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FULL LENGTH ARTICLE

Protective role of α-tocopherol on two *Vicia faba* cultivars against seawater-induced lipid peroxidation by enhancing capacity of anti-oxidative system

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Abstract To examine the effect of seawater stress on growth, yield, physiological and antioxidant responses of faba bean plant and whether the exogenous application with vitamin E could mitigate the adverse effect of salinity stress or not, a pot experiment was carried out during 2011/12 winter season under green house of the National Research Centre, Dokki, Cairo, Egypt. Two faba bean cultivars (Giza 3 and Giza 843) irrigated with diluted seawater (Tap water, 3.13 or 6.25 dS m^{-1}) and α-tocopherol (0, 50 or 100 mg L^{-1}) were used. At 75 days after sowing, growth sample was taken for vegetative growth measurement, proline, carotenoids, antioxidant enzyme activities (SOD, CAT, POX and PAL), lipid peroxidation, and inorganic ions as well as seed yield and yield attributes were determined. The results revealed that seawater triggered significant inhibitory effects on faba bean growth and yield especially for Giza 3 cultivar with obvious increments in MDA and Na^+ ion contents. Foliar application with α-tocopherol at rate of 100 mg L^{-1} followed by 50 mg L^{-1} on faba bean plants exerted certain alleviative effects on these indices in particular on Giza 843. α-Tocopherol could play an important role in alleviation of injury of faba bean irrigated with diluted seawater through the enhancement of the protective parameters such as antioxidant enzymes, proline, carotenoids, and inorganic ions (K^+ and Ca^{2+}) to be effective in decreasing MDA content, lessening the harmful effect of salinity, and improving faba bean growth, seed yield and seed yield quality.

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1. Introduction

Salt tolerance in plants is a complex trait, which varies widely among closely related species and between different varieties (Ashraf, 2002). Differences between closely related plants are

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particularly interesting to identify a small number of factors responsible for salt tolerance (Gehlot et al., 2005). Salinity stress has been studied in relation to regulatory mechanisms of osmotic and ionic homeostasis (Ashraf and Harris, 2004). The response of plants to a salinity stress may vary with the genotype, nevertheless some general reactions occur in all genotypes. Salinity can affect plant physiological processes resulting in reduced growth and yield (Yamaguchi and Blumwald, 2005). Increased tolerance to salinity stress in crop plants is necessary in order to increase productivity with limited water supplies and high salinity.

Salinity stress is known to trigger oxidative stress in plant tissues through the increase in reactive oxygen species (Apel and Hirt, 2004). Chloroplasts are the major organelles producing the reactive oxygen species (ROS) such as, the superoxide radical ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2) and singlet oxygen ($O_1^{\cdot}$) during photosynthesis (Asada, 1992). The production of ROS can be particularly high, when plants are exposed to salinity stress (Ashraf, 2009). ROS cause chlorophyll degradation and membrane lipid peroxidation. So, malondialdehyde (MDA) accumulation is an oxidative stress indicator that is a tested tool for determining salt tolerance in plants (Yildrimin et al., 2008).

Removal of the toxic oxygen radicals rapidly is of prime importance in any defense mechanism. Plants protect cells and sub-cellular systems from the cytotoxic effects of these active oxygen radicals with both non-enzymatic and enzymatic antioxidant systems such as carotenoids, ascorbic acid, α-tocopherol, proline, SOD, peroxidase (POX) and catalase (CAT) (Munne-Bosch and Alegre, 2000; Sairam and Srivastava, 2001; Mishra et al., 2009). There are several reports that underline the intimate relationship between antioxidant enzyme activities and increased tolerance to environmental stress (Abd El-Motty and Orabi, 2013; Orabi et al., 2013). Differences in the accumulation patterns of Na^+ and K^+ were found under salinity stress. The salt tolerant plants maintained a high K^+ content and higher K^+:Na^+ ratio compared with the salt sensitivity plants (Azooz et al., 2004). High K^+:Na^+ ratio is more important for many species than simply maintaining a low concentration of Na^+ (Cuin et al., 2003).

Faba beans (*Vicia faba* L.) are popular legume food with high yield capacity and high protein content (30% of their dry weight) which contain most of the necessary amino acids for human and animal nutrition and low sulfur amino acid concentrations (Gaber et al., 2000). In recent years, the importance of carotenoids and tocopherols has been increasingly recognized due to the emerging knowledge of their health benefits. Because humans can synthesize neither carotenoids nor tocopherols, they rely on their uptake through diet for the production of vitamin A and the supply with vitamin E (Fraser and Bramley, 2004). Tocopherols are a group of compounds synthesized only by photosynthetic organisms and are involved in the quenching and scavenging of $^1O_2$ (Neely et al., 1988) and act as highly efficient recyclable chain reaction terminators for the removal of polyunsaturated fatty acid (PUFA) radical species generated during Lipid peroxidation (Munne-Bosche and Falk, 2004). Furthermore, tocopherols contribute to membrane stability by influencing its fluidity and permeability (Fryer, 1999) and might participate in protection of the D1 protein against high light (Trebst et al., 2002). Tocopherols are believed to protect chloroplast membranes in plants from photo oxidation and help to provide an optimal environment for the photosynthetic machinery, their accumulations also occur as a response to variety of abiotic stress including high light, drought, salt and cold and may provide an additional line of protection from oxidative damage (Munné-Bosch and Algere, 2002). The major tocochromanol in leaves is α-tocopherol, whereas seeds accumulate higher levels of tocotrienols (Grusak and Dellapenna, 1999).

