C., Whitney, D.A., 1988. *Measurement of soil salinity*. In: Dahnke, W.C. (Ed.), Recommended chemical soil test procedures for the north central region, vol. 221, pp. 32-34, North Dakota Agric. Exp. St. Bulletin. 499, North Central Regional Publications.
- Delgado, M.J., Ligeró, F., Lluch, C., 1994. *Effects of salt stress on growth and nitrogen fixation by pea, faba bean, common bean, and soybean plants*. Soil Biology and Biochemistry, 26: 371-376.
- Eraslan, F., Inal, A., Gunes, A., Alpaslan, M., 2007. *Impact of exogenous salicylic acid on the growth, antioxidant activity and physiology of carrot plants subjected to combined salinity and boron toxicity*. Scientia Horticulturae, 113: 120-128.
- Glass, A.D.M., 1974. *Influence of phenolic acid on ion uptake*. III. Inhibition of potassium absorption. Journal of Experimental Botany, 25: 1104-1113.
- Gomez, K.A., Gomez, A.A., 1984. *Statistical Procedures for Agricultural Research*, 2nd ed., p. 680, John Wiley & Sons, Singapore.
- Gunes, A., Inal, A., Alpaslan, M., Eraslan, F., Bagci, E.G., Cicek, 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*. Journal of Plant Physiology, 164: 728-736.
- Guo, R., Zhou, J., Hao, W., Gu, F., Liu, Q., Li, H., Xia, X., Mao, L., 2014. *Germination, Growth, Chlorophyll Fluorescence and Ionic Balance in Linseed Seedlings Subjected to Saline and Alkaline Stresses*. Plant Production Science, 17(1): 20-31.
- Hafez, A.R., Mikkelsen, D.S., 1981. *Colorimetric determination of nitrogen for evaluating the nutritional status of rice*. Communications in Soil Science and Plant Analysis, 12: 61-69.
- Hayat, S., Ali, B., Hasan, S.A., Ahmad, A., 2007. *Brassinosteroid enhanced the level of antioxidants under cadmium stress in Brassica juncea*. Environmental and Experimental Botany, 60: 33-41.
- Idrees, M., Naeem, M., Aftab, T., Khan, M.M.A., Moinuddin, 2011. *Salicylic acid mitigates salinity stress by improving antioxidant defence system and enhances vincristine and vinblastine alkaloids production in periwinkle [Catharanthus roseus (L.) G. Don]*. Acta Physiologiae Plantarum, 33: 987-999.
- Irigoyen, J.J., Emerich, D.W., Sanchez-Diaz, M., 1992. *Water stress induced changes in the concentrations of proline and total soluble sugars in nodulated alfalfa (Medicago sativa) plants*. Physiologia Plantarum, 8: 455-460.
- Jackson, M.L., 1967. *Soil Chemical Analysis*, pp. 144-197, 326-338, Prentice Hall of India Pvt. Ltd, New Delhi, India.
- Khan, W., Prjithivira, B., Smith, A., 2003. *Photosynthetic responses of corn and soybean to foliar application of salicylates*. Journal of Plant Physiology, 160: 485-492.
- Klein, A., Keyster, M., Ludidi, N., 2015. *Response of soybean nodules to exogenously applied caffeic acid during NaCl-induced salinity*. South African Journal of Botany, 96: 13-18.
- Manaa, A., Gharbi, E., Mimouni, H., Wasti, S., Aschi-Smiti, S., Lutts, S., Ben Ahmed, H., 2014. *Simultaneous application of salicylic acid and calcium improves salt tolerance in two contrasting tomato (Solanum lycopersicum) cultivars*. South African Journal of Botany, 95: 32-39.
- Mass, E.V., Hoffman, G.J., 1977. *Crop salt tolerance: current assessment*. American Society of Civil Engineers, 103: 115-134.
- Matysik, J., Alia, B.B., Mohanty, P., 2002. *Molecular mechanisms of quenching of reactive oxygen species by proline under stress in plants*. Current Science, 82: 525-532.
- Maxwell, K., Johnson, G.N., 2000. *Chlorophyll fluorescence - a practical guide*. Journal of Experimental Botany, 51: 659-668.
- Munns, R., Tester, M., 2008. *Mechanisms of salinity tolerance*. Annual Review of Plant Biology, 59: 651-681.
- Nazar, R., Iqbal, N., Masood, A., Syeed, S., Khan, N.A., 2011. *Understanding the significance of sulphur in improving salinity tolerance plants*. Environmental and Experimental Botany, 70: 80-87.
- Page, A.I., Miller, R.H., Keeny, D.R., 1982. *Methods of soil analysis*. Part II, Chemical and

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- Microbiological Methods, pp. 225-246, 2nd ed. Amer. Soc. Agron., Madison, WI, USA.
