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RESPONSE OF ANTIOXIDANTS AND LIPID PEROXIDATION TO EXOGENOUS APPLICATION OF ALPHA-TOCOPHEROL IN SUNFLOWER (*HELIANTHUS ANNUUS* L.) UNDER SALT STRESS

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Abstract

Alpha-tocopherol is an antioxidant mainly responsible for detoxification of reactive oxygen species produced during prolonged abiotic stresses as reported in the previous studies. A pot experiment was carried out to study the response of antioxidants and lipid peroxidation to alpha-tocopherol seed soaking treatment in saline conditions. Seeds of sunflower cvs. FH-[572 and 621] were immersed for 16 hours in four alpha-tocopherol levels, [0 (dist. H_2O), 100, 200, 300] mg L^{-1}. Thirty five days after seed sowing plants were irrigated with 0 m*M* and 120 m*M* (two levels) of salt (NaCl) till final harvest. Leaves were sampled 61 days after seed sowing for biochemical analyses and yield at maturity. Salt stress (120 m*M* NaCl) caused significant reduction in shoot and root dry weight, total phenolic contents and superoxide dismutase activity and had non-significant effect on all yield related parameters. Salt stress showed substantial increase in the activity of enzymatic antioxidants such as catalase, peroxidase and glutathione reductase. Seed soaking with alpha-tocopherol significantly increased shoot and root dry weight, 100 achene weight and total achene weight plant^{-1}, catalase and peroxidase activity, total phenolic content, ascorbic acid and showed non-significant effect on leaf total soluble proteins and hydrogen peroxide. Alpha-tocopherol pre-sowing seed treatment caused significant reduction in lipid peroxidation. Cultivar FH-621 showed higher salt tolerance and alpha tocopherol 200 and 300 mg L^{-1} levels effectively increased its yield and dry matter production respectively under salt stress.

Key words: Sunflower; Alpha-tocopherol; Salt stress; Antioxidants; Lipid peroxidation.

Introduction

Saline soils in continents especially Australia, Africa, America and Asia are a menace, accelerating @ 10% each year and expected to expand by 2050 over 50 % cultivated land (Jamil *et al*., 2011; Rengasamy, 2016). Salt stress obstructs plant growth and reduce yield by reallocating energy to plant's stress defense mechanism (Krasensky & Jonak, 2012; Munns & Gilliham, 2015). Contingent upon the nature, severity and period of stress a variety of reactive oxygen species and lipid peroxidation products are produced acting as key biomarkers of oxidative stress (Hamed *et al*., 2014; Anjum *et al*., 2015). Reactive oxygen species (ROS) increase membrane permeability, causing K^+ leakage and cellular dysfunction (Demidchik, 2015). Previous studies reported increased levels of hydrogen peroxide (H_2O_2), malondialdehyde and electrolyte leakage in wheat, cotton and barley upon exposure to salt stress (Qiu *et al*., 2014; Liu *et al*., 2014; Agami, 2014). Plants detoxify ROS by altering activities of various enzymatic and non-enzymatic antioxidants especially alpha-tocopherol, ascorbic acid, phenolics etc. have the capacity to extinguish singlet oxygen and hydroxyl radical (Bose *et al*., 2014; Vardhini & Anjum, 2015).

Alpha-tocopherol besides a non-enzymatic antioxidant also physiologically modulates processes like germination, translocation of photo-assimilates and leaf senescence (Shao *et al*., 2008; Szarka *et al*., 2012). Alpha-tocopherol by reducing oxidative damage and increasing nutrients and water availability sustains growth and yield of plants under abiotic stresses (Foyer & Noctor, 2005; Shao *et al*., 2008). Some previous studies revealed that foliar application of alpha-tocopherol played crucial role in abiotic stress mitigation by quenching ROS and reducing lipid peroxidation and ion leakage (Sattler *et al*., 2006; Jin & Daniell, 2014). On the contrary, there is little information about the role of alpha-tocopherol's seed soaking treatment in glycophytes defense against salt stress.

