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Foliar-applied α-tocopherol enhances salt-tolerance in *Vicia faba* L. plants grown under saline conditions

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ABSTRACT

The effect of foliar-applied α-tocopherol (αTOC) on salt tolerance in two varieties of faba beans (i.e., Giza 40 and Giza 429) grown under saline soil conditions was investigated. Salinity stress caused a significant reduction in growth traits, physiological attributes, yields and anatomy of the two faba bean varieties. αTOC-treated plants, grown under the abovementioned adverse conditions, had enhanced all growth parameters (i.e., shoot length, numbers of leaves and branches, leaf area, and shoot fresh and dry weights) and yield and its components (i.e., number of dry pod per plant, average 100-seed weight, and dry seed yield per plant and per hectare) of both varieties compared to control plants. In addition, performance index, relative water content, membrane stability index, nutrients and their relations, and anatomy of stem and leaf were significantly improved in αTOC-treated plants compared to control plants. Giza 429 was generated better growth and yield reflecting more salt-tolerance than Giza 40. Results of this study suggested that αTOC as antioxidant could activate the antioxidants in plants to enable them to alleviate the oxidative damage leading to improvements in physiological attributes in plants grown under the adverse conditions of newly-reclaimed saline soils.

1. Introduction

Faba beans (*Vicia faba* L.) are popular legume foods and consumed worldwide as an important protein source for human and animal nutrition (Cazzato et al., 2012). Faba bean seeds are rich in carbohydrates (51–68% of dry matter; Nachi and Guen, 1996) and proteins (28–30% of dry matter; Burbano et al., 1995), which are considerable among vegetables. Legumes are considered either sensitive or only moderately tolerant to salinity (Subbarao and Johansen, 1993). However, Saxena et al. (1993) reported that there is a considerable variability in salinity tolerance among legumes. The *V. faba* plants are proved to be moderately sensitive to salinity (Delgado et al., 1994), showing 50% reduction in plant growth under 6.7 dS m^{-1} salinity (Mass and Hoffman, 1977). In Egypt, breeding faba beans for salt tolerance has usually been limited by the lack of reliable traits for selection. Therefore, it is necessary to determine the differences in resistance mechanisms between genotypes and to incorporate characteristics, which improve genotype tolerance into reasonably high yielding backgrounds (Noble and Rogers, 1992). The differences in salt tolerance of faba bean genotypes were observed by Gaballah and Gomaa (2005) based on their growth and enzymatic activity. In addition, Abdelhamid et al. (2010) found that the broomrape-tolerant faba bean cultivars are more salinity-tolerant than broomrape-susceptible cultivars.

In the near future, there is a tendency for the agricultural expansion of many crops, including faba beans in newly-reclaimed soils in Egypt. However, most of these soils are affected by salinity. Salinity stress is one of the abiotic stresses, which negatively affect crop production in different regions, particularly in arid and semi-arid regions. There are over 800 million hectares of land worldwide are affected by salinity (Munns, 2005). High salinity causes ion toxicity and osmotic stress, leading to the excessive production of reactive oxygen species (ROS) in plant cells, including superoxide radicals, hydrogen peroxide, hydroxyl anions, and singlet oxygen. These ROS cause damage to lipids, proteins and DNA (Yasar et al., 2006). Salinity stress causes osmotic stress, ion imbalance and direct toxic effects of ions on the metabolic processes, which are the most important and widely studied physiological deterioration (Munns et al., 2006). These effects under salinity stress are usually created by the increased Na^+ and Cl^- levels in soils. Salinity stress adversely affects the morphological, physiological and biochemical responses of plants (Nazar et al., 2011). It reduces photosynthetic attributes (Khan et al., 2009; Saha et al., 2010) and disturbs plant growth and development (Sairam and Tyagi, 2004). Several mechanisms are being developed by plants to induce tolerance to overcome the adverse effects of salinity, including antioxidants.

Efforts have been made to control salinity by various means, including foliar application of plants with some antioxidants, for developing

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the sustainable agriculture. α-Tocopherol (αTOC) is a low molecular weight lipophilic membrane-located antioxidant. It protects cell membranes from oxidative damage (Asada, 1999) and polyunsaturated fatty acids from lipid peroxidation (Krieger-Liszkay and Trebst, 2006), and improves membrane stability and permeability. It helps also to provide an optimal environment for the photosynthetic machinery (Wise and Naylor, 1987). There was a positive correlation between αTOC and shoot/root growth in two grass species; tall fescue and creeping bentgrass (Zhang and Schmidt, 2000).

Up till now, there are no Egyptian cultivars of faba bean produced as salinity tolerant that could be recommended for cultivation in the newly-reclaimed and salt-affected soils. Therefore, the aim of this study is to evaluate the salt tolerance of two Egyptian faba bean cultivars and their different responses to different levels of αTOC under the adverse conditions of a reclaimed saline soil with regard to growth characteristics, physiological attributes, accumulation of ions and their relations and the obtained yield.