Comparing the response among genotypes of the same species to salinity provides a convenient and useful tool for un-veiling basic mechanisms involved in salt tolerance. The mechanism of salt tolerance is still not fully understood (Gharsa et al., 2008). Therefore, this work was conducted to compare the effect of salinity stress on growth, yield parameters, physiological and antioxidant responses of two faba bean (*V. faba* L.) genotypes differing in salt tolerance and to examine whether exogenous application with vitamin E could mitigate the adverse effect of salinity stress.

2. Materials and methods

2.1. Experimental procedures

A pot experiment was conducted at the green house of the National Research Centre, Dokki, Cairo, Egypt during the winter season of 2011/12 to study the effect of foliar spray of α-tocopherol (Vitamin E) on faba bean grown under salinity conditions. Daytime temperatures ranged from 14.5 to 30.2 °C with an average of 23.2 ± 3.8 °C whereas temperatures at night were 12.4 ± 1.8 °C, with minimum and maximum of 8.0 and 17.0 °C, respectively. Daily relative humidity averaged 57.7 ± 9.6% in a range from 38.1% to 78.7%.

Two Faba bean (*V. faba* L.) cultivars were used in this experiment, namely Giza 3 (G3, *Orobanche*-susceptible) and Giza 843 (G843, *Orobanche*-tolerant) were obtained from Agricultural Research Centre, Ministry of Agriculture and Land Reclamation, Egypt. Faba bean seeds were selected for uniformity by choosing those of equal size and with the same color. The selected seeds were washed with distilled water, sterilized with 1% sodium hypochlorite solution for about 2 min and thoroughly washed again with distilled water. Ten seeds were sown on November 22, 2011 along a centre row in each pot at 30-mm depth in plastic pots, each filled with about 7.0 kg clay soil mixed with sandy soil in a proportion of 3:1 (V:V), respectively in order to reduce compaction and improve drainage. Saline water was prepared by mixing fresh water (0.23 dS m^{-1}) with seawater (51.2 dS m^{-1}). Concentration of EC, pH, cations and anions of irrigation water and soils used on the pot experiment are shown in Table 1. At sowing, granular commercial rhizobia were incorporated into the top 30-mm of the soil in each pot with the seeds. Granular ammonium sulfate 20.5% N at a rate of 40 kg N ha^{-1}, and single super phosphate (15% P_2O_5) a rate of 60 kg P_2O_5 ha^{-1} were added to each pot. The N and P fertilizers were mixed thoroughly into the soil of each pot immediately before sowing.

The experiment was laid out in factorial design using three factors (cultivars, seawater, and α-tocopherol) with five replications. Seedlings were thinned after 10 days after sowing (DAS) to leave four seedlings per pot till harvest and irrigated with equal volumes of tap water until 15 DAS. Starting from 16th day, all pots were irrigated either with tap water (S0) or different dilutions of seawater namely 3.13 or

Table 1 EC, pH, and concentration of cations and anions of irrigation water and soil used in the pot experiment.

	EC (dS m^{-1})	pH	Cations (meq l^{-1})				Anions (meq l^{-1})			
			Ca^{2+}	Mg^{2+}	Na^{+}	K^{+}	HCO_3^-	CO_3^{2-}	SO_4^{2-}	Cl^-
Water										
Tap water	0.23	7.35	1.00	0.50	2.40	0.20	0.10	0.00	1.30	2.70
Sea water	51.2	7.76	43.20	15.12	454.57	1.51	6.05	0.00	76.36	432.00
Soil										
Sandy	0.14	8.11	2.60	2.40	1.31	0.21	1.13	0.00	4.22	0.70
Clay	1.40	7.59	5.60	1.90	5.90	0.37	1.50	0.00	6.77	5.50

6.25 dS m^{-1} which considered as S1, and S2, respectively. Faba bean plants were sprayed with three levels of α-tocopherol at the rate of 0, 50, or 100 mg L^{-1} twice at 45 and 60 DAS. The α-tocopherol levels of 0, 50, or 100 mg L^{-1} were considered as Toc0, Toc1, or Toc2, respectively. Faba bean plant growth sample was taken at 75 DAS, for determining some growth traits (plant height and shoot dry weight) and some biochemical measurements in leaves. At harvest time (140 DAS), pods were collected from each replicate and some yield criteria such as seed weight, seed numbers and seed index were determined.