Sunflower (*Helianthus annuus* L.) is ranked 5th among oilseed and food security crops (Anon., 2016; Seiler *et al*., 2017). During years 2014-2015 global estimated production of sunflower was 23.2 million ha (Anon., 2016b). In advanced countries, 10-20% achenes of sunflower are used for baked goods, one fourth for bird's food and 60% for oil extraction (Seiler & Gulya, 2015). Sunflower has two growing seasons per year and requires 90-110 days for its life cycle completion (Moghanibash *et al*., 2013). Pakistan's expenditure on the import of edible oil is increasing every year (Badar *et al*., 2002). Due to zero cholesterol and high polyunsaturated fatty acids, demand for sunflower oil is constantly increasing (Anuradha, 2014). Sunflower is less prone to moderate salt stress, can bear nearly 50 m*M* of salt stress and survives better in reduced number of irrigations (Francois 1996; Riaz *et al*., 2012; Kumar *et al*., 2014). Nevertheless, higher accumulation of salts in soil caused drastic reduction in yield of sunflower (Wang *et al*., 2017). However, it is assumed that alpha-tocopherol through regulation of antioxidant defense mechanism could protect sunflower from damaging effects of salt stress. The aim of the present research was to study modulations in lipid peroxidation and antioxidant defense mechanism of sunflower in response to alpha-tocopherol pre-sowing seed treatment and its impact on dry weight and yield related attributes.

Materials and Methods

Pot experiments with sand culture were performed in University of agriculture, Faisalabad (Botanical Garden) from February to June, 2015 and 2016 for which achenes of sunflower cultivars were obtained from Ayub Agricultural Research Institute (Oilseed Research Section), Faisalabad, Pakistan. Hundred healthy achenes of sunflower cvs. FH-[572 and 621] were immersed in four alpha-tocopherol (α-Toc) concentrations such as [0 (dist. H_2O), 100, 200, 300] mg L^{-1} for 16 hours and dried before planting. Factorial CRD statistical design with four repetitions was used for this purpose. Initially 10 achenes per pot (plastic pots of 11 inches depth and 10 inches diameter) were sown and after thinning at three leaves seedling stage only six plants were maintained pot^{-1}. Salt stress (0 m*M* and 120 m*M*) NaCl concentration was applied for 35 days after seed sowing along with Hoagland's nutrient solution every two weeks till final harvest @ two liters per pot. In order to determine root and shoot dry weight, lipid peroxidation and activity of antioxidants leaves were sampled 61 days after seed sowing and data for yield attributes were recorded at the completion of the experiment.

Pre-chilled mortar and pestle were used for grinding (0.5 g) fresh leaves while adding (50 m*M*) 10 ml (pH 7.8) while maintaining temperature at 4°C. Homogenized mixture was then centrifuged for 20 minutes at 12000 × *g*. Supernatant was extracted and stored at -20°C for determining the activity of enzymatic antioxidants. Activities of all enzymatic antioxidants such as catalase, peroxidase, superoxide dismutase and glutathione reductase were evaluated on protein basis.

Giannopolitis & Ries (1977) method was used to determine superoxide dismutase activity. Phosphate buffer 250 µL of 50 m*M* strength having pH 7.8 was added in 400 µL distilled H_2O in a cuvette. In this mixture methionine (100 µL), NBT (50 µL), enzyme extract (50 µL) and riboflavin (50 µL) were supplemented and retained for 15 min. underneath fluorescent lamp. Absorbance (at 560 nm) of this fusion was measured using spectrophotometer (Ultraviolet visible) (Model: U2020 IRMECO). The amount of enzyme responsible for 50% inhibition in NBT photo-reduction compared to the control (mixture deprived of enzyme extract) is considered as a unit superoxide dismutase activity.

Catalase and peroxidase enzyme activities were determined by the method of Chance & Maehly (1955). In a cuvette, one ml H_2O_2 was added to 1.9 ml (5.9 m*M*) phosphate buffer (pH 7.8). Reaction started upon adding 0.1 ml enzyme extract to the mixture. Change in the absorbance of the reaction mixture was observed at 20 seconds intervals for two minutes in order to determine catalase activity.

In a cuvette, 100 µL enzyme extract was added to a mixture of (750 µL) phosphate buffer, (100 µL) guaiacol (20 m*M*) and 100 µL hydrogen peroxide (40 m*M*). Peroxidase activity was determined by recording change in absorbance at twenty seconds intervals for 3 minutes at 470 nm. One unit catalase and peroxidase was considered equivalent to 0.01 units/minute change in absorbance.