2. Materials and methods

2.1. 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 the Faculty of Agriculture, Fayoum University, Southeast Fayoum (29° 17′N; 30° 53′E), Egypt. In the 2011/2012 season, the daily temperatures averaged 21.2° ± 2.6 °C, and the daily relative humidity averaged 58.4 ± 5.1%. In the 2012/2013 season, the daily temperatures averaged 22.1° ± 2.8 °C, and the daily relative humidity averaged 60 ± 4.9%.

Healthy seeds of two varieties (i.e., Giza 40 and Giza 429) of faba bean (*V. faba* L.) were sown on 22 October 2011, and on 25 October 2012. Seeds were obtained from Horticulture Research Institute, Agricultural Research Centre, Giza, Egypt, and were sown at the equivalent of 120 kg ha^{-1} to achieve the recommended planting density. Faba bean seeds were selected for uniformity by choosing those of equal size and of the same color. 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 × 3.5 m plots. Thinning was done before the first irrigation to produce 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. These recommendations were for 450 kg ha^{-1} calcium superphosphate (15.5% P_2O_5), 250 kg ha^{-1} ammonium sulfate (20.5% N), and 120 kg ha^{-1} potassium sulfate (48% K_2O) during seed-bed preparation. Irrigation water was added to 100% of the reference crop evapotranspiration (ETo), values from the Fayoum Meteo Station. Seven irrigations were applied in each season, with total water rates of about 2800 m^3 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 according to Black et al. (1965) and Jackson (1973). 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 the 2011/2012 and 2012/2013, respectively]. The other main characteristics of the soil in the two growing seasons were: pH [at a soil:water (w/v) ratio of 1:2.5], 7.74 and 7.80; EC (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, respectively. Based on the above EC values classed the soil as being strongly saline according to Dahnke and Whitney (1988). The experiments were arranged in a randomized complete block design, with four levels of αTOC (0, 0.25, 0.50 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), 0.25, 0.50 and 1.0 mM αTOC, and then the sprays were repeated at 30 and 40 DAS. The concentration of Vit-E (Yongyi Chemicals Group Co., Ltd., China), and the number and timing of sprays were based on results from a preliminary pot trial (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.

2.2. Plant growth and yield analyses

Fifty-day-old faba bean 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, and then placed in an oven at 80 °C for 24 h. The dried shoots were weighed to record their dry weights. Leaf areas were measured manually using a graph sheet, where the squares covered by the leaf were counted to note the leaf area. At the end of each experiment (2 April 2012 and 3 April 2013), all the dry pods on each plant in each experimental plot were collected and counted. The dry seeds of faba bean were then extracted from the pods and weighed to calculate the average 100-seed weight, and seed yield per plant and per hectare.

2.3. Measurement of proline and total soluble sugar concentrations

Proline contents (in mg 100 g^{-1} DW of leaf) were measured using the rapid colorimetric method outlined by Bates et al. (1973). Proline 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 ×*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 then extracted with 5 ml of toluene and 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 then 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 sugars (TSS) were extracted and determined as suggested by Irigoyen et al. (1992). A sample of 0.2 g dried leaves 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 ×*g* for 10 min and the supernatant was stored at 4 °C prior to measurement. TSS 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 Bauschand Lomb-2000 Spectronic Spectrophotometer.

2.4. Measurement of chlorophyll fluorescence

Chlorophyll fluorescence was measured on two different sunny days using a portable fluorometer (Handy PEA, Hansatech Instruments Ltd, Kings Lynn, UK). One leaf (the same age) was chosen per plant from three plants in each experimental plot of each treatment (n = 9). Fluorescence measurements included: Maximum quantum yield of PS II F_v/F_m was calculated as; $F_v/F_m = (F_m - F_o)/F_m$ (Maxwell and Johnson, 2000). Performance index of photosynthesis based on the

Table 1
Effect of exogenously applied α-tocopherol (αTOC; mM) on growth characteristics of two varieties of *Vicia faba* L. grown in reclaimed-saline calcareous soil.

