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Foliar-applied α-tocopherol enhances salt-tolerance in onion plants by improving antioxidant defence system

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Abstract

α-Tocopherol (αTOC) is a vitamin and antioxidant compound that plays a crucial role in amelioration of biotic and abiotic stresses. It has been found that it improves salt-tolerance in some plant species. Two field experiments were conducted in 2013/14 and 2014/15 to evaluate the potential effects of foliar applications with 0.5 and 1.0 mM αTOC on growth, yield, plant water relations, osmoprotectants and the activity of antioxidant system of two onion varieties (i.e., Giza 20 and Giza ^{Red}) "classified as salt-sensitive" under saline soil condition (EC_e = 7.94 - 8.81 dS m^{-1}). Exogenous application of αTOC significantly improved salt stress tolerance in onion plants by reducing the endogenous H_2O_2 and lipid peroxidation, and increasing enzymatic (i.e., superoxide dismutase, catalase, ascorbate peroxidase, and glutathione reductase) and non-enzymatic (i.e., ascorbic acid and glutathione) antioxidant activity. However, Giza 20 was more sensitive to salinity, while it was more responsive to αTOC treatment. Moreover, αTOC application significantly affected photosynthesis efficiency and plant water status as evaluated by relative water content and membrane stability index. These results were positively reflected in plant growth, productivity and water use efficiency under salt stress conditions, indicating that αTOC may participate in the enzymatic and non-enzymatic antioxidants. Therefore, foliar application of αTOC could be used to induce salt-tolerance in onion plants.

Keywords: abiotic stresses; antioxidant system; α-tocopherol; growth; physiology; yield.
Abbreviations: αTOC_ α-Tocopherol; H_2O_2_ Hydrogen peroxide; ROS_ Reactive oxygen species; MDA_ Malondialdehyde; GR_ Glutathione reductase; APX_ Ascorbate peroxidase; CAT_ Catalase; SOD_ Superoxide dismutase; GSH_ Glutathione; AsA_ Ascorbic acid; IAA_ Indole-3-acetic acid; TSS_ Total soluble sugars; RWC_ Relative water content; MSI_ Membrane stability index; WUE_ Water use efficiency.

Introduction

Onion (*Allium cepa* L.) is since ancient Egypt times a valuable vegetable crop for people all over the world. On the list of worldwide cultivated vegetable crops, onions rank second only preceded by tomatoes, the average annual production in the last five years in Egypt is put at 2,113,749 tons (FAOSTAT, 2015). Onions are rated as a salt sensitive crop, bulbs yield severely declines for every unit increase in soil salinity (ECe) (Shannon and Grieve, 1999). In many areas, particularly in arid and semi-arid regions, salt stress considers one of the major limiting factors to plant growth and crop production. Salt stress deleteriously affects plant morphology and physiology through osmotic and ionic stress, and changes biochemical responses in plants (Khan et al., 2013). It causes physiological drought problem-induced osmotic stress that adversely affects water relations and ion balance in plants, leading to ion toxic effects on the metabolic processes (Munns et al., 2006). These problems-induced salinity occur due to triggering oxidative stress in plant tissues through an excessive generation of reactive oxygen species (ROS), damaging lipids, proteins and DNA (Yasar et al., 2006). Chloroplasts are the major organelles producing the ROS such as the superoxide radical ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2) and singlet oxygen ($O_1^{\bullet}$) during photosynthesis (Asada, 1992). ROS cause chlorophyll degradation and membrane lipid peroxidation, namely the accumulation of malondialdehyde (MDA). MDA accumulation is an oxidative stress indicator that is a tested tool to determine the salt tolerance in plants (Yildrimin et al., 2008). The harmful effects generated under salt stress are usually caused by elevated Na^+ and Cl^- concentrations in soil or in water irrigation. Salt stress decreases photosynthetic attributes, plant growth and development, and stimulates the activity of antioxidant system (Sairam and Tyagi, 2004; Rady, 2011; Rady et al., 2013; Semida et al., 2014; Semida and Rady, 2014). To alleviate these adverse effects of salt stress, plants develop several mechanisms to induce their tolerance, protecting their cells and sub-cellular systems from the cytotoxic effects of the ROS with both non-enzymatic and enzymatic antioxidant systems (Sairam and Srivastava, 2001; Mishra et al., 2009), including ion homeostasis, osmotic adjustments, stress damage control and repair, and growth regulation (Zhu, 2002). In addition to the internal mechanisms of plants, efforts have been made to control salinity by various means, including plant foliar application

with some antioxidants, to develop 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), between αTOC and growth characteristics and yield (Semida et al., 2014; Rady et al., 2015) and between αTOC and antioxidant system; enzymatic and non-enzymatic antioxidants (Orabi and Abdelhamid, 2014).

Comparing the response among genotypes of the same species to salinity provides a convenient and useful tool to elucidate the basic mechanisms involved in salt tolerance. The mechanism of salt tolerance is still not fully understood (Gharsa et al., 2008). Therefore, the present study was conducted to investigate if αTOC could be used to ameliorate the harmful effects of salinity stress on two selected onion cultivars. To achieve this aim, the parameters measured in this study included growth, yields, photosynthetic pigments, osmoprotectants, water relations, membrane stability. The anti-oxidative defense system (enzymatic and non-enzymatic antioxidants) that could ameliorate stress generated by soil salinity (7.94 - 8.81 dS m^{-1}) was also determined.

Results

Growth traits of onion plants as affected by α-tocopherol (αTOC) and saline soil conditions

The data in Table 4 show that under saline conditions (EC = 7.94 - 8.81 dS m^{-1}), growth parameters of the two cultivars of onion plants [i.e., shoot length, number of leaves per plant, leaf area per plant, shoot fresh weight (FW) and shoot dry weight (DW)] were significantly increased by the spray applications of αTOC compared to water-applied control plants. The level of 0.5 mM αTOC was found to be more effective, increasing shoot length, number of leaves, leaf area, shoot FW and shoot DW by 11.1 and 57.1%, 24.4 and 71.4%, 62.7 and 155.0%, 83.1 and 273.8%, and 62.9 and 155.1% in the Giza^{Red} and Giza 20 cultivars, respectively compared to those in water-applied controls. The data also indicate that the Giza 20 cultivar was more sensitive to soil salinity, while it was more responsive to the spray applications of αTOC.