Glutathione reductase activity was determined using Foyer & Helliwell (1976) protocol. In this method (0.5 g) leaf (freshly sampled) was crushed in a (pre-chilled) mortar-pestle while adding (10 ml) (pH 7.8) phosphate buffer. Maintaining temperature at 4 ºC, the mixture was centrifuged at 12000 x *g* for 15 minutes. Enzyme extract (100 µL) and 100 µL NADPH (1.2 m*M*) were added to the mixture of 700 µL phosphate buffer and 100 µL oxidized glutathione (5 m*M*) contained in a cuvette. Change in absorbance at 340 nm was recorded every 20 seconds for 2 minutes. UV-visible spectrophotometer (Model: IRMECO U2020) was used for recording of absorbance.

Bradford (1976) method was followed to determine (total) soluble proteins (TSP). Leaf (0.5 g) (freshly sampled) was pulverized using mortar-pestle and (50 m*M*) 10 ml phosphate buffer was added (pH 7.8). Mixture was centrifuged (temperature 4ºC) at 12000 × *g* for 20 min. To 0.5 ml supernatant in a test tube 1 ml Bradford reagent was supplemented and kept for 30 minutes in dark at 32ºC and absorbance was measured at 595nm for determination of the total soluble proteins using spectrophotometer. Different concentrations of bovine serum albumin were used to prepare standard curve.

Ascorbic acid was determined according to Mukherjee & Choudhri (1983) method. Using mortar and pestle (0.25 g) fresh leaves were pounded in (10 ml) (6%) trichloroacetic acid (TCA) and centrifuged (4ºC temperature) at 1000 × *g* for 10 min. One drop thiourea (10% in 70% ethanol) was supplemented in a blend of supernatant (4 ml) and 2% dinitrophenyl hydrazine (2 ml) and (using water bath) boiled for 20 minutes. Test tubes were placed on ice for quick cooling and 5 ml sulphuric acid (80% v/v) was added at 0ºC. Absorbance of the mixture was recorded at 530 nm.

Julkenen-Titto (1985) protocol was applied for determining total phenolic. Leaves (freshly sampled) (0.1 g) were homogenized in 80% acetone (2 ml) and centrifuged at 10,000 x *g* for 15 minutes. Supernatant was stored at -20°C. Distilled water (2 ml) and phenol (Folin–Ciocalteau) 0.5 ml was added to 100 µL leaf extract (supernatant) taken in a test tube. Mixture was vigorously shaked for 5-10 seconds after adding 20% Na_2CO_3 (2.5 ml) and 5 ml distilled water. After 20 minutes, absorbance of the mixture at 750 nm was measured.

Velikova *et al*., (2000) procedure was followed for determining hydrogen peroxide. Trichloroacetic acid (TCA) (0.1% w/v) 5 ml was added to 0.5 g ground fresh leaf and centrifuged (at 12,000 x *g*) for 15 min. Phosphate buffer (0.5 ml) (pH maintained at 7.0) and (1 ml) KI was added to 0.5 ml supernatant in a test tube, vortexed and absorbance of the mixture was measured at 390 nm.

Carmak & Horst (1991) method was used to determine leaf malondialdehyde content**.** 0.5 g fresh leaf was homogenized in (0.1% w/v) TCA (10 ml) and centrifuged for 10 minutes at 12000 x *g*. In supernatant (1 ml), thiobarbituric acid (0.5%) 4 ml prepared in 20% TCA was added and kept at 95°C in a water bath for 30 minutes. Upon cooling in ice the absorbance was measured at 600 and 532 nm.

The data for yield related parameters of sunflower such as total achene weight plant^{-1}, number of achene plant^{-1} and 100 achene weight were recorded upon sunflower ripening.

The data collected for dry weight, biochemical and yield attributes of two years experiment were combined and significance among mean square values were statistically analyzed (three-way analyses of variance) with Snedecor and Cochran (1980) software package (Cohort software Berkeley, California).