2.2. Measurements

Extraction of the antioxidant enzymes such as superoxide Dismutase (SOD), catalase (CAT), and peroxidase (POX) was performed with 5 g of frozen leaf tissues homogenized in pre-chilled mortar in the presence of 10 ml of 59 mM potassium phosphate buffer (pH7) with 1% (W/V) insoluble polyvinyl pyrrolidone (PVP) and 0.1 mM EDTA. The extraction procedures were repeated twice and supernatants were pooled, raised to a certain volume, referred as crude enzyme extract, all operations were carried out at 4C for further analysis. The activity of SOD (EC 1.15.1.1) was determined following Dhindsa et al. (1981). One unit of SOD was defined as the amount of enzyme that inhibits by 50% the rate of NADH oxidation observed in blank. The activity of CAT (EC 1.11.1.6) was determined according to Aebi (1983). The activity of catalase was estimated by the decrease of absorbance at 240 nm for 1 min as a consequence of H_2O_2 consumption. The activity of peroxidase (POX, EC 1.11.1.7) was determined according to Nakano and Asada (1981). The activity of peroxidase was estimated by the increase of absorbance due to the formation of tetraguaiacol at 470 nm due to the oxidation of guaiacol in the presence of H_2O_2. The activity of phenylalanine ammonia lyase (PAL, EC 4.3.1.5) was estimated according to the method adopted by Beaudoin-Egan and Thorpe (1985). The activity of PAL is defined as the amount of enzyme forming 1 m mol of trans-cinnamic acid from the substrate phenylalanine per min. Lipid peroxidation was determined by estimating the malondialdehyde (MDA) content as described by Dhindsa et al. (1982). Proline concentration was determined by the method of Bates et al. (1973). Carotenoid content was determined according to Jensen (1978). Mineral ion content was measured in dry samples according to the method described by Chapman and Pratt (1978).

2.3. Statistical analysis of the data

All data were subjected to an analysis of variance (ANOVA) for a factorial design, after testing for the homogeneity of error variances according to the procedure outlined by Gomez and Gomez (1984). Statistically significant differences between means were compared at $P \leqslant 0.05$ using Least Significant Difference (LSD) test.

3. Results and discussions

3.1. Growth and yield

The data presented in Table 2 indicated that plant height and shoot dry weight (shoot DW) of both cultivars G3 and G843 were significantly decreased at all levels of seawater especially at 6.25 dS m^{-1}. Foliar application with α-tocopherol at concentrations of 50 or 100 mg L^{-1} recorded significant increments in growth parameters compared with control. Although seawater salinity stress reduced the growth traits of the two faba bean cultivars, there were major differences in their reduction, which was judged with the ability of G843 to enhance its tissue water contents, whereas the opposite was appeared in G_3. Accordingly, plant salt tolerance is determined by genotypes and biochemical pathways that facilitate retention of water and synthesis of osmotically active metabolites (Sarwat and El-Sherif, 2007). Salinity stress limits plant growth by adversely affecting various physiological and biochemical processes including photosynthesis, antioxidant capacity and homeostasis (Ashraf, 2004) resulting in damaging growth cells so that they cannot perform their functions (Chen and Murata, 2000). The reduction in growth parameters under seawater salinity conditions was obtained in several other plant species (Dogan, 2011; Abd El-Samad et al., 2011; Orabi et al., 2013). α-Tocopherol foliar application at 50 or 100 mg L^{-1} improved growth parameters even at a higher level of seawater of 6.25 dS m^{-1}. Supporting these results, Sakr and El-Metwally (2009) reported that α-tocopherol significantly increased dry matter accumulation in the stem and leaves of wheat plants compared with untreated plants in the soil salt areas, where it could counteract the harmful effect of high soil salinity stress level on growth of wheat plants. In addition, α-tocopherol at 50 and 100 mg L^{-1} significantly increased all tested morphological parameters (plant height, number of branches, leaves/plant, fresh cut of shoots and roots) of *Hibiscus rosa sinensis* L. plants grown under new reclaimed lands of Noubaria and the highest values were obtained at 100 mg L^{-1} application

Table 2 Effect of α-tocopherol on plant height, shoot dry weight (shoot DW), seed yield per plant, seed number per plant and weight per seed (seed index) in two faba bean cultivars grown under seawater saline conditions.[#]

Treatments			Growth and yield traits				
Cv	Seawater	Toc	Plant height (cm)	Shoot DW (g plant^{-1})	Seed yield (g plant^{-1})	Seed No. plant^{-1}	Seed index (g)
G3	S0	Toc0	66.5	2.23	4.20	6.7	0.63
		Toc1	68.0	2.80	5.70	7.7	0.75
		Toc2	70.7	3.10	6.30	8.2	0.77
	S1	Toc0	62.2	2.01	3.60	5.7	0.64
		Toc1	65.8	2.56	4.81	7.0	0.69
		Toc2	67.5	2.70	5.33	7.7	0.70
	S2	Toc0	60.5	1.62	2.30	4.0	0.58
		Toc1	63.7	2.05	3.63	5.8	0.63
		Toc2	64.5	2.16	3.91	6.2	0.63
G843	S0	Toc0	64.8	3.04	6.55	9.0	0.73
		Toc1	66.0	3.45	8.55	10.7	0.80
		Toc2	68.8	3.80	9.10	11.3	0.80
	S1	Toc0	59.5	2.36	5.61	7.7	0.73
		Toc1	63.3	3.01	7.23	9.7	0.75
		Toc2	64.2	3.30	8.11	10.3	0.79
	S2	Toc0	58.0	1.91	3.61	6.0	0.60
		Toc1	61.0	2.41	5.05	7.7	0.66
		Toc2	62.5	2.55	5.48	8.1	0.68
LSD 0.05			1.6	0.15	0.42	0.7	0.08