Bulb yields and water use efficiency (WUE) of onion plants as affected by α-tocopherol (αTOC) and saline soil conditions

The data in Table 5 reveal that under saline conditions (EC_e = 7.94 - 8.81 dS m^{-1}), bulb yields, particularly the bulb size of 5.0 – 7.5 cm that consumer preferences, total yield and WUE of the two cultivars of onion plants were significantly increased by the spray treatments of αTOC compared to water-applied control plants. The applied level of 0.5 mM αTOC was more effective, increasing total yield and WUE by 94.5 and 70.1%, and 94.0 and 70.2% in the Giza^{Red} and Giza 20 cultivars, respectively compared to those in water-applied controls. The data also show that the Giza^{Red} cultivar had more yield and WUE than the Giza 20 cultivar which was more sensitive to soil salinity.

Leaf photosynthetic pigments and chlorophyll fluorescence of onion plants as affected by α-tocopherol (αTOC) and saline soil conditions

The data in Table 6 show that under saline conditions (EC_e = 7.94 - 8.81 dS m^{-1}), the spray applications of αTOC significantly increased total chlorophyll, total carotenoids, F_v/F_m, F_v/F_0 and PI of the two cultivars of onion plants compared to those of water-applied control plants. The exogenously-applied level of 0.5 mM αTOC was more effective, increasing the above attributes by 10.0 and 13.3%, 20.0 and 16.0%, 9.5 and 11.0%, 87.8 and 38.4%, and 117.2 and 109.9% in the Giza^{Red} and Giza 20 cultivars, respectively compared to those in water-applied controls.

Osmoprotectants, membrane stability index (MSI) and relative water content (RWC) as affected by α-tocopherol (αTOC) and saline soil conditions

The data in Table 7 show that under saline conditions (EC_e = 7.94 - 8.81 dS m^{-1}), the spray application of αTOC reduced the concentrations of free proline and total soluble sugars (TSS), but significantly increased MSI and RWC in the two cultivars of onion plants compared to water-applied control plants. The applied level of 0.5 mM αTOC was found to be more effective, reducing the concentrations of free proline and TSS by 7.1 and 17.6%, and 14.0 and 35.4%, and increasing MSI and RWC by 23.7 and 15.9%, and 20.8 and 22.5% in the Giza^{Red} and Giza 20 cultivars, respectively compared to those in water-applied controls.

Non-enzymatic antioxidants, hydrogen peroxide (H_2O_2) and lipid peroxidation (MDA) as affected by α-tocopherol (αTOC) and saline soil conditions

The data in Table 8 show that under saline conditions (EC_e = 7.94 - 8.81 dS m^{-1}), the spray applications of αTOC significantly increased the concentrations of ascorbic acid (AsA) and glutathione (GSH), but significantly reduced the concentrations of H_2O_2 and MDA in the two cultivars of onion plants compared to water-applied control plants. The applied level of 0.5 mM αTOC was found to be more effective, increasing the concentrations of AsA and GSH by 48.3 and 34.9%, and 20.5 and 19.4%, and reducing H_2O_2 and MDA by 60.0 and 67.7%, and 45.6 and 45.8% in the Giza^{Red} and Giza 20 cultivars, respectively compared to those in water-applied controls.

Antioxidant enzyme activities as affected by α-tocopherol (αTOC) and saline soil conditions

The activities of superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX) and glutathione reductase (GR) are shown in Table 9. Under saline conditions (EC_e = 7.94 - 8.81 dS m^{-1}), the spray application of αTOC significantly increased the activities of SOD, CAT, APX and GR in the two cultivars of onion plants compared to water-applied control plants. The level of 0.5 mM αTOC was more effective, increasing the activities these enzymes by 20.3 and 14.8%, 34.9 and 24.7%, 20.9 and 20.4%, and 35.6 and 25.5% in the Giza^{Red} and Giza 20 cultivars, respectively compared to those in water-applied controls.

Discussion

In the arid and semiarid regions, salt stress adversely affects different processes during seed germination, growth and flowering that reflects in plant productivity. These negative

Table 1. Chemical composition of irrigation water.

Ionic concentration (Meq/l)								EC[a]	pH	SAR[b]
CO_3^{2--}	HCO_3^-	Cl^-	SO_4^{2-}	Ca^{++}	Mg^{2+}	Na^+	K^+	(dS/m)		
2013/14										
0.00	4.35	16.73	6.82	7.34	6.84	12.4	1.32	2.67	7.46	5.38
2014/15										
0.00	4.21	15.32	6.41	6.42	5.39	12.71	1.42	2.53	7.41	7.40

[a]EC means the average electrical conductivity, [b]SAR means sodium adsorption ratio.

Table 2. Some initial physical properties of the experimental soil.

Layer (m)	Particle size distribution				Bulk density g/cm^3	K_{sat} m/h	FC (%)	WP (%)	AW (%)
	Sand %	Silt %	Clay %	Texture class					
2013/14									
0 – 0.3	79.2	10. 0	10.8	LS	1.60	0.02	25.33	9.73	15.60
0.3 – 0.6	77.2	10.1	10.7	LS	1.55	0.015	22.19	12.13	10.06
2014/15									
0 – 0.3	78.27	9.80	10.55	LS	1.67	0.025	24.50	9.73	15.60
0.3 – 0.6	77.75	11.70	11.93	LS	1.61	0.015	22.09	11.21	13.25

F.C=Field capacity, W.P = wilting point, A.W= Available water, LS= loamy sand and K_{sat}=Hydraulic conductivity.

Table 3. Some initial chemical properties of the experimental soil.