Treatment		Shoot length (cm)	Leaves no.	Leaves area (dm^2)	Branches no.	Shoot FW (g)	Shoot DW (g)
Var.	αTOC						
G 40	0	30.7 ± 0.6c	12.3 ± 0.3c	5.8 ± 0.3c	1.0 ± 0.1d	28.4 ± 3.1d	4.4 ± 0.2d
	0.25	49.0 ± 1.0b	16.0 ± 0.5b	10.0 ± 0.4b	2.0 ± 0.2c	63.0 ± 6.2c	9.0 ± 0.6c
	0.50	56.0 ± 2.5a	18.7 ± 0.8a	15.7 ± 0.6a	3.3 ± 0.2a	103.4 ± 8.1a	14.5 ± 0.9a
	1	55.0 ± 1.7a	16.0 ± 0.5b	10.9 ± 0.5b	2.7 ± 0.1b	91.8 ± 7.5b	11.2 ± 0.8b
	Means	**47.7B**	**15.8B**	**10.6B**	**2.3B**	**71.7B**	**9.8B**
G 429	0	36.7 ± 0.8c	14.7 ± 0.4d	6.2 ± 0.1d	2.0 ± 0.1c	57.1 ± 3.3d	7.6 ± 0.3b
	0.25	51.7 ± 1.4b	17.7 ± 0.6b	13.1 ± 0.7b	3.0 ± 0.2b	89.3 ± 6.8b	11.3 ± 0.6a
	0.50	59.3 ± 2.8a	19.0 ± 0.8a	16.1 ± 1.0a	4.3 ± 0.2a	113.3 ± 8.4a	13.4 ± 0.7a
	1	57.0 ± 1.9a	16.0 ± 0.5c	11.6 ± 0.4c	3.3 ± 0.1b	80.9 ± 6.1b	11.1 ± 0.6a
	Means	**51.2A**	**16.9A**	**11.8A**	**3.2A**	**85.2A**	**10.9A**

Mean values (n = 9) in the same column for each trait with the same lower small or upper bold-case letters are not significantly different by Duncan's multiple range test at $P \leq 0.05$.
mM = millimole; cm = centimeter; dm = decimeter; FW = fresh weight; DW = dry weight; Var. = variety; G 40 = Giza 40; G 429 = Giza 429.
Measurements were made in 50-day-old plants.

equal absorption (PI_{ABS}) was calculated as reported by Clark et al. (2000).

2.5. Determination of membrane stability index and relative water content

Membrane stability indices (MSI) were estimated, in 9 samples for each treatment, using duplicate 0.2 g samples of fully-expanded leaf tissue (Rady, 2011). One 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. The electrical conductivity (C_1) of the solution was recorded using a conductivity bridge. The second sample was boiled at 100 °C for 10 min, and the conductivity was also measured (C_2). The formula: MSI (%) = $[1 - (C_1 \div C_2)] \times 100$ was applied for calculating the MSI.

Excluding the midrib, fresh 2 cm-diameter fully-expanded leaf discs (n = 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 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: RWC (%) = $[(FM - DM) \div (TM - DM)] \times 100$.

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

Leaf N contents (in mg g^{-1} DW) were determined (Hafez and Mikkelsen, 1981) using the Orange-G dye method. 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. Ground plant material (0.2 g leaf tissue) 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, 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 contents were calculated using the formulae:

$$N(\%) = 0.39 + 0.954 \times \text{Dye absorbed}(g/100\ g) \text{ and}$$
$$\text{Dye absorbed}(g/100\ g) = (a–b/a)(cfv/w) \times 100$$

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 color method (Jackson, 1967), in sulphuric acid (with reduction to exclude arsenate), was used to determine P contents (in mg g^{-1} DW). Sulphomolybdic acid (molybdenum blue), diluted sulphomolybdic acid, and 8% (w/v) sodium bisulphite-H_2SO_4 solution were used as reagents. Leaf K^+ and Na^+ ion contents (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 Ca^{2+} was determined using a Perkin-Elmer Model 3300 Atomic Absorption Spectrophotometer (Chapman and Pratt, 1961).

Table 2
Effect of exogenously applied α-tocopherol (αTOC; mM) on physiological attributes of two varieties of *Vicia faba* L. grown in reclaimed-saline calcareous soil.

Treatment		Free proline (mg g^{-1} DW)	Total soluble sugars (mg g^{-1} DW)	F_v/F_m	PI	MSI (%)	RWC (%)
Var.	αTOC						
G 40	0	0.61 ± 0.02a	6.7 ± 0.3a	0.81 ± 0.03	3.3 ± 0.2c	48.8 ± 3.2c	42.7 ± 1.5c
	0.25	0.38 ± 0.01b	4.4 ± 0.2b	0.82 ± 0.03	3.7 ± 0.3b	55.8 ± 3.5b	48.3 ± 1.9b
	0.50	0.30 ± 0.01c	3.5 ± 0.2c	0.84 ± 0.06	5.1 ± 0.4a	61.0 ± 4.2a	53.7 ± 2.3a
	1	0.38 ± 0.01b	5.7 ± 0.2b	0.82 ± 0.04	3.8 ± 0.3b	54.9 ± 3.2b	48.0 ± 1.7b
	Means	**0.42A**	**5.1A**	**0.82A**	**4.1B**	**55.1B**	**46.2B**
G 429	0	0.76 ± 0.02a	6.1 ± 0.3a	0.81 ± 0.02	3.4 ± 0.2c	51.7 ± 3.1c	47.0 ± 1.8c
	0.25	0.33 ± 0.01b	5.3 ± 0.2b	0.82 ± 0.03	4.7 ± 0.2b	61.1 ± 3.8b	55.0 ± 2.3b
	0.50	0.27 ± 0.01c	4.5 ± 0.1c	0.83 ± 0.05	7.6 ± 0.4a	65.7 ± 4.0a	60.7 ± 2.4a
	1	0.34 ± 0.01b	5.2 ± 0.2b	0.81 ± 0.02	5.1 ± 0.3b	59.0 ± 3.4b	54.7 ± 2.1b
	Means	**0.43A**	**5.3A**	**0.82A**	**5.2A**	**59.4A**	**52.9A**