Results and Discussions

Enzymatic antioxidants: Salt stress considerably reduced activity of superoxide dismutase ($p\leq0.001$) in sunflower cultivars (Table 1; Fig. 1). Whereas, seed priming with α-Toc (vitamin E) had not influenced SOD activity significantly. Strong interaction ($p\leq0.001$) within salt stress and cultivars exhibited accelerated SOD activity under saline condition in FH-621 compared to FH-572. Substantial interaction within cultivars and α-Toc seed priming ($p\leq0.01$) exhibited the effectiveness of α-Toc 100 mg L^{-1} level in increasing activity of SOD in cv. FH-572 only. Imposition of salt stress speed up the activity of catalase (CAT) enzyme considerably ($p\leq0.001$) in both sunflower cultivars (Table 1; Fig. 1). Overall increase in CAT activity was more ($p\leq0.05$) in FH-621 cultivar compared to FH-572. Seed treatment with [100 and 200] mg L^{-1} α-Toc substantially increased ($p\leq0.01$) activity of CAT in FH-572 and FH-621 cultivars respectively, under salt stress. Sunflower cultivars responded differentially to salt stress ($p\leq0.05$) in case of peroxidase (POD) activity (Table 1; Fig. 1). Sunflower cultivar FH-572 showed increase and cv. FH-621 showed decrease in POD activity under saline condition. α-Toc seed treatment with 200 mg L^{-1} level markedly increased ($p\leq0.05$) POD activity under saline conditions in both sunflower cultivars. Salt stress considerably enhanced glutathione reductase activity ($p\leq0.001$) in FH-[572 and 621] cultivars (Table 1; Fig. 1). However, α-Toc seed treatment had non-significant influence on glutathione reductase (GR) activity. Remarkably strong interaction within cultivars (cvs.) and α-Toc seed priming ($p\leq0.001$) exhibited that under both non-saline and saline conditions, all levels of α-Toc were responsible for accelerated GR activity in FH-572 cultivar only. However, interaction in α-Toc and salt stress ($p\leq0.05$) revealed the effectiveness of 100 mg L^{-1} concentration in increasing activity of GR in both sunflower cultivars upon salt stress imposition.

Table 1. Mean squares from analyses of variance of data for antioxidant, lipid peroxidation (MDA), H_2O_2, total soluble proteins and yield related attributes of sunflower (*Helianthus annuus* L.) plants raised from α-tocopherol treated seeds (16h).

Source of variations	Df	SOD	CAT	POD	GR	Ascorbic acid
Cultivars (Cvs)	1	0.662ns	0.943*	0.846*	78.934ns	1.241***
Salinity (S)	1	13.379***	5.953***	0.591*	1434.97***	0.272ns
α-tocopherol (α-Toc)	3	0.387ns	0.946**	0.463*	114.42ns	0.583***
Cvs x S	1	7.290***	0.081ns	0.189ns	184.917ns	0.296ns
Cvs x α-Toc	3	4.597**	0.306ns	0.075ns	542.626***	0.410**
S x α-Toc	3	0.483ns	0.256ns	0.248ns	219.22*	0.149ns
Cvs x S x α-Toc	3	0.947ns	0.485ns	0.025ns	21.563ns	0.113ns
Error	48	0.497	0.191	0.143	54.647	0.074
Source of variations	**Df**	**Total phenolics**	**H_2O_2**	**MDA**	**Shoot dry weight**	**Root dry weight**
Cultivars (Cvs)	1	0.104***	9726.62**	5263.2***	57.95***	0.389ns
Salinity (S)	1	0.019*	2943.445ns	145.619ns	54.94***	1.597***
α-tocopherol (α-Toc)	3	0.031***	637.751ns	163.452*	28.99***	0.611**
Cvs x S	1	0.008ns	2455.912ns	33.841ns	0.018ns	0.197ns
Cvs x α-Toc	3	0.009ns	1406.571ns	171.321*	17.10**	0.131ns
S x α-Toc	3	0.010ns	306.318ns	15.564ns	0.911ns	1.105ns
Cvs x S x α-Toc	3	0.015*	3315.738*	13.826ns	6.987ns	1.108ns
Error	48	0.004	794.611	49.448	3.866	0.116
Source of variations	**Df**	**Total soluble protein**	**Number of achene plant^{-1}**	**Total achene weight**	**100 achene weight**	
Cultivars (Cvs)	1	0.415ns	135884.39***	62.114***	0.072ns	
Salinity (S)	1	0.342ns	166.015ns	0.032ns	0.012ns	
α-tocopherol (α-Toc)	3	0.938ns	20341ns	20.806**	1.710***	
Cvs x S	1	2.365*	6460.14ns	14.774*	1.279*	
Cvs x α-Toc	3	1.106ns	20154.31ns	10.706*	0.941**	
S x α-Toc	3	0.627ns	3993.256ns	5.385ns	0.263ns	
Cvs x S x α-Toc	3	0.592ns	10049.64ns	5.871ns	0.086ns	
Error	48	0.410	7350.182	3.266	0.202	