Properties	Value	
	2013/14	2014/15
pH [at a soil: water(w/v) ratio of 1:2.5]	7.75	7.86
ECe (dS/m; soil – paste extract)	7.94	8.81
CEC($cmol_e$ /kg)	11.15	11.10
$CaCO_3$ (%)	4.83	4.81
Organic matter (%)	1.20	1.10
ESP(exchangeable sodium percentage)	13.46	14.62
Soluble ions:		
Ca^{2+} (meq/l)	21.31	25.32
Mg^{2+} (meq/l)	20.14	22.14
Na^+ (meq/l)	37.12	40.32
K^+ (meq/l)	1.23	1.34
CO_3^{2-} (meq/l)	0.00	0.00
HCO_3^- (meq/l)	3.40	4.38
Cl^- (meq/l)	39.21	44.25
$SO4^{2-}$ (meq/l)	37.19	40.49
Exchangeable cations:		
Ca^{2+} (meq/100g soil)	5.16	5.11
Mg^{2+} (meq/100g soil)	2.83	2.78
Na^+ (meq/100g soil)	1.64	1.61
K^+ (meq/100g soil)	1.56	1.51
Available nutrients:		
N (%)	0.005	0.004
P (mg/kg soil)	530.20	523.80
K (mg/kg soil)	71.20	69.90
Fe (mg/kg soil)	3.60	3.40
Mn (mg/kg soil)	10.64	10.60
Zn (mg/kg soil)	0.72	0.70
Cu (mg/kg soil)	0.53	0.50

Table 4. Effects of foliar applications with α-tocopherol (αTOC; mM) on growth traits of two varieties of onion plants grown on a saline soil (EC = 8.81 dS m^{-1}).

Treatment Variety	αTOC	Shoot length (cm)	Number of Leaves	Leaf Area (dm^2)	Shoot FW (g)	Shoot DW (g)
$Giza^{Red}$	0	72.2 ± 2.5c	8.2 ± 0.6c	16.1 ± 1.2b	72.6 ± 6.5c	7.53 ±0.61b
	0.5	80.2 ± 3.9a	10.2 ± 0.7b	26.2 ± 2.1a	132.9 ± 9.6a	12.27 ±0.97a
	1	77.4 ± 1.7ab	8.8 ± 0.5c	25.2 ± 2.5a	124.7 ± 8.7a	11.82 ±1.01a
Giza 20	0	51.8 ± 2.5d	7.0 ± 0.3d	10.0 ± 1.6c	34.3 ± 2.8d	4.68 ±0.35c
	0.5	81.4 ± 3.2a	12.0 ± 0.9a	25.5 ± 2.0a	128.2 ± 9.6a	11.94 ±0.96a
	1	76.2 ± 3.5b	11.4 ± 0.9ab	23.1 ± 1.9a	113.9 ± 7.2b	10.83 ±0.88a

[#]Values are means ± SE (n = 6). Mean values in each column followed by a different lower-case-letter are significantly different by Fisher's least-significant difference test (LSD) at $P \leq 0.05$.

Table 5. Effects of foliar applications with α-tocopherol (αTOC; mM) on yields and water use efficiency (WUE) of two varieties of onion plants grown on a saline soil (EC = 8.81 dS m^{-1}).

Treatment		Yield (t/ha) of different sizes			Total yield (t/ha)	WUE
Variety	αTOC	< 5.0 cm	5.0–7.5 cm	>7.5 cm		
Giza^{Red}	0	2.0 ± 0.2d	13.8 ± 0.3b	14.9 ± 0.5c	30.7 ± 2.1c	3.51 ± 0.12c
	0.5	5.4 ± 0.4b	18.1 ± 1.0a	36.2 ± 2.1a	59.7 ± 3.2a	6.81 ± 0.30a
	1	4.0 ± 0.3c	20.3 ± 2.5a	25.7 ± 2.6b	50.0 ± 4.3b	5.71 ± 0.28b
Giza 20	0	6.8 ± 0.7a	11.6 ± 1.5b	18.4 ± 1.9c	36.8 ± 3.1c	4.20 ± 0.26c
	0.5	5.0 ± 0.4b	17.9 ± 2.0a	39.7 ± 2.0a	62.6 ± 4.5a	7.15 ± 0.37a
	1	5.5 ± 0.6b	19.8 ± 1.1a	35.7 ± 2.6a	61.0 ± 4.8a	6.97 ± 0.25a

#Values are means ± SE (n = 6). Mean values in each column followed by a different lower-case-letter are significantly different by Fisher's least-significant difference test (LSD) at $P \leq 0.05$.

Table 6. Effects of foliar applications with α-tocopherol (αTOC; mM) on leaf photosynthetic pigments and chlorophyll fluorescence of two varieties of onion plants grown on a saline soil (EC = 8.81 dS m^{-1}).

Treatment		Total chlorophyll	Total carotenoids (mg	F_v/F_m	F_v/F_0	PI
Variety	αTOC	(mg g^{-1} FW)	g^{-1} FW)			
Giza^{Red}	0	3.0 ±0.1c	0.25 ±0.01b	0.74 ±0.02b	2.29 ±0.31b	2.09 ±0.60d
	0.5	3.3 ±0.2ab	0.30 ±0.02a	0.81 ±0.02a	4.30 ±0.12a	4.54 ±0.61bc
	1	3.0 ±0.2c	0.29 ±0.02a	0.80 ±0.02a	4.07 ±0.27a	3.85 ±0.33cd
Giza 20	0	3.0 ±0.1c	0.25 ±0.01b	0.73 ±0.01b	3.31 ±0.28b	3.03 ±1.01d
	0.5	3.4 ±0.2a	0.29 ±0.02a	0.81 ±0.03a	4.58 ±0.28a	6.36 ±1.46a
	1	3.1 ±0.1bc	0.28 ±0.02a	0.81 ±0.02a	4.37 ±0.35a	5.54 ±0.94ab

#Values are means ± SE (n = 6). Mean values in each column followed by a different lower-case-letter are significantly different by Fisher's least-significant difference test (LSD) at $P \leq 0.05$.