Mean values (n = 9) in the same column for each trait with the same lower small or upper bold-case letters are not significantly different by Duncan's multiple range test at $P \leq 0.05$.
mM = millimole; mg = milligram; DW = dry weight; Var. = variety; G 40 = Giza 40; G 429 = Giza 429; 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.
Measurements were made in 50-day-old plants.

2.7. Anatomical study

For anatomical study, stem and leaf samples were taken 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. Thereafter, samples were washed in 50% ethyl alcohol, dehydrated and cleared in tertiary butyl alcohol series, embedded in paraffin wax of 54–56 °C m.p. Cross sections, 20 μ thick, were cut by a rotary microtome, adhesived by Haupt's adhesive and stained with the crystal violet–erythrosine combination (Sass, 1961), cleared in carbol xylene and mounted in Canada balsam. The sections observed and documented using an upright light microscope (AxioPlan, Zeiss, Jena, Germany). Measurements were done, using a micrometer eye-piece and an average of five readings were calculated.

2.8. Statistical analysis

All data were subjected to analysis of variance (ANOVA) for a randomized complete block design, after testing for homogeneity of error variances according to the procedure outlined by Gomez and Gomez (1984). Combined analysis of data of the two seasons was conducted and significant differences between treatments were compared at $P \leq 0.05$ by Duncan's multiple range test.

3. Results

3.1. Growth characteristics of V. faba L. varieties

Foliar applications of α-tocopherol (αTOC) at different levels (i.e., 0.25, 0.50 and 1.0 mM) to two varieties of faba bean (i.e., Giza 40 and Giza 429) significantly increased all tested growth traits such as 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 level of 0.50 mM αTOC was found to be most effective, increasing the above growth characteristics by 82.4, 52.0, 170.7, 230.0, 264.1 and 229.5%, respectively for Giza 40, and by 61.6, 29.3, 159.7, 115, 98.4 and 76.3%, respectively for Giza 429 compared to the controls. For varieties, there are significant increases of Giza 429 growth traits compared to Giza 40.

3.2. Physiological attributes of V. faba L. varieties

Except F_v/F_m, all three tested levels of αTOC significantly reduced free proline and total soluble sugars, while increased significantly PI, MSI and RWC compared to the controls in the two faba bean cultivars (Table 2). Regarding PI, MSI and RWC, αTOC level of 0.50 mM was most effective, exceeding the control values by 54.5 and 123.5%, 25.0 and 27.1%, and 25.8 and 29.1% for Giza 40 and Giza 429, respectively. Giza 429 was better than Giza 40 in tolerating the adverse effects of reclaimed saline soil conditions.

Table 4
Effect of exogenously applied α-tocopherol (αTOC; mM) on relations of Ca and/or K with Na of two varieties of *Vicia faba* L. grown in reclaimed-saline calcareous soil.

Treatment		Ca:Na ratio	K:Na ratio	K + Ca:Na
Var.	αTOC			
G 40	0	0.24 ± 0.01	1.40 ± 0.05	1.64 ± 0.07
	0.25	0.45 ± 0.02	2.19 ± 0.07	2.64 ± 0.10
	0.50	1.56 ± 0.06	4.58 ± 0.15	6.14 ± 0.27
	1	0.76 ± 0.04	3.05 ± 0.11	3.81 ± 0.17
	Means	**0.75B**	**2.81B**	**3.56B**
G 429	0	0.51 ± 0.02	2.24 ± 0.08	2.75 ± 0.12
	0.25	0.96 ± 0.04	3.19 ± 0.14	4.15 ± 0.19
	0.50	2.04 ± 0.08	5.52 ± 0.17	7.56 ± 0.27
	1	0.99 ± 0.05	4.34 ± 0.15	5.33 ± 0.22
	Means	**1.13A**	**3.82A**	**4.95A**

Mean values (n = 9) in the same column for each trait with the same lower small or upper bold-case letters are not significantly different by Duncan's multiple range test at $P \leq 0.05$.
mM = millimole; Var. = variety; G 40 = Giza 40; G 429 = Giza 429; K = potassium; Ca = calcium; Na = sodium.
Measurements were made in 50-day-old plants.