* = $p\leq0.05$, ** = $p\leq0.01$, *** = $p\leq0.001$, ns = Non-significant, df = Degrees of freedom; ns = Non-significant; df = Degrees of freedom; CAT = Catalase; POD = Peroxidase; SOD = Superoxide dismutase; GR = Glutathione reductase; H_2O_2 = Hydrogen peroxide; MDA = Malondialdehyde

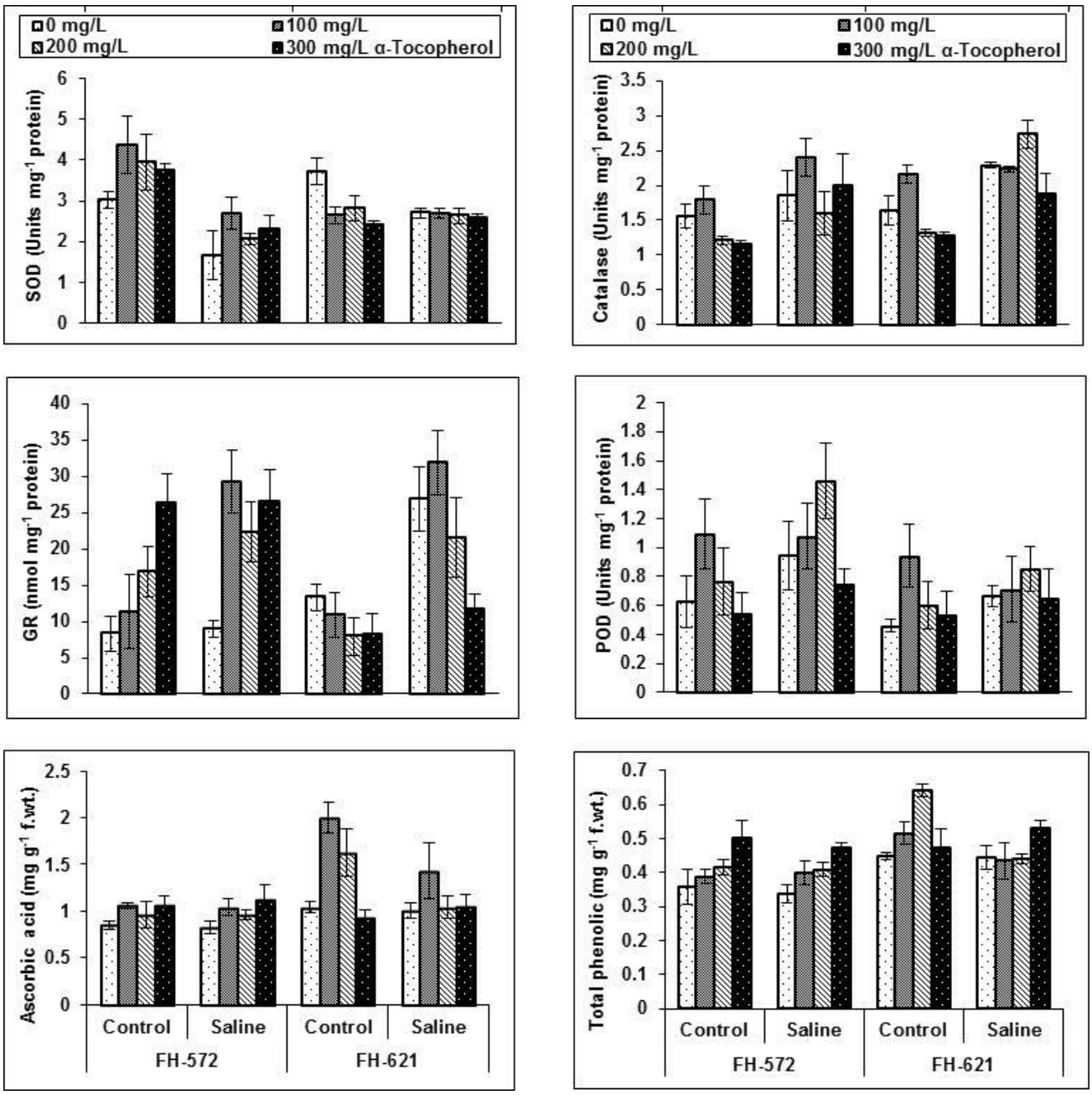

Fig. 1. Antioxidant activities of sunflower (*Helianthus annuus* L.) plants raised from α-tocopherol treated seeds (16h) under saline and non-saline regimes.