Table 7. Effects of foliar applications with α-tocopherol (αTOC; mM) on leaf free proline, total soluble sugars (TS sugars), membrane stability index (MSI) and relative water content (RWC) of two varieties of onion plants grown on a saline soil (EC = 8.81 dS m^{-1}).

Treatment		Free proline	TS sugars	MSI (%)	RWC (%)
Variety	αTOC	(mg g^{-1} DW)	(mg g^{-1} DW)		
Giza^{Red}	0	0.14 ± 0.008b	3.35 ± 0.13ab	55.6 ± 1.6b	71.3 ± 1.6c
	0.5	0.13 ± 0.007b	2.88 ± 0.16bc	68.8 ± 1.2a	86.1 ± 1.9a
	1	0.13 ± 0.006b	2.33 ± 0.14d	66.9 ± 2.7a	80.2 ± 1.8b
Giza 20	0	0.17 ± 0.009a	3.87 ± 0.10a	57.2 ± 1.1b	71.9 ± 1.6c
	0.5	0.14 ± 0.007b	2.50 ± 0.11cd	66.3 ± 2.2a	88.1 ± 1.7a
	1	0.14 ± 0.007b	2.97 ± 0.12b	64.7 ± 2.3a	78.1 ± 1.9b

#Values are means ± SE (n = 6). Mean values in each column followed by a different lower-case-letter are significantly different by Fisher's least-significant difference test (LSD) at $P \leq 0.05$.

Table 8. Effects of foliar applications with α-tocopherol (αTOC; mM) on the activity of leaf antioxidants; ascorbic acid (AsA), glutathione (GSH), hydrogen peroxide (H_2O_2) and malondialdehyde (MDA) of two varieties of onion plants grown on a saline soil (EC = 8.81 dS m^{-1}).

Treatment		AsA (ng ascorbate	GSH (nmol GSH	H_2O_2 (µmol g	MDA (nmol of
Variety	αTOC	mg protein^{-1})	mg protein^{-1})	FW^{-1})	MDA g FW^{-1})
Giza^{Red}	0	515.4 ± 7.3c	28.07 ± 0.12c	8.84 ±0.04b	1.49 ±0.03a
	0.5	764.1 ± 9.8a	33.83 ± 0.19a	3.54 ±0.02c	0.81 ±0.01c
	1	681.7 ± 8.2b	31.33 ± 0.17b	3.62 ±0.02c	0.94 ±0.02b
Giza 20	0	527.4 ± 7.6c	28.40 ± 0.12c	9.90 ±0.04a	1.55 ±0.04a
	0.5	711.4 ± 9.7a	33.90 ± 0.18a	3.20 ±0.03c	0.84 ±0.02c
	1	685.6 ± 8.5b	31.63 ± 0.17b	3.71 ±0.02c	0.89 ±0.02bc

#Values are means ± SE (n = 6). Mean values in each column followed by a different lower-case-letter are significantly different by Fisher's least-significant difference test (LSD) at $P \leq 0.05$.

Table 9. Effects of foliar applications with α-tocopherol (αTOC; mM) on the activity of leaf antioxidant enzymes; superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX) and glutathione reductase (GR) of two varieties of onion plants grown on a saline soil (EC = 8.81 dS m^{-1}).

Treatment		SOD (nmol NO_2 mg	CAT (nmol H_2O_2 mg	APX (nmol ascorbate	GR (nmol NADPH
Variety	αTOC	protein^{-1} min^{-1})	protein^{-1} min^{-1})	oxidized mg protein^{-1} min^{-1})	oxidized mg protein^{-1} min^{-1})
Giza^{Red}	0	78.2 ± 0.7c	19.5 ± 0.2d	50.7 ± 0.5d	20.8 ± 0.2d
	0.5	94.1 ± 0.8a	26.3 ± 0.3a	61.3 ± 0.7a	28.2 ± 0.4a
	1	86.2 ± 0.6b	23.1 ± 0.3c	56.3 ± 0.6c	25.1 ± 0.3c
Giza 20	0	77.2 ± 0.6c	19.4 ± 0.2d	49.4 ± 0.5d	21.2 ± 0.2d
	0.5	88.6 ± 0.7b	24.2 ± 0.4b	59.5 ± 0.6b	26.6 ± 0.4b
	1	87.2 ± 0.7b	23.0 ± 0.3c	58.9 ± 0.6b	25.6 ± 0.3bc

#Values are means ± SE (n = 6). Mean values in each column followed by a different lower-case-letter are significantly different by Fisher's least-significant difference test (LSD) at $P \leq 0.05$.

effects of salinity occur by stimulating the overproduction of reactive oxygen species (ROS) through various organelles and enzymes (Sairam and Tyagi, 2004). To avoid these effects, plants adopt several strategies, including ion homeostasis, osmotic adjustment, and enhancing the antioxidant defense system (Xiong and Zhu, 2002). In the current study, the reduction in plant growth (Table 4) under the adverse conditions of the tested saline soil (EC = 7.94 - 8.81 dS m^{-1}) could be attributed to the osmotic effect of salt stress, increasing the growth inhibitors (i.e., abscisic acid), reducing the growth promoters [i.e, indole-3-acetic acid (IAA) and gibberellins] and causing a disturbance in the water balance of the stressed plants. This leads to stomatal closure, ionic imbalance, reduction in photosynthesis, accumulation of toxic ions and consequently inhibition of growth (Qiu et al., 2007; Rady, 2011; Rady et al., 2013; Semida and Rady, 2014; Semida et al., 2014).