3.3. Nutrient status of V. faba L. varieties

Plants of the two faba bean varieties sprayed with 0.25, 0.50 and 1.0 mM αTOC had significantly increased contents of the tested nutrients (i.e., N, P, K and Ca), while contained reduced contents of Na compared to the water-sprayed control plants (Table 3). The relations between useful nutrients (K and Ca) and harmful elements (Na) were higher in favor of αTOC levels compared to the controls (Table 4). The best αTOC level was 0.50 mM that increased N, P, K and Ca nutrients in the Giza 40 and Giza 429 varieties by 49.6 and 37.2%, 53.2 and 68.8%, 61.7 and 47.8%, and 216.5 and 142.7%, respectively, and reduced Na contents by 50.7 and 48.5%, respectively compared to the controls. There is a clear superiority of Giza 429 in collecting the useful nutrients and excluding the harmful elements compared to Giza 40.

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

All αTOC levels tested (i.e., 0.25, 0.50 and 0.1 mM) 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). The αTOC level of 0.50 mM was most effective,

Table 3
Effect of exogenously applied α-tocopherol (αTOC; mM) on nutrient status of two varieties of *Vicia faba* L. 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)
Var.	αTOC					
G 40	0	23.8 ± 0.5c	0.62 ± 0.02d	11.5 ± 0.3c	2.00 ± 0.08d	8.24 ± 0.28a
	0.25	28.2 ± 0.6b	0.71 ± 0.01c	16.3 ± 0.5b	3.33 ± 0.11c	7.44 ± 0.24b
	0.50	35.6 ± 0.7a	0.95 ± 0.01a	18.6 ± 0.6a	6.33 ± 0.21a	4.06 ± 0.16d
	1	29.3 ± 0.6b	0.80 ± 0.01b	16.6 ± 0.6b	4.33 ± 0.13b	5.44 ± 0.19c
	Means	**29.2B**	**0.77B**	**15.8B**	**4.00B**	**6.30A**
G 429	0	26.6 ± 0.3c	0.64 ± 0.03d	13.4 ± 0.4c	3.02 ± 0.12d	6.97 ± 0.15a
	0.25	31.9 ± 0.4b	0.84 ± 0.02c	16.9 ± 0.6b	5.10 ± 0.18b	5.29 ± 0.14b
	0.50	36.5 ± 0.7a	1.08 ± 0.02a	19.8 ± 0.7a	7.33 ± 0.23a	3.59 ± 0.13d
	1	32.5 ± 0.4b	0.88 ± 0.02b	17.9 ± 0.6b	4.09 ± 0.15c	4.12 ± 0.13c
	Means	**31.9A**	**0.86A**	**17.0A**	**4.89A**	**4.74B**

Mean values (n = 9) in the same column for each trait with the same lower small or upper bold-case letters are not significantly different by Duncan's multiple range test at $P \leq 0.05$.
mM = millimole; mg = milligram; DW = dry weight; Var. = variety; G 40 = Giza 40; G 429 = Giza 429; N = nitrogen; P = phosphorus; K = potassium; Ca = calcium; Na = sodium.
Measurements were made in 50-day-old plants.

Table 5
Effect of exogenously applied α-tocopherol (αTOC; 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 yield plant^{-1} (g)	Dry yield hectare^{-1} (ton)
Var.	αTOC				
G 40	0	7.8 ± 0.2d	66.1 ± 0.5d	21.5 ± 1.5d	1.20 ± 0.05c
	0.25	15.9 ± 0.3c	70.6 ± 0.5c	37.1 ± 1.9b	1.30 ± 0.05b
	0.50	23.8 ± 0.5a	81.7 ± 0.7a	48.9 ± 2.5a	1.42 ± 0.06a
	1	21.6 ± 0.4b	76.0 ± 0.6b	26.3 ± 1.8c	1.33 ± 0.06b
	Means	**17.3B**	**73.6B**	**33.5B**	**1.31B**
G 429	0	7.3 ± 0.3d	72.5 ± 0.6c	21.9 ± 1.2d	1.26 ± 0.06c
	0.25	23.7 ± 0.7b	76.6 ± 0.6b	46.6 ± 2.3b	1.39 ± 0.07b
	0.50	26.7 ± 0.7a	81.9 ± 0.7a	51.2 ± 2.6a	1.58 ± 0.09a
	1	18.0 ± 0.5c	76.9 ± 0.6b	32.0 ± 1.7c	1.43 ± 0.07b
	Means	**18.9A**	**77.0A**	**37.9A**	**1.42A**

Mean values (n = 9) in the same column for each trait with the same lower small or upper bold-case letters are not significantly different by Duncan's multiple range test at $P \leq 0.05$.
mM = millimole; g = gram; Var. = variety; G 40 = Giza 40; G 429 = Giza 429.
Measurements were made in 50-day-old plants.