[#] S0 (tap water); S1 (3.13 dS m^{-1}); S2 (6.25 dS m^{-1}); Toc0 (0 mg L^{-1}); Toc1 (50 mg L^{-1}); Toc2 (100 mg L^{-1}). Measurements (plant height and shoot DW) were made at 75 d after sowing (DAS), while seed yield, seed number and seed index were made at harvest. Cultivars of faba bean used were Giza 3 (G3) and Giza 843 (G843).

compared with those obtained by low level and untreated plants (El-Quesni et al., 2009).

Seawater salinity caused significant decreases in seed weight, seed number and seed index especially those of sensitive G3 compared with G843 (Table 2). These results are in agreement with those reported by Abdelhamid et al. (2010), Kumar et al. (2012), El-Lethy et al. (2013), and Orabi et al. (2013) on different plant species. These decreases in yield and yield components might be attributed to the decreases in plant growth, photosynthetic pigments and disturbance in the nutrient balance. On the other hand, α-tocopherol at rate of 50 or 100 mg L^{-1} caused marked increases in yield of the two faba bean cultivars either irrigated with tap water or seawater saline solution compared with the corresponding controls. The enhancement effects of α-tocopherol on faba bean yield were proved earlier by other researchers on different plant species (Sakr and El-Metwally, 2009; Soltani et al., 2012; Sadak and Dawood, 2014).

3.2. Total carbohydrates and total crude proteins

Table 3 shows that seawater salinity caused significant decreases in total carbohydrate and total crude protein contents of seeds of the two faba bean cultivars compared with controls. The reduction in the biosynthesis of carbohydrates might be due to the inhibitory effect of salinity on chlorophyll synthesis (Sadak and Dawood, 2014). The reduction in protein content under seawater salinity stress might be due to the disturbance in nitrogen metabolism or inhibition of nitrate absorption as reported by El-Zeiny et al. (2007). Meanwhile, foliar application of α-tocopherol at 50 or 100 mg L^{-1} showed opposite trends to salinity effects mainly at 100 mg L^{-1}, causing significant increases in the two parameters not only relative to corresponding stressed plants but also to the untreated plants irrigated with tap water. However, Sadak et al. (2010) demonstrated that application of α-tocopherol on sunflower plants led to the accumulation of total carbohydrates, stimulation of protein synthesis and delaying senescence of sunflower plant. As for the role of α-tocopherol, it could be concluded that its supplementation could alleviate the harmful effect of ROS caused by salt salinity, through its powerful antioxidant properties (Bughdadi, 2013) and these antioxidant activity of α-tocopherol is mainly due to their ability to donate their phenolic hydrogens to lipid free radicals (Bagheri and Sahari, 2013), involving in both electron transport of PSII and antioxidizing system of chloroplasts and act as membrane stabilizers and multifaceted antioxidant that scavenge oxygen free radicals, lipid peroxy radicals and singlet oxygen (Diplock et al., 1989), reacting with peroxy radicals formed in the bilayer as they diffuse to the aqueous phase, scavenging cytotoxic H_2O_2 and reacts non-enzymatically with other ROS: singlet oxygen, superoxide radical and hydroxyl radical and stabilize membrane structures (Blokhina et al., 2003) modulating membrane fluidity in a similar manner to cholesterol and also membrane permeability to small ions and molecules (Foyer, 1992) and decreasing the permeability of digalactosyldiacyl glycerol vesicles for glucose and protons (Berglund et al., 1999).

3.3. Protectant (proline and carotenoids) concentrations

3.3.1. Proline concentration

The effects of α-tocopherol on proline content as one of the osmotic solutes in the leaves of faba bean plants grown under seawater salinity are shown in Table 4. Irrigation of faba bean plants with diluted seawater (3.13 or 6.25 dS m^{-1}) caused significant and gradual increments in proline content in the

Table 3 Effect of α-tocopherol on total carbohydrate (TC), and total crude protein (TCP) in seed yield of two faba bean cultivars grown under seawater saline conditions[#].

Treatments			Seed traits	
Cv	Seawater	Toc	TCP	TC
			(%)	
G3	S0	Toc0	25.0	49.9
		Toc1	27.0	53.2
		Toc2	29.0	57.6
	S1	Toc0	22.3	42.8
		Toc1	23.3	45.0
		Toc2	23.7	46.2
	S2	Toc0	21.0	39.1
		Toc1	22.7	41.8
		Toc2	23.3	42.6
G843	S0	Toc0	25.5	51.0
		Toc1	27.5	54.3
		Toc2	29.6	58.8
	S1	Toc0	22.8	43.7
		Toc1	23.8	45.9
		Toc2	24.1	47.2
	S2	Toc0	21.4	39.9
		Toc1	23.1	42.7
		Toc2	23.8	43.5
LSD 0.05			1.5	1.9

[#] S0 (tap water); S1 (3.13 dS m^{-1}); S2 (6.25 dS m^{-1}); Toc0 (0 mg L^{-1}); Toc1 (50 mg L^{-1}); Toc2 (100 mg L^{-1}). Measurements were made at harvest. Cultivars of faba bean used were Giza 3 (G3) and Giza 843 (G843).