Salt stress limits plant growth by its adverse effect on various physiological and biochemical processes, including photosynthesis, antioxidant capacity and ion homeostasis (Orabi and Abdelhamid, 2014), resulting in damaging growth cells which, therefore, cannot perform their functions (Chen and Murata, 2000). Spraying the onion seedlings with 0.5 mM αTOC significantly improved all plant growth characteristics, resulting in the increase in the final yields of both Giza^{Red} and Giza 20 (Tables 4 and 5). Sakr and El-Metwally (2009) reported that αTOC alleviated the harmful effect of high soil salinity stress on growth of wheat plants and increased plant dry matter accumulation in the soil salt areas. αTOC is the major vitamin E compound located in the envelope, thylakoid membranes and plastoglobuli of the leaf chloroplasts. αTOC as an antioxidant, deactivates photosynthesis-derived reactive oxygen species (ROS), and prevents the increase in lipid peroxidation by scavenging lipid peroxyl radicals in thylakoid membranes. The levels of αTOC change differentially in response to environmental restrictions, depending on the magnitude of the stress and species-sensitivity to stress. αTOC considers an important part of the plant defense machinery, which maintains the integrity and normal function of the photosynthetic apparatus (Liu et al., 2008). This photosynthetic apparatus confirms the significant increase in the performance index (PI) and an increase in Fv/Fm and Fv/F0 by the application of αTOC under salt stress (Table 6). Foyer and Noctor (2005) reported that αTOC acts directly to neutralize superoxide radicals or singlet oxygen in plant cells. αTOC also affects positively many physiological processes under saline conditions such as the regulation of growth, differentiation and metabolism of plants and the increase in the physiological availability of water and nutrients (Azooz et al., 2002; Barakat 2003). Some works used αTOC (Cvelkorska et al., 2005; Pourcel et al., 2007; Shao et al., 2008), reported that it 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, and consequently obtaining healthy plant growth and satisfactory yield under soil salinity conditions.

Salt stress partially inhibited photosynthesis by the reduction in photosynthetic pigments (chlorophylls and carotenoids) and chlorophyll fluorescence (Fv/Fm, Fv/F0 and PI; Table 6), while αTOC application increased these parameters. αTOC as an antioxidant protected photosynthetic machinery from salt-induced ROS. In the present study, chlorophyll and carotenoid concentrations and chlorophyll fluorescence decreased under salt stress through the reduction in intermediates of chlorophyll biosynthesis (Khan, 2003; Abd El-Mageed and Semida, 2015b), leading to a reduced absorption of light by the chloroplast, indirectly impairing photosynthesis (Ashfaque et al., 2014; Semida et al., 2015). In addition, salinity stress caused deleterious effects on leaf carotenoids that have been reported in several investigations (Sadak et al., 2010; Rady et al., 2013; Abdelhamid et al., 2013; Orabi and Abdelhamid, 2014). Foliar application of αTOC significantly increased carotenoid concentrations under salt stress. In a previous study under salinity stress, αTOC significantly increased the concentrations of carotenoids, which might play a key role as a free radical scavenger (Sakr and El-Metwally, 2009), controlling the cellular level of free radicals and peroxides (Apel and Hirt, 2004) and enhancing plant capacity to reduce the damage caused by ROS, which in turn increased chlorophyll contents in plants (Orabi and Abdelhamid, 2014). The F_v/F_m, F_v/F_0 and PI were used as a noninvasive method to determine the functional state of the photosynthetic machinery. They reduced significantly by salt stress, while αTOC application significantly improved these components in leaves of salt-stressed plants (Table 6). The highest F_v/F_m, F_v/F_0 and PI were observed in the leaves of salt-stressed plants sprayed with 0.5 mM αTOC. The reduction in the F_v/F_m, F_v/F_0 and PI provides an indicator of photo-inhibitory damage caused by the incident photon flux density when plants are subjected to a wide range of environmental stresses (Bjorkman and Demming, 1987), including salinity, however, αTOC application was a remedy for plants.

The decreased growth and yields of onion plants Giza^{Red} and Giza 20) grown under salt stress have been associated with the reduction in water potential that decreased the water use efficiency (WUE; Tables 4 and 5), while the application of αTOC invalidated these adverse effects and increased WUE, which may be related to the increase in relative water content (RWC; Table 7). The αTOC application enabled the plant leaves to maintain a high level of RWC by regulating the leaf osmolality, alleviating the negative effects of salt stress. The increase in water potential and osmotic potential might help stabilization of protein and increases photosynthesis (Ashfaque et al., 2014). Under salt stress, soil salts trigger the osmotic stress, and the over-accumulation of salts in plant cells causes ionic stress. These stresses individually affect the physiological status of plant (Ueda et al., 2007). However, exogenous application of αTOC showed amelioration of the deleterious salt effects and increased the membrane stability index (MSI) and RWC, maintaining cells turgid for the healthy metabolic processes and membranes integrity. In addition, Sairam et al. (2005) and Abd El-Mageed and Semida (2015a) reported a decrease in relative water content (RWC) and membrane stability index (MSI) under the effect of salt stress. This result confirms our results (Table 7).

Although some researchers have reported a higher proline content with foliar application of αTOC acting as a solute for intercellular osmotic adjustment (Orabi and Abdelhamid, 2014) and a further important factor of adaptation to salinity (Taie et al., 2013), the present study reported a reduction in free proline and total soluble sugars by αTOC application (Table 7). This may be attributed to the crucial role of αTOC as an antioxidant in mitigating the deleterious salt effects. The antioxidant activity of αTOC is mainly attributed 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.

Malondialdehyde (MDA), produced under stress, is the decomposition product of poly unsaturated fatty acids of cell

membranes. The rate of lipid peroxidation in terms of MDA can therefore be used as an indicator to evaluate plant tolerance or sensitivity to oxidative stress (Jain et al., 2001). Increase in lipid peroxidation level in onion plants exposed to soil salinity shows that the enzyme activities (superoxide dismutase; SOD, catalase; CAT, ascorbate peroxidase; APX and glutathione reductase; GR, Table 9) might have not been enough to prevent the peroxidation of membrane lipids. However, reductions have recorded as a result of αTOC application assuring that plant tolerance would be gained to scavenge ROS generated under salinity. The increased contents of H_2O_2 in the salt-stressed onion plants were significantly decreased when plants sprayed with αTOC (Table 8). This is accompanied by the increased contents of antioxidants such as AsA and GSH (Table 8), and the antioxidative enzymes such as SOD, CAT, APX and GR (Table 9), which enable onion plants to overcome salt stress by limiting the ROS damages.