Table 7
Effect of exogenously applied α-tocopherol (αTOC; 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
Var.	αTOC					
G 40	0	420	800	200	200	15
	0.25	450	800	240	150	26
	0.50	490	820	250	160	38
	1	430	840	230	150	32
	Means	**448**	**815**	**230**	**165**	**28**
G 429	0	430	730	250	200	21
	0.25	410	750	220	180	18
	0.50	600	1050	280	250	25
	1	400	740	210	170	20
	Means	**460**	**818**	**240**	**200**	**21**

Mean values (n = 1).
mM = millimole; μ = micrometer; Var.= variety; G 40 = Giza 40; G 429 = Giza 429.
Measurements were made in 50-day-old plants.

exceeding the control values in Giza 40 and Giza 429 by 205.1 and 265.8%, 23.6 and 13.0%, 127.4 and 133.8%, and 18.3 and 25.4% for the abovementioned yield and its components, respectively. Giza 429 was generated more yield and its components than Giza 40.

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

Plants of the two faba bean varieties sprayed with 0.25, 0.50 and 1.0 mM αTOC had improved stem and leaf anatomy compared to the anatomy of tap water-sprayed control plants (Tables 6, 7 and Figs. 1, 2). In general, the best αTOC level was 0.50 mM that increased stem length, stem width, stem pith diameter, leaf blade thickness, leaf vascular bundle length and number of leaf xylem vessels in the Giza 40 and Giza 429 varieties by 28.6 and 47.0%, 26.8 and 36.4%, 38.7 and 47.4%, 16.7 and 39.5%, 25.0 and 12.0%, and 153.3 and 19.0%, respectively compared to the controls. There is a better anatomy in favor of Giza 429 than that of Giza 40.

4. Discussion

Agricultural productivity faces a great problem due to salinity worldwide, particularly in the arid and semiarid areas in which salinity could be caused by many factors. Low rainfall, high evaporation rate, poor irrigation water that contains considerable amounts of salts, poor water management, 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 due to excessive evaporation are examples of the factors that could cause salinity related problems in these areas (Rady et al., 2013). In this study, the reduction in plant growth under the adverse conditions of the tested newly-reclaimed saline soil could be attributed to the osmotic effect resulting from salt stress, which causes increases in growth inhibitors (i.e., abscisic acid), reductions in growth promoters [i.e., indole-3-acetic acid (IAA) and gibberellins] and disturbances in the water balance of the stressed plants, leading to stomatal closure, ionic imbalance, reduction in photosynthesis, accumulation of toxic ions and consequently inhibition of growth (Shalata and Neumann, 2001; Qiu et al., 2007; Rady, 2011; Rady et al., 2013; Semida and Rady, 2014).

Spraying plants with αTOC significantly improved plant growth characteristics of faba beans, particularly at 0.50 mM. Bosch (1995) reported that a wide range of plant reactions exist to circumvent the potentially harmful effects caused by biotic or abiotic stresses. αTOC is the major vitamin E compound found in leaf chloroplasts, where it is located in the chloroplast envelope, thylakoid membranes and plastoglobuli. This antioxidant deactivates photosynthesis-derived reactive oxygen species (ROS) (mainly $^1O_2$ and OH), and prevents the propagation of lipid peroxidation by scavenging lipid peroxyl radicals in thylakoid membranes. αTOC levels change differentially in response to environmental constraints, depending on the magnitude of the stress and species-sensitivity to stress.

Under the adverse conditions of the tested soil (i.e., EC = 8.88–8.95 dS m^{-1} and pH = 7.74–7.80), foliar application of αTOC was significantly effective in increasing plant growth traits. The magnitude of increases was more pronounced particularly at 0.50 mM αTOC as represented in our data (Table 1). αTOC considers an important part of the plant defense machinery maintaining the integrity and normal function of the photosynthetic apparatus (Liu et al., 2008) that confirm the significant increase in performance index (PI) and a slightly increment in F_v/F_m by the application of αTOC under salt stress (Table 2). αTOC acts directly to neutralize superoxide radicals or singlet oxygen in plant cells (Foyer and Noctor, 2005). It has effects on many physiological processes including the regulation of growth, differentiation and metabolism of plants under saline conditions and increasing physiological availability of water and nutrients (Azooz et al., 2002; Barakat, 2003). In addition, αTOC protects metabolic processes against H_2O_2 and other toxic derivatives of oxygen, affects many enzyme activities, minimizes the damage caused by oxidative processes through synergic function with other antioxidants and stabilizes membranes (Cvelkorska et al., 2005; Pourcel et al., 2007; Shao et al., 2008), and consequently