Alpha-tocopherol (α-Toc) plays important role in antioxidant defense system's regulation upon exposure to stress conditions in plants. Previous researchers reported accelerated activities of antioxidant enzymes (superoxide dismutase, catalase, glutathione reductase and ascorbate peroxidase) in response to α-Toc in wheat (Sakr & El-Metwally, 2009), faba beans (Orabi & Abdelhamid, 2016) and onion (Semida *et al*., 2016). Srinieng *et al*., (2015) described that superoxide dismutase (SOD) enzyme by converting superoxide anion into H_2O_2 acts as first line of defense against oxidative stress. Several studies on glycophytes revealed either negative or positive correlation between salt stress and SOD activity (Maksimovic *et al*., 2013). In our research, salt stress showed remarkable decrease in SOD activity and considerable increase in glutathione reductase (GR) activity whereas, both enzymes showed non-significant response to alpha-tocopherol seed treatment. Ozgur *et al*., (2013) reported GR as an antioxidant and redox regulatory enzyme in plants. In present investigation, α-Toc seed treatment mitigated the toxic influence of salt (NaCl) stress by increasing activity of (enzymatic) antioxidant especially catalase and peroxidase which might be responsible for reducing reactive oxygen species level. Catalases and peroxidases detoxify H_2O_2 by its conversion into oxygen and water mainly in peroxisomes and apoplast respectively (Mittler, 2002; Fagerstedt *et al*., 2010) and protect plant from oxidative damage by providing phenols for elimination of ROS (Zabalza *et al*., 2007). Enhanced activity of catalase in faba beans and cotton (Orabi & Abdelhamid, 2016; Hussien *et al*., 2015), catalase and peroxidase in tomato, wheat and eggplant

(Shalata & Neumann, 2001; Fraouk, 2011; Shaheen *et al*., 2013), have reported influential role of these enzymatic antioxidants in protection of plants against oxidative damage. In our case, increased salt tolerance of cv. FH-621 was due to increased peroxidase and glutathione reductase activity. Gill *et al*., (2013) revealed in a study that glutathione reductase (reduced form) reduces dehydroascorbate through glutathione ascorbate cycle.

Antioxidants (non-enzymatic): On the whole, FH-621 cultivar indicated (both in saline and non-saline condition) increase ($p \leq 0.001$) in leaf ascorbic acid (AsA) concentration than cv. FH-572 (Table 1; Fig. 1). However, salt stress had non-significant influence on amount of leaf AsA. Seed priming with [100 and 300] mg L^{-1} α-Toc concentrations considerably raised AsA ($p \leq 0.001$) amount under salt stress in leaves of FH-[621 and 572] cultivars, respectively. Remarkably strong ($p \leq 0.01$) interaction in α-Toc seed priming and cultivars revealed that 100 mg L^{-1} α-Toc concentration was more significant in maximizing AsA production in the leaves of FH-621 cultivar only. Imposition of salt stress caused reduction in phenolic contents ($p \leq 0.05$) of both sunflower cultivars (Table 1; Fig. 1). Overall (saline and non-saline condition) increase in the amount of total phenolic was more ($p \leq 0.001$) in the leaves of FH-621 than cv. FH-572. Seed priming with 300 mg L^{-1} (α-Toc) level substantially raised ($p \leq 0.001$) the amount of total phenolic in the leaves of both sunflower cultivars under salt stress.

Our results showed that seed treatment with (vitamin E) α-Toc mitigated negative impact of salt (NaCl) stress in sunflower (cultivars) by raising level of non-enzymatic antioxidants (total phenolic content and ascorbic acid) more in cv. FH-621. Among non-enzymatic antioxidant ascorbic acid (AsA) is effective in quenching reactive oxygen species (Ashraf, 2009). Helliwell *et al*., (1995) and Jang *et al*., (2007) reported that phenols prevent protein oxidation and lipid peroxidation by providing phenolic hydrogen. Increase in phenolic content and AsA have been reported in wheat upon vitamin C (ascorbic acid) and alpha-tocopherol application (Farouk, 2011). Semida *et al*., (2014) reported rise in total phenols in *Vicia faba* upon application of α-Toc exogenously. In our study, salt stress reduced phenolic contents in both sunflower cultivars but did not effect AsA content. Ashraf *et al*., (2013) and Kanwal *et al*., (2013) observed diminished phenolic (contents) in wheat and mungbean in response to salt stress. However, a previous research on eggplant showed no (significant) effect of salt (NaCl) stress on AsA (Shaheen *et al*., 2013).