Several studies have indicated that the oxidative damage generated during salt stress is due to the overproduction of ROS such as $O_2^{\bullet-}$ and H_2O_2 and antioxidant activity alterations (Rady et al., 2013). To avoid the damage caused by the oxidative stress, plants have developed many antioxidant systems, including SOD that constitutes the first line of defence against ROS (Alscher et al., 2002) by reducing the $O_2^{\bullet-}$ radical to H_2O_2. H_2O_2 can serve as a substrate for numerous enzymes such as CAT, which is located in the peroxysomes where the H_2O_2 concentration is very high, and thus H_2O_2 is eliminated by peroxidases. CAT together with SOD considers the most effective antioxidant enzymes in preventing cellular damage (Scandalios, 1993). In addition, APX considers one of the most important enzymes in the reduction of H_2O_2 (Feierabend, 2005). The regenerating enzymes DHAR and GR as a fundamental part of the Halliwell–Asada cycle form a part of the regeneration of AsA from DHA using GSH as a reducing power (Foyer and Noctor, 2009). The GSH consumed can be, in turn, regenerated from its oxidized form (GSSG) by the reaction of GR (Foyer et al., 1991). Data of the present study show that αTOC application increased the activities of SOD, CAT, APX and GR compared to the salt stressed-control plants (Table 9). These increased activities of antioxidant enzymes may contribute advantages to onion plants and help to perform better in various aspects of growth and metabolism as they defend against the harmful effect of salinity stress mainly through the increase in these enzyme activities together with the increase of some antioxidant substances. The AsA and GSH (the substrates of the Halliwell–Asada cycle) act also as antioxidants in an isolated way on being involved in the direct reduction of ROS during different types of stress (Del Río et al., 2006). This situation is reflected in the total contents of AsA and GSH in this study. They are increased by the application of αTOC under salt stress, alleviating the accumulation of $O_2^{\bullet-}$. The AsA can directly eliminate $O_2^{\bullet-}$ and H_2O_2 in a non-enzymatic way (Foyer et al., 1991).

αTOC has appeared to play a major role in chloroplastic antioxidant network of plants, contributing to preserve an adequate redox station in chloroplasts, and to maintaining thylakoid membrane structure and function during plant development and in plant responses to stress (Munne´-Bosch and Alegra, 2002; Munne´-Bosch, 2005). Similar to our results, Sakr and El-Metwally (2009) and Orabi and Abdehamid (2014) recorded increases in the antioxidant enzymes in response to αTOC application on wheat and faba bean plants against oxidative stress. Salt stress tolerance in onion plants, in this study, was improved by the application of αTOC that was effective in alleviating the soil salinity stress by better chlorophyll, enzymatic and non-enzymatic antioxidants, and plant growth and productivity. This might be attributed to cytokinin mediated stay green effect. Findings of this study suggested that the exogenous application of αTOC, particularly at the level of 0.5 mM, promotes the expression of stress–response genes and increases salt stress tolerance in onion plants. In addition, inducing the expression of ROS-related stress–response genes by αTOC application is an effective means of enhancing resistance to subsequent stress, and we recommend performing additional studies of the potential application of αTOC for plant production under stress.

Materials and methods

Plant material and growth conditions

Tow field experiments were conducted in 2013/14 and 2014/15 in Sennoris District, Fayoum, Southwest Cairo, Egypt between latitudes 29° 29´ 41" N and longitudes 30° 52´ 30" E. The climatic data of studied area indicate that the total rainfalls does not exceed 7.5 mm/year and the mean minimum and maximum annual temperatures are 14.5 and 31.0°C in January and June, respectively. The evaporation rates coincide with temperatures where the lowest evaporation rate (1.9 mm day^{-1}) was recorded in January while the highest value (7.3 mm day^{-1}) was recorded in June. According to the aridity index (Ponce et al., 2000). The area is located under hyper-arid climatic condition. These landforms are characterized by less than 3.5% surface slopes with an elevation vary from 49 m below sea level to 26 m above sea level.

Healthy seeds of two varieties [i.e., Giza 20 and $Giza^{Red}$], the most common varieties of onion (*Allium cepa* L.) in Egypt were sown on 30 and 25 September 2013 and 2014, respectively. Transplants were transported and replanted on the 7^{th} and 10^{th} of December respectively and harvested on 6 May for both seasons. The experimental layout was a randomized complete block design with three replications. Total surface area used for the experiment was 550 m^2 divided into 18 experimental plots of 16.5 m^2 each (1.1 m wide × 15 m long). Each experimental unites were separated by two guard rows to protect against border effects. Each plot included 12 planting rows placed 10 cm apart with a distance of 15 cm between plants. These plant densities are a typical practice of producers. The soil was supplemented with a full dose of NPK fertilizer according to the recommendations of the Ministry of Agriculture and Land Reclamation, Egypt. These recommendations were for 400 kg ha^{-1} calcium super-phosphate (15.5% P_2O_5), 450 kg ha^{-1} ammonium nitrate (33.5% N), and 150 kg ha^{-1} potassium sulphate (48% K_2O).
The onion plants were irrigated 2 d intervals using the amounts of applied water as 100% in a drip irrigation method. The daily ETo was calculated from weather data according to the following equation of FAO-PM (Allen et al. 1998):

$$ETo = \frac{0.408\ \Delta (R_n - G) + \gamma \frac{900}{T_{mean} + 273} u_2 (e_s - e_a)}{\Delta + \gamma (1 + 0.34\, u_2)}$$

Where: *ETo* is the reference evapotranspiration (mm day^{-1}), Δ is the slope of the saturation vapor pressure curve at air temperature (kPa C^{-1}), R_n is the net radiation at the crop surface (MJm^{-2} d^{-1}), *G* is the soil heat flux density (MJm^{-2} d^{-1}), γ is the psychometric constant = ($0.665 \times 10^{-3} \times P$), kPa C^{-1} (Allen et al., 1998), P is the atmospheric pressure (kPa), U_2 is the wind speed at 2 m height (m s^{-1}), e_s is the

saturation vapor pressure (kPa), e_a actual vapor pressure (kPa) ($e_s - e_a$) is the saturation vapor pressure deficit (kPa), and T_{mean} is the mean daily air temperature at 2 m height (°C).