Table 6
Effect of exogenously applied α-tocopherol (αTOC; 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)
Var.	αTOC	Length	Width			
G 40	0	3125	3175	40	1875	30
	0.25	3875	4000	50	2350	45
	0.50	4000	4025	60	2600	47
	1	3700	3760	55	2325	50
	Means	**3675**	**3740**	**51**	**2288**	**43**
G 429	0	2925	3300	50	1950	40
	0.25	4125	4150	100	2625	40
	0.50	4300	4500	94	2875	43
	1	4000	4050	69	2675	38
	Means	**3838**	**4000**	**78**	**2531**	**40**

Mean values (n = 1).
mM = millimole; μ = micrometer; Var.= variety; G 40 = Giza 40; G 429 = Giza 429.
Measurements were made in 50-day-old plants.

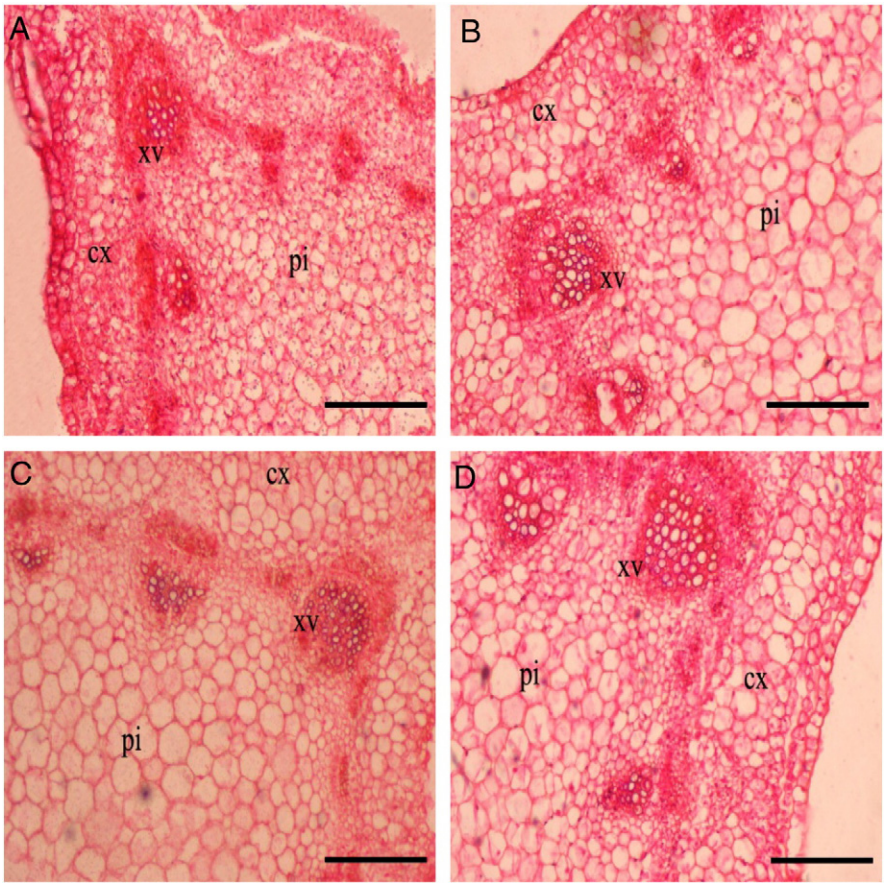

Fig. 1. Effect of exogenously applied α-tocopherol (αTOC; mM) on stem anatomy of two varieties of *Vicia faba* L. grown in reclaimed-saline calcareous soil. A: Control (Giza 40). B: Control (Giza 429). C: 0.50 Mm αTOC (Giza 40). D: 0.50 Mm αTOC (Giza 429). (cx = cortex, xv = xylem vessel, Pi = Pith). Scale bar = 350 μ.