Lipid peroxidation (MDA) and hydrogen peroxide (H_2O_2): Seed treatment with α-Toc significantly reduced ($p \leq 0.05$) malondialdehyde (product of lipid peroxidation) content (Table 1; Fig. 2). While, malondialdehyde (MDA) showed insignificant response to salt stress. FH-621 (sunflower) cultivar on the whole (under stressed and non-stressed conditions) experienced less lipid peroxidation (diminished MDA amount) ($p \leq 0.001$) compared to FH-572 cultivar. Interaction was significant ($p \leq 0.05$) in α-Toc and cultivars showing that 300 mg L^{-1} α-Toc (vit. E) conc. was influential only in reducing MDA content in cv. FH-621. α-Toc seed treatment and salt (NaCl) stress had no (significant) influence on concentration of hydrogen peroxide (H_2O_2) (Table 1; Fig. 2). However, overall production (under stressed and non-stressed conditions) of H_2O_2 was less ($p \leq 0.01$) in sunflower FH-621 cultivar compared to FH-572. Interaction among salinity, α-Toc and cultivars was significant ($p \leq 0.05$) showing increase and decrease in H_2O_2 content in FH-572 and FH-621 cultivars respectively, under salt stress and (200 and 300) mg L^{-1} α-Toc concentrations were responsible for considerable reduction of H_2O_2 content in FH-[572 and 621] cultivars respectively, under (NaCl) stress.

Seckin *et al*., (2009) reported malondialdehyde (MDA) as an indicator of abiotic stress (produced in consequence of lipid peroxidation). In our study, overall lesser production of MDA and H_2O_2 in cv. FH-621 than cv. FH-572 was observed. Similar to our finding that plant grown from alpha-tocopherol treated seeds showed lesser production of leaf MDA, Jie *et al*., (2008) also observed the same in *Leymus chinensis* seedlings. Furthermore, overexpression of α-Toc and decrease in MDA content in (*TMT*) tobacco leaves suggested role of α-Toc in membrane protection and mitigation of salt stress (Jin & Daniell, 2014). Farouk (2011) observed lesser MDA and H_2O_2 production upon ascorbic acid and α-Toc exogenous application in wheat plants. Salt stress induced increase in MDA and H_2O_2 have been observed by researchers in onion (Semida *et al*., 2016), wheat (Qiu *et al*., 2014), mung bean (Orabi & Abdelhamid, 2016) and citrus (Kostopoulou *et al*., 2014). On the contrary, the current investigation showed non-significant response of H_2O_2 and MDA to salt stress.

Dry weight: Salt stress significantly reduced dry weight ($p \leq 0.001$) of shoot in both (sunflower) cultivars (Table 1; Fig. 2). Overall (under stressed and non-stressed conditions), shoot dry weight reduction was more (significant) in FH-572 cultivar ($p \leq 0.001$) compared to FH-621 cultivar. Seed treatment with 300 mg L^{-1} concentration of α-Toc remained effective (significantly) in increasing dry weight of shoot ($p \leq 0.001$) under salt stress compared to control (dist. H_2O treatment) in FH-[572 and 621] cultivars. Strong interaction in cultivars and (vit. E) α-Toc exists showing highly significant response of cv. FH-621 to α-Toc seed treatment ($p \leq 0.01$). Imposition of salt stress significantly reduced ($p \leq 0.001$) root's dry weight in both cultivars (Table 1; Fig. 2). Seed priming with (100 and 300) mg L^{-1} concentrations of α-Toc, considerably increased dry weight of root ($p \leq 0.01$) in FH-[572 and 621] cultivars respectively, under salt stress.

Salt stress reduced dry weight of root and shoot in present study might be due to restricted growth as a result of rapid osmotic effect and restricted cell elongation and division (Godfery *et al*., 2004; Jampeetong & Brix, 2009). Previous studies also reported salt stress induced inhibition in sunflower growth (Riaz *et al*., 2012; Kumar *et al*., 2014). Alpha-tocopherol seed priming ameliorated toxic impact of salt stress by improving dry weight of (stem and root) FH-621 cultivar (more significantly). Similar to our findings, Al-Qubaie (2012) reported improvement in growth attributes of sunflower, wheat (Kumar *et al*., 2013), Shoe flower (El-Quesni *et al*., 2009) and mung bean (Orabi & Abdelhamid, 2016) upon exogenous application of antioxidants and they attributed these results to less amount of ROS production due to enhanced accumulation of antioxidants and improved ion homeostasis.