The average daily *ETo* in Fayoum region was estimated using the monthly mean weather data for a 15-year period (January 1994 – December 2008) of Etsa station. The average daily *ETo* was 2.17, 1.64, 2.29, 3.35 and 5.02 ETo mm day^{-1} in December, January, February, Marsh and April, respectively.

The crop water requirements (*ETc*) were estimated using the crop coefficient according to equation:

$$ETc = ETo \times Kc$$

Where: *ETc* is the crop water requirement (mm day^{-1}) and *Kc* is the crop coefficient.

The lengths of the different crop growth stages were 15, 25, 70, and 40 days for initial, crop development, mid-season and late season stages, respectively. The crop coefficients (*Kc*) of initial, mid and end stages were 0.7, 1.05 and 0.75, respectively according to Allen et al. (1998).

The amount of irrigation water applied was calculated according to the following equation:

$$IWA = \frac{A \times ETc \times Ii \times Kr}{Ea \times 1000 \times (1 - LR)}$$

Where: *IWA* is the irrigation water applied (m^3), *A* is the plot area (m^2), *ETc* is the crop water requirements (mm day^{-1}), *Ii* is the irrigation intervals (d), *Ea* is the application efficiency (%) (*Ea* = 85) and *LR* is the leaching requirements.

All other recommended agricultural, disease and pest management practices were followed as recommended by the Ministry of Agriculture and Land Reclamation.

Analysis of soil and water was conducted according to Klute (1986) and Page et al. (1982) and its results are shown in Tables (1 – 3). Based on the EC values shown in Table (3), the soil is classed as being strongly saline according to Dahnke and Whitney (1988). In addition, scale the used irrigation water lies within the second categories for salinity and sodicity levels (C_2S_1, ECiw = 0.75 – 3.00 dS m^{-1} and SAR < 6.0; Ayers and Wesctcot, 1985). The experiment was arranged in a randomized complete block design, with 3 levels of α-tocopherol (αTOC; 0, 0.5 and 1.0 mM) for two onion cultivars, with three replicate plots.

Twenty days after transplanting, seedlings in each plot were sprayed to run-off with 0 (tap water as a control), 0.5 and 1.0 mM αTOC. Sprays were then repeated at 20 and 40 days later. The concentrations of αTOC, and the number and timing of sprays were based on 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.

Growth traits, bulb yields and water use efficiency (WUE) measurements

Thirteen-week-old 3 onion plants were carefully removed from each experimental plot (n = 9). The lengths of their shoots were measured using a meter scale, and the numbers of leaves per plant were counted. Leaf areas were measured manually using a graph sheet, where the squares covered by the leaf were counted to note the leaf area. The shoots of plants were weighed to record their fresh weights, and then placed in an oven at 70ºC till the constant weight and the dry weights were recorded. At the end of experiment, all onion plant in each experimental plot were removed to estimate the bulb yield in 3 sizes; < 5.0 cm, 5 – 7.5 cm and >7.5 cm, which were then collected together to measure the total bulb yield.

WUE values of applied water were calculated (as kg yield per m^3 water) for different treatments after harvest according to the following equation (Jensen, 1983):

$$WUE = \frac{\text{Bulb yield (Kg ha}^{-1})}{\text{water applied (m}^3\text{ ha}^{-1})}$$

Determination of leaf photosynthetic pigments and chlorophyll fluorescence

Total chlorophyll and carotenoids were extracted and determined (in mg g^{-1} FW) in 0.2 g fresh leaf (Arnon, 1949). Samples were homogenized in 50 ml 80% (v/v) acetone, and then centrifuged at 10,000 × *g* for 10 min. The absorbance of the acetone extract was measured at 663, 645, and 470 nm using a UV-160A UV-visible recording spectrometer (Shimadzu, Kyoto, Japan).

On two different sunny days, chlorophyll fluorescence was measured using a portable fluorometer (Handy PEA, Hansatech Instruments Ltd, Kings Lynn, UK). One leaf (at the same age) was chosen per plant to conduct the fluorescence measurements. Maximum quantum yield of PS II F_v/F_m was calculated using the formulae; $F_v/F_m = (F_m - F_0) / F_m$ (Maxwell and Johnson, 2000). F_v/F_0 reflects the efficiency of electron donation to the PSII RCs and the rate of photosynthetic quantum conversion at PSII RCs. F_v/F_0 was calculated using the formulae; $F_v/F_0 = (F_m - F_0) / F_0$ (Spoustová et al., 2013). Performance index of photosynthesis based on the equal absorption (PI_{ABS}) was calculated as reported by Clark et al. (2000).

Osmoprotectants , membrane stability index (MSI) and relative water content (RWC) determination

Proline was extracted and determined (in mg per g of leaf DW) in 0.5 g dried leaf tissue as illustrated by Bates et al. (1973). TSS were extracted and determined in 0.2 g dried leaf according to Irigoyen et al. (1992). The MSI was determined using duplicate 0.2 g samples of fully-expanded leaf tissue (Rady, 2011). RWC was estimated using 2 cm-diameter fully-expanded leaf discs as reported by Hayat et al. (2007).