Table 4 Effect of α-tocopherol on proline and carotenoids in the leaves of two faba bean cultivars grown under seawater saline conditions.[#]

Treatments			Protectant concentrations	
Cv	Seawater	Toc	Proline (μmol g^{-1} FW)	Carotenoids (mg g^{-1} FW)
G3	S0	Toc0	8.9	0.187
		Toc1	9.5	0.197
		Toc2	11.1	0.217
	S1	Toc0	9.7	0.220
		Toc1	12.1	0.253
		Toc2	13.4	0.263
	S2	Toc0	10.5	0.207
		Toc1	13.0	0.227
		Toc2	14.9	0.240
G843	S0	Toc0	10.7	0.227
		Toc1	11.3	0.250
		Toc2	12.2	0.267
	S1	Toc0	11.6	0.250
		Toc1	14.0	0.287
		Toc2	15.6	0.330
	S2	Toc0	12.9	0.257
		Toc1	15.0	0.297
		Toc2	17.1	0.337
LSD 0.05			0.7	N.S

[#] S0 (tap water); S1 (3.13 dS m^{-1}); S2 (6.25 dS m^{-1}); Toc0 (0 mg L^{-1}); Toc1 (50 mg L^{-1}); Toc2 (100 mg L^{-1}). Measurements were made at 75 d after sowing (DAS). Cultivars of faba bean used were Giza 3 (G3) and Giza 843 (G843).

two studied cultivars especially G843 compared with the corresponding plants irrigated with tap water (So). The response was ascertained after α-tocopherol application at 100 mg L^{-1} followed by 50 mg L^{-1}, suggesting an excellent mechanism of plants to decrease the osmotic potential. These results support the hypothesis that proline accumulation is a part of physiological response of the plant to intense stress (Ain-Lhout et al., 2001). The accumulation of proline may be through an increase in its synthesis constantly with inhibition of its catabolism (Jaleel et al., 2007). In this regard, some researchers have reported that high proline content is a sign of stress (Rai et al., 2003), while others suggest that proline at high concentrations acts as a solute for intercellular osmotic adjustment (Silveira et al., 2003). In this regards, higher proline accumulation can be appreciated as a further important factor of adaptation to salinity as reported in number of species (Ashraf and Harris, 2004; Hameed and Ashraf, 2008; Azooz et al., 2011; Taie et al., 2013).

3.3.2. Carotenoid concentration

Data in Table 4 show a significant and gradual increase in carotenoid content with increasing seawater levels from 0 to 6.25 dS m^{-1} and the response was more obvious in G843 (the tolerant cultivar) than in G3 (the susceptible cultivar). In this concern, in chickpea leaves, the carotenoids were measured and effective elevation over control was observed under salinity stress (Mishra et al., 2009). Moreover, deleterious effects of salinity stress on leaf carotenoids have been reported in several crops such as soybean (Dogan, 2011), peanut (Hossain et al., 2011), maize (Abd El-Samad et al., 2011), common bean (Abdelhamid et al., 2013) and faba bean (Abd El-Samad et al., 2011; Taie et al., 2013). Foliar application of α-tocopherol recorded mostly higher increments in carotenoid content compared with the untreated plants. Parallel to this study, α-tocopherol significantly increased the contents of carotenoids in two wheat genotypes under salinity stress (2840 and 6080 mg L^{-1}) compared with the corresponding controls, consequently there was a progressive decline in total chlorophyll:carotenoid ratio, carotenoids might play a role as a free radical scavenger (Sakr and El-metwally, 2009). Therefore, increase of carotenoids in genotypes treated with salinity could enhance their capacity to reduce the damage caused by ROS, which in turn increased chlorophyll content of such plants (Azooz, 2009), where carotenoids play a key role in controlling the cellular level of free radicals and peroxides (Apel and Hirt, 2004).

3.4. Antioxidant enzyme activities

Data in Table 5 revealed that the salinity tolerant faba bean cultivar G843 showed relatively higher activities of the enzymes such as superoxide Dismutase (SOD), catalase (CAT), and phenylalanine ammonia lyase (PAL). As salinity level increased, significant increases in the activities of these antioxidant enzymes were obtained. Similar results were obtained by other researchers (Costa et al., 2005; Azooz, 2009; Azooz et al., 2009; Weisany et al., 2012). In the present study, the antioxidant activities were clearly increased after application of α-tocopherol at 100 mg L^{-1} followed by 50 mg L^{-1}, this may contribute advantages to faba bean plants especially Cv. G843 and helped Cv. G3 to perform better in

Table 5 Effect of α-tocopherol on superoxide dismutase (SOD), catalase (CAT), phenylalanine ammonia lyase (PAL), and peroxidase (POX) enzyme activities in the leaves of two faba bean cultivars grown under seawater saline conditions.[#]