healthy plant growth and satisfactory yield under moderate salinity. El Hariri et al. (2010) found an increase in IAA content in flax plants by the foliar application of αTOC, and attributed this increased IAA to the role of αTOC in activating the biosynthesis of endogenous hormone, reflecting in stimulating cell division and/or the cell enlargement that lead to the improvement in plant growth under stress. The same authors proved an increase in total phenols by αTOC application, which play a mechanism in regulation of plant metabolic processes, act as a substrate for many antioxidant enzymes, so, it mitigate the salinity stress injuries (Khattab, 2007). These phenols protect cells from potential oxidative damage and increase stability of cell membranes (Randhir et al., 2003; Burguieres et al., 2006). Sairam et al. (2005) indicated that salinity stress decreased relative water content (RWC) and membrane stability index (MSI) that was confirmed by our results (Table 2). The application of αTOC, particularly at 0.50 mM significantly increased RWC and MSI, maintaining cells turgid for the healthy metabolic processes and membrane integrity. The reduction in free proline and total soluble sugars by αTOC application in this study (Table 2) may be attributed to the crucial role of αTOC in mitigating the negative salt effects. Our results show a promotion in Na^+ uptake under salinity that was accompanied by corresponding declines of K^+ and Ca^{2+} contents, showing an apparent antagonism between K^+ and/or Ca^{2+} and Na^+. In contrast, the application of αTOC reversed the status of these ions, since increased K^+ and Ca^{2+} contents, reduced Na^+ content and increased their relations (i.e., K^+/Na^+ and Ca^{2+}/Na^+) that positively reflected in plant growth and yield. This promotion in nutrient contents may be attributed to the role of antioxidants, including αTOC in increasing osmotolerance and/or regulating various processes, including absorption of nutrients from soil solution and improving 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 αTOC in modifying K^+/Na^+ and Ca^{2+}/Na^+ selectivity under salt stress. All enhanced parameters (i.e., growth traits, plant water relations, contents of nutrients and their relations, and final yields) by the foliar application of αTOC were accompanied with the improved stem and leaf anatomy (Tables 6 and 7), which gave an opportunity to a good 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. Our data also show that, the variety Giza 429 represented better growth, water relations, nutrient contents, yield and anatomy than the variety Giza 40, concluding that Giza 429 was more salt-tolerant compared to Giza 40.

5. Conclusion

αTOC is a major compound of vitamin E that can play different roles in plant metabolism and can play important roles in amelioration of biotic and abiotic stresses. αTOC enabled faba bean plants to tolerate soil salinity at different levels and produce satisfactory yield. Therefore, our study recommends using αTOC at the level of 0.50 mM for the faba bean variety Giza 429 when grown under the newly-reclaimed saline soils.

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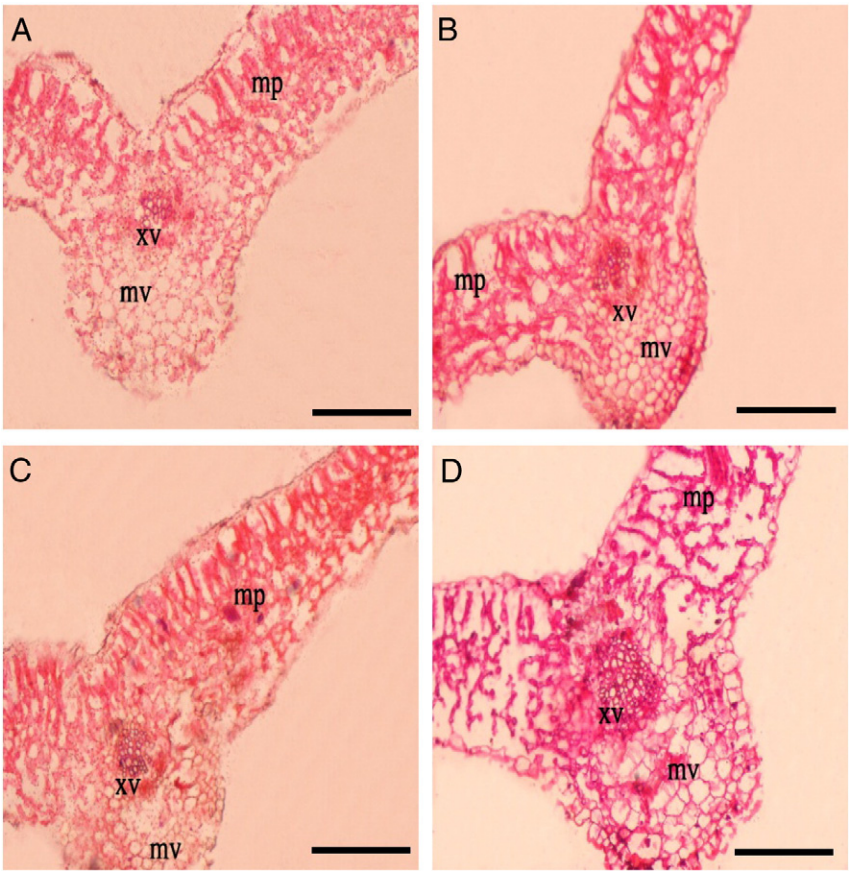

Fig. 2. Effect of exogenously applied α-tocopherol (αTOC; mM) on leaf anatomy of two varieties of *Vicia faba* L. grown in reclaimed-saline calcareous soil. A: Control (Giza 40). B: Control (Giza 429). C: 0.50 Mm αTOC (Giza 40). D: 0.50 Mm αTOC (Giza 429). (xv = xylem vessel, mp = mesophyll and mv = midvein). Scale bar = 350 μ.

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