- Pérez-López, U., Robredo, A., Lacuesta, M., Muñoz-Rueda, A., Mena-Petite, A., 2010. *Atmospheric CO₂ concentration influences the contributions of osmolytes accumulation and cell wall elasticity to salt tolerance in barley cultivars.* Journal of Plant Physiology, 167: 15-22.
- Rady, M.M., 2011. *Effect of 24-epibrassinolide on growth, yield, antioxidant system and cadmium content of bean (Phaseolus vulgaris L.) plants under salinity and cadmium stress.* Scientia Horticulturae, 129: 232-237.
- Rady, M.M., Bhavya Varma, C., Howladar, S.M., 2013. *Common bean (Phaseolus vulgaris L.) seedlings overcome NaCl stress as a result of presoaking in Moringa oleifera leaf extract.* Scientia Horticulturae, 162: 63-70.
- Rafique, N., Raza, S.H., Qasim, M., Iqbal, N., 2011. *Presowing application of ascorbic acid and salicylic acid to seed of pumpkin and seedling response to salt.* Pakistan Journal of Botany, 43: 2677-2682.
- Saha, P., Chatterjee, P., Biswas, A.K., 2010. *NaCl pre-treatment alleviates salt stress by enhancement of antioxidant defense system and osmolyte accumulation in mungbean (Vigna radiata L. Wilczek).* Indian Journal of Experimental Biology, 48: 593-600.
- Sairam, R.K., Tyagi, A., 2004. *Physiology and molecular biology of salinity stress tolerance in plants.* Current Science, 86: 407-421.
- Sass, J.A., 1961. *Botanical Microtechnique*, 3rd ed., The Iowa State Univ. Press, Ames, Iowa, USA.
- Saxena, N.P., Johansen, C., Saxena, M.C., Silim, S.N., 1993. *Selection for drought and salinity tolerance in cool-season food legumes.* In: Singh, K.B., Saxena, M.C. (Eds.), *Breeding for Stress Tolerance in Cool-Season Food Legumes*, pp. 245-270, John Wiley & Sons, New York.
- Semida, W.M., Rady, M.M., 2014a. *Presoaking application of propolis and maize grain extracts alleviates salinity stress in common bean (Phaseolus vulgaris L.).* Scientia Horticulturae, 168: 210-217.
- Semida, W.M., Rady, M.M., 2014b. *Pre-soaking in 24-epibrassinolide or salicylic acid improves seed germination, seedling growth, and anti-oxidant capacity in Phaseolus vulgaris L. grown under NaCl stress.* Journal of Horticultural Science and Biotechnology, 89: 338-344.
- Semida, W.M., Rady, M.M., Abd El-Mageed, T.A., Howladar, S.M., Abdelhamid, M.T., 2015. *Alleviation of cadmium toxicity in common bean (Phaseolus vulgaris L.) plants by the exogenous application of salicylic acid.* Journal of Horticultural Science and Biotechnology, 90: 83-91.
- Semida, W.M., Taha, R.S., Abdelhamid, M.T., Rady, M.M., 2014. *Foliar-applied α-tocopherol enhances salt-tolerance in Vicia faba L. plants grown under saline conditions.* South African Journal of Botany 95: 24-31.
- Shakirova, F.M., Sakhabutdinova, A.R., Bezrukova, M.V., Fathudinova, R.A., Fathutdinova, D.R., 2003. *Changes in hormonal status of wheat seedlings induced by salicylic acid and salinity.* Plant Science, 164: 317-322.
- Shi, D.C., Yin, L.J., 1993. *Difference between salt (NaCl) and alkaline (Na₂CO₃) stresses on Puccinellia tenuiflora (Griseb.) Scribn et Merr. plants.* Acta Botanica Sinica, 35: 144-149.
- Subbarao, G.V., Johansen, C.J., 1993. *Potential for genetic improvement in salinity tolerance in legumes.* In: Pessarakli, M. (Ed.), *Handbook of Plant and Crop Stress*, pp. 581-591, Marcel Dekker, New York.
- Xu, Q., Xu, X., Zhao, Y., Jiao, K., Herbert, J.S., Hao, L., 2008. *Salicylic acid, hydrogen peroxide and calcium-induced salinity tolerance associated with endogenous hydrogen peroxide homeostasis in naked oat seedlings.* Plant Growth Regulation, 54: 249-259.
- Yasar, F., Kusvuran, S., Elialtiolu, S., 2006. *Determination of antioxidant activities in some melon (Cucumis melo L.) varieties and cultivars under salt stress.* Journal of Horticultural Science and Biotechnology, 81: 627-630.
- Yasuta, Y., Kokubun, M., 2014. *Salinity Tolerance of Super-Nodulating Soybean Genotype En-b0-1.* Plant Production Science, 17(1): 32-40.
- Yuan, S., Lin H.H., 2008. *Role of salicylic acid in plant abiotic stress.* Zeitschrift für Naturforschung C, 63: 313-320.
- Zhu, J.K., 2002. *Salt and drought stress signal transduction in plants.* Annual Review of Plant Biology, 53: 247-273.