Treatments			Enzyme activities			
Cv	Seawater	Toc	SOD	CAT	PAL	POX
			(μmol g^{-1} FW)			
G3	S0	Toc0	68.6	63.7	23.9	6.4
		Toc1	74.8	68.8	26.0	8.1
		Toc2	72.7	71.0	29.0	8.8
	S1	Toc0	73.3	72.6	31.7	10.2
		Toc1	91.2	79.3	38.0	11.2
		Toc2	85.7	83.1	41.6	12.9
	S2	Toc0	79.6	74.5	36.3	12.2
		Toc1	101.3	88.2	41.8	13.3
		Toc2	110.7	90.8	43.9	15.2
G843	S0	Toc0	80.4	71.0	31.7	6.1
		Toc1	86.0	77.1	33.0	6.9
		Toc2	89.0	78.4	35.9	7.0
	S1	Toc0	93.8	80.3	40.5	9.6
		Toc1	114.8	93.3	44.6	10.7
		Toc2	119.5	95.3	48.9	12.1
	S2	Toc0	99.1	90.8	45.5	11.7
		Toc1	138.4	108.0	50.9	12.5
		Toc2	140.6	112.1	55.1	14.0
LSD 0.05			3.9	5.5	1.9	1.9

[#] S0 (tap water); S1 (3.13 dS m^{-1}); S2 (6.25 dS m^{-1}); Toc0 (0 mg L^{-1}); Toc1 (50 mg L^{-1}); Toc2 (100 mg L^{-1}). Measurements were made at 75 d after sowing (DAS). Cultivars of faba bean used were Giza 3(G3) and Giza 843 (G843).

various aspects of growth and metabolism as they defend against the harmful effect of salinity stress mainly through the increase in activities of SOD, CAT, PAL, and peroxidase (POX) enzymes together with the increase of some antioxidant substances. Salt provokes a dose-dependent increase in SOD activity, which could represent a defense mechanism against salt-induced $O_2^{\cdot-}$ generation. SOD catalyzes the conversion of the super oxide anion to H_2O_2. It was clear that G843 cultivar has a higher dismutating capacity under moderate and high doses of seawater salinity. These results are in a good agreement with those reported by Wang et al. (2009), and Wang and Han (2009), who found higher constitutive and induced level of SOD in tolerant alfalfa cultivar under salinity stress. Similarly, Koca et al. (2006) reported higher activities of SOD in wild salt-tolerant tomato species than in the cultivated salt sensitive one. Orabi et al. (2013) studied the harmful effect of seawater salinity and recorded higher activities and specific activities of SOD enzymes in the tolerant cultivar Giza 429 of faba bean plants. In the same line, El-Lethy et al. (2013) reported significant increases in SOD enzyme activity in wheat plants irrigated with NaCl saline solutions. CAT eliminates H_2O_2 by breaking it down directly to form water and Oxygen. Thus, this enzyme does not require a reducing power and has a high reaction rate but a low affinity for H_2O_2 (Willekens et al., 1997). CAT together with SOD considered the most effective antioxidant enzymes in preventing cellular damage (Scandalios, 1993). Increases in the activity of CAT, have been reported in alfalfa (Wang and Han, 2009; Wang et al., 2009), soybean (Comba et al., 1998), Tobacco (Bueno et al., 1998) and mulberry (Chinta et al., 2001) under salinity stress. Significant roles of POX have been suggested in plant development processes (Gaspar et al., 1985). Guaiacol peroxidase is among the enzymes that scavenge H_2O_2 in chloroplasts which is produced through dismutation of $^{-}O_2$ catalyzed by SOD. Increased peroxidase (POX), or sometime referred as POD activity has been reported in salt-tolerant and sensitive species of alfalfa (Wang et al., 2009; Wang and Han, 2009), tomato (Koca et al., 2006) and rice (Dionisio-Sese and Tobita, 1998). Increased POX in salt sensitive cultivar G3 and relatively in salt-tolerant faba bean G843 may be attributed to increased activity of POX encoding genes or increased activation of already existing enzymes as suggested by Dionisio-Sese and Tobita (1998) who reported an increase in peroxidase activity in salt sensitive rice varieties, Hitomebore and IR28, in response to salinity stress and showed an increase in lipid peroxidation and electrolyte leakage as well as Na^+ accumulation in the leaves under saline conditions. In this regard, in a study by Sadak et al. (2010) on the tolerant faba bean cultivar G843 under salinity stress, the tolerance was attained with lowering the level of POX activity. In the same line, Siegal et al. (1982) concluded that change in peroxidase activity level in response to salinity is not a reliable criterion for screening for tolerance in Brassica species. More specifically, guaiacol peroxidase enzyme has been related to the appearance of physiological injuries caused by oxidative stress. Phenylalanine ammonia-lyase (PAL) is considered to be the principal enzyme of the phenylpropanoid pathway (Kacperska, 1993), catalyzing the transformation by deamination of L-phenylalanine into trans-cinnamic acid, which is the prime intermediary in the biosynthesis of phenolics (Levine et al., 1994). This enzyme is considered by most authors as one of the main lines of cell acclimation against stress (Kacperska, 1993; Leyva et al., 1995) where plants could accumulate phenolic compounds in response to oxidative stress (Rivero et al., 2001; Ali et al., 2007). α-Tocopherol has appeared to play a major role in chloroplastic antioxidant network of plants. Therefore, it contributes to preservation of an adequate redox station in chloroplasts, and to maintaining thylakoid membrane structure and function during plant development and in plant responses to stress (Munné-Bosch and Alegra, 2002; Munne-Bosch, 2005). Similar to our results (Sakr and El-Metwally, 2009) recorded increments in the antioxidant enzymes in response to α-tocopherol application on wheat against oxidative stress.