Determination of total ascorbic acid (AsA), glutathione (GSH), hydrogen peroxide (H_2O_2), and lipid peroxidation levels

Extraction of AsA from leaf samples and its determination were conducted according to the method of Kampfenkel et al. (1995). Fresh leaf sample (1 g) was homogenized in liquid N_2 and extracted with 5% (w/v) TCA. At 4°C, the homogenate was centrifuged for 5 min at 15,600 × *g*. The supernatant was then transferred to a new reaction vessel and immediately assayed for the ascorbate concentration in a reaction mixture containing supernatant, 10 mM DTT, 0.2 M phosphate buffer (pH 7.4), 0.5% NEM, 10% TCA, 42% H_3PO_4, 4% 2,2′-dipyridyl and 3% $FeCl_3$.

The level of GSH was determined as described by the method of De Kok et al. (1986). GSH was extracted from filtered FW in 2 volumes of extracting buffer (2% sulfosalicylic acid, 1 mM Na_2EDTA and 0.15% AsA) and homogenized. The homogenate was centrifuged at 12,000 × *g* for 5 min. An aliquot of supernatant was then used for the measurement of GSH in leaf samples by GSH assay kit (Sigma Chemical Co., USA).

H_2O_2 level was determined as the described method of Mukherjee and Choudhuri (1983) with a slight modification. The mixture of 2 ml of ice-cold acetone extract of the sample,

titanium reagent (0.2 ml of 20% titanic tetrachloride in concentrated HCl, v/v), 0.4 ml of NH_4OH to form hydroperoxide-titanium complex, was centrifuged at 12,000 ×g for 10 min. The precipitate was dissolved in 2 ml of 2 M H_2SO_4 and absorbance of the solution was measured at 415 nm against a reagent blank. Concentration of H_2O_2 (in µmol per g of fresh leaf) was determined using the standard curve plotted with known concentration of H_2O_2.

By measuring the level of malondialdehyde (MDA), the level of lipid peroxidation was determined according to Madhava Rao and Sresty (2000). MDA is a product of lipid peroxidation by thiobarbituric acid reaction. Fresh leaf samples (1 g) were mixed with 1 ml of 10% TCA and 1 ml of 0.67% thiobarbituric acid (TBA), and heated in a boiling water bath for 15 min. MDA was determined spectophotometrically by absorbance at 535 nm and expressed as nmol of MDA per g fresh leaf samples.

Antioxidant enzyme assays

Enzymes were extracted from 1 g of fresh onion leaves. The biomass was filtered and homogenized in liquid N_2 with 0.05 M phosphate buffer (pH 7.0) containing 0.1 M EDTA and 1% PVP at 4 °C. The leaf biomass:extraction buffer (w/v) proportion was 1:2, respectively. At 4°C, the homogenate was centrifuged for 10 min at 15,000 ×*g*. The supernatant was then dialyzed overnight in phosphate buffer. The activity determination of the selected enzymes was performed. In the homogenate, protein concentration was also determined according to Lowry et al. (1951).

The activity of superoxide dismutase (E.C. 1.15.1.1) was assayed according to the method of Beauchamp and Fridovich (1971). The ability of enzyme to inhibit the photochemical reduction of nitroblue tetrazolium (NBT) and the change in absorbance was measured at 560 nm. The reaction mixture consisted of 25 mM phosphate buffer (pH 7.8), 65 µM NBT, 2 µM riboflavin, enzyme extract, and TEMED and the reaction mixture was exposed to light of 350 µmol m^{-2} s^{-1} for 15 min. The activity of enzyme was expressed as nmol of H_2O_2 per mg of soluble protein per min. The activity of catalase (EC 1.11.1.6) was determined as outlined in the method of Aeby (1984). The rate of H_2O_2 decomposition at 240 nm was measured spectrophotometrically and calculated using a molar extension coefficient ε = 45.2 mM^{-1} cm^{-1}. The reaction mixture consisted of phosphate buffer, 0.1 mM H_2O_2 and enzyme extract. One unit of catalase activity was presumed as the amount of enzyme that decomposed 1 nmol of H_2O_2 per mg of soluble protein per min at 30 °C.

The activity of total ascorbate peroxidase (APX; EC 1.11.1.11) was determined using the method described by Nakano and Asada (1981). The reaction mixture consisted of phosphate buffer, 5 mM sodium ascorbate, 0.1 mM H_2O_2 and enzyme extract. The activity of APX was determined as a reduction in the absorbance of ascorbate at 290 nm and calculated using a molar extension coefficient ε = 2.8 mM^{-1} cm^{-1}. The activity of enzyme was calculated as the amount of the enzyme that oxidized 1 nmol of ascorbate consumed per mg of soluble protein per min at 30°C. The activity of enzyme was expressed as nmol ascorbate oxidized per mg of soluble protein per min.

The activity of glutathione reductase (EC 1.6.4.2) was determined according to Jablonski and Anderson (1978). The reaction mixture consisted of 10 mM GSSG, 1 mM Na_2EDTA, 200 mM phosphate buffer and enzyme extract was pre-incubated at 25°C for 5 min. The reaction was initiated by the addition of 1 mM NADPH and the rate of oxidation of NADPH was measured spectrophotometrically at 340 nm. The activity of enzyme was expressed as nmol NADPH oxidized per mg of soluble protein per min.

Statistical analysis

All data were subjected to analysis of variance (ANOVA) for a randomized complete block design with three replications, 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.

Conclusions

The αTOC is a major compound of vitamin E, which can play different roles in plant metabolism and can play important roles in amelioration of biotic and abiotic stresses. Therefore, this study suggests spraying onion plants (either cv. $Giza^{Red}$ or cv. Giza 20) with 0.5 mM αTOC to improve the response of plants to soil salinity stress (8.81 dS m^{-1}), because αTOC significantly increased the activity of the antioxidant system, including enzymatic and non-enzymatic antioxidants, increased the plant water relations and decreased the endogenous H_2O_2 and MDA concentrations. Accordingly, our study considered αTOC as a beneficial foliar application at the level of 0.50 mM to help plants to overcome the deleterious effects of salt stress conditions.

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