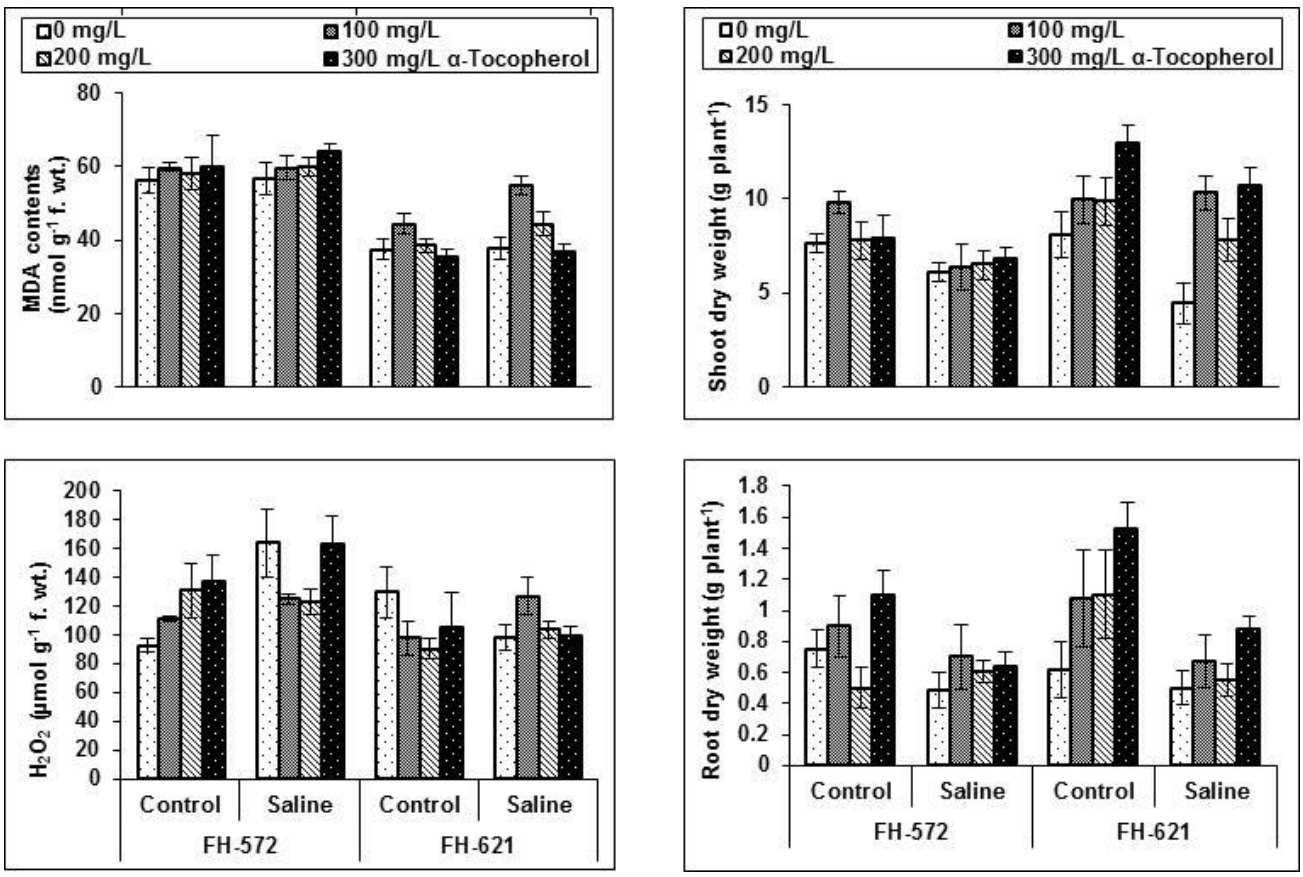

Fig. 2. Leaf malondialdehyde (MDA), hydrogen peroxide (H_2O_2), shoot and root dry weight of sunflower (*Helianthus annuus* L.) plants raised from α-tocopherol treated seeds (16h) under saline and non-saline regimes.