3.5. Lipid peroxidation

Data in Fig. 1 show that seawater treatments gradually increased MDA contents especially to 6.25 dS m^{-1} which gave the highest significant content of lipid peroxidation. Meanwhile, α-tocopherol treatments decreased significantly MDA content, and the response reached the maximum at 100 mg L^{-1} with G843. MDA is the decomposition product of poly unsaturated fatty acids of plant membranes under stress. The rate of lipid peroxidation in terms of MDA can therefore be used as an indicator to evaluate plant tolerance to oxidative stress as well as the sensitivity of plants to salinity stress (Jain et al., 2001). Increase in lipid peroxidation level in faba bean plants exposed to seawater salinity shows that the enzyme activities might have not been enough to

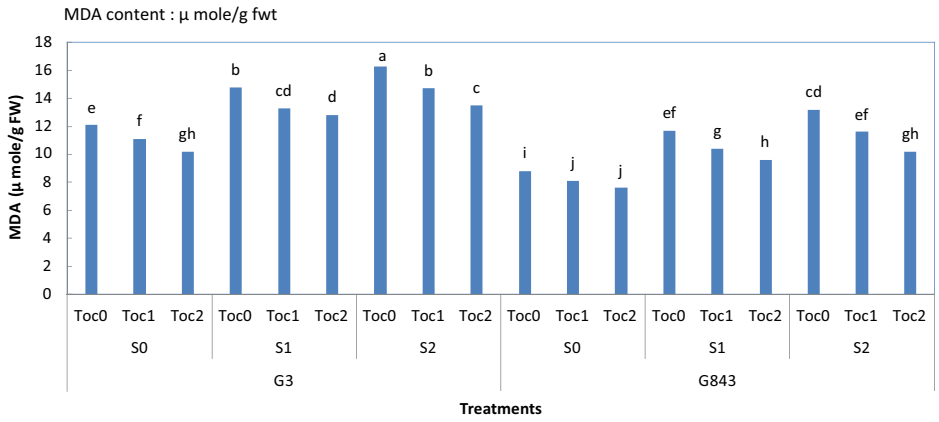

Figure 1 Effect of α-tocopherol on malondialdehyde (MDA) in the leaves of two faba bean cultivars grown under seawater saline conditions. The vertical bars with different letters are significantly different from each other at $P \leqslant 0.05$ according to Least Significant Difference (LSD) test. S0 (tap water); S1 (3.13 dS m^{-1}); S2 (6.25 dS m^{-1}); Toc0 (0 mg L^{-1}); Toc1 (50 mg L^{-1}); Toc2 (100 mg L^{-1}). Measurements were made at 75 d after sowing (DAS). Cultivars of faba bean used were Giza 3 (G3) and Giza 843 (G843).

prevent the peroxidation of membrane lipids caused by high concentrations of seawater salinity or in other words, the increase in MDA level especially in the susceptible cultivar G3 might also be correlated with inadequate activities of the studied antioxidant enzymes (SOD, CAT, POX and PAL) to scavenge ROS produced in faba bean leaves. Variations in MDA contents were found in cultivars differing in water deficit stress (Masoumi et al., 2011). On the other hand, decrements have occurred as a result of α-tocopherol application ascertains that plant tolerance would be attained to scavenge ROS produced under salinity.

3.6. Mineral ion content

Data in Table 6 show that mineral ion concentration (K^+ and Ca^{2+}) was significantly higher in G843 than G3 cultivar. However, seawater salinity levels (S_1 or S_2) caused significant and gradual reductions in K^+ and Ca^{+2} concentrations, as well as in the K^+:Na^+ and Ca^{+2}:Na^+ ratios, accompanied by gradual and significant increases in Na^+ concentrations compared with control plants (So). These results are similar to those results reported by Doğan (2011), Abdelhamid et al. (2010), Abd El-Samad et al. (2011) and El-Lethy et al. (2013)

Table 6 Effect of α-tocopherol on ion concentrations (K^+, Ca^{2+} and Na^+) and K^+:Na^+ and Ca^{+2}:Na^+ ratios in the leaves of two faba bean cultivars grown under seawater saline conditions[#].

Treatments			K	Ca	Na	K^+:Na^+	Ca^{+2}:Na^+
Cv	Seawater	Toc	(%)			Ratio	
G3	S0	Toc0	1.74	2.33	0.31	5.68	7.56
		Toc1	1.91	2.45	0.28	6.86	8.80
		Toc2	2.02	2.56	0.25	8.08	10.25
	S1	Toc0	1.29	2.01	0.42	3.09	4.83
		Toc1	1.38	2.17	0.39	3.58	5.64
		Toc2	1.54	2.32	0.37	4.25	6.36
	S2	Toc0	1.18	1.82	0.52	2.26	3.51
		Toc1	1.27	1.91	0.49	2.61	3.94
		Toc2	1.32	1.99	0.48	2.77	4.16
G843	S0	Toc0	1.83	2.76	0.24	7.57	11.39
		Toc1	2.01	2.93	0.20	10.29	15.02
		Toc2	2.12	3.06	0.21	10.36	14.98
	S1	Toc0	1.35	2.41	0.33	4.12	7.38
		Toc1	1.45	2.60	0.31	4.65	8.41
		Toc2	1.62	2.78	0.30	5.50	9.44
	S2	Toc0	1.24	2.18	0.42	2.92	5.14
		Toc1	1.33	2.29	0.39	3.44	5.92
		Toc2	1.39	2.42	0.37	3.75	6.53
LSD 0.05			0.18	0.17	0.17	0.95	1.25

[#] S0 (tap water); S1 (3.13 dS m^{-1}); S2 (6.25 dS m^{-1}); Toc0 (0 mg L^{-1}); Toc1 (50 mg L^{-1}); Toc2 (100 mg L^{-1}). Measurements were made at 75 d after sowing (DAS). Cultivars of faba bean used were Giza 3 (G3) and Giza 843 (G843).

from different plant species. Seawater salinity enhances the content of Na^+ as reported by Gunes et al. (2007) and the excess of Na^+ might cause problems with membranes, enzyme inhibition, and disturbance in metabolism which disorganize cell division, elongation and structure (Abo Kassem, 2006). In this connection, Kiarostami et al. (2010) suggested that increased accumulation of sodium (Na^+) and chloride (Cl^-) ions in the tissues inhibits biochemical processes related to photosynthesis through direct toxicity leading to low water potential. The promotion of Na^+ uptake by salinity was accompanied by a corresponding decline in K^+ concentration, showing an apparent antagonism between K^+ and Na^+ (Cuin et al., 2009). The selectivity of high K^+:Na^+ ratio in plants is an important content mechanism and selection criterion for salt tolerance (Wenxue et al., 2003; Ashraf and Harris, 2004). Cuin et al. (2003) concluded that high K^+:Na^+ ratio is more important for many species than simply maintaining a low Na^+ concentration that is reflected in lowering membrane damage and high water content in both genotypes especially under salinity stress (Azooz, 2009). Ashraf and Orooj (2006) reported that the maintenance of higher K^+:Na^+ ratio in shoot of *Trachyspermum ammi* L. could be an important component of its salt tolerance. Gharsa et al. (2008) concluded that the better tolerance of plant to salinity stress was primarily due to better K^+ assimilation, resulting in higher K^+:Na^+ ratio. Faba bean plants subjected to seawater salinity took up high amounts of Na^+, whereas the uptake of K^+ and Ca^{2+} was considerably reduced (Abdelhamid et al., 2010), that low Ca^{2+}:Na^+ ratio in a saline medium plays a significant role in growth inhibition in addition to causing significant changes in morphology and anatomy of plants (Cramer et al., 1991). The maintenance of Ca^{2+} acquisition and transport under salinity stress is an important determinant of salinity tolerance. Ca^{2+} is known to play a crucial role in maintaining the structural and functional integrity of plant membranes in addition to its considerable roles in cell wall stabilization, regulation of ion transport, and selectivity and activation of cell wall enzymes (Marschner, 1995). The reduction in Ca^{2+} uptake under salinity stress conditions might be due to the suppressive effect of Na^+ and K^+ on this cation or due to reduction of transport of Ca^{+2} and Mg^{+2} ions (Asik et al., 2009). On the other hand, plants of both cultivars irrigated either with tap water or saline solution at different levels and exogenously applied with α-tocopherol exhibited decreases in Na^+ whereas increments observed in K^+ and Ca^{2+} relative to their corresponding control. Thus, α-tocopherol mostly at 100 mg L^{-1} followed by 50 mg L^{-1} partially mitigated the adverse effect of salinity stress on mineral content in faba bean leaves. Application of α-tocopherol led to an increase in the contents of ions in the leaf through their role in increasing osmotolerance and/or through regulating various processes including absorption of nutrients from soil solution (Buschmann and Lichtenthaler, 1979; Sadak and Dawood, 2014).

4. Conclusion

It could be concluded that faba bean cv Giza843 is more tolerant to salinity stress than cv Giza 3. It is possible that better resistance to salinity of cv Giza 843 was related to its ability to maintain higher levels of antioxidant enzyme activity, proline and carotenoid contents resulting in lower H_2O_2 production and lipid peroxidation associated with diminishing oxidative injury and consequently improved faba bean plant growth and yield. α-Tocopherol could play an important role in alleviation of injury of faba bean irrigated with diluted seawater, through the enhancement of the protective parameters such as antioxidant enzymes, proline, carotenoids, and inorganic ions (K^+, Ca^{2+}) to be effective in decreasing MDA content, lessening the harmful effect of salinity, and improving faba bean growth, seed yield and seed yield quality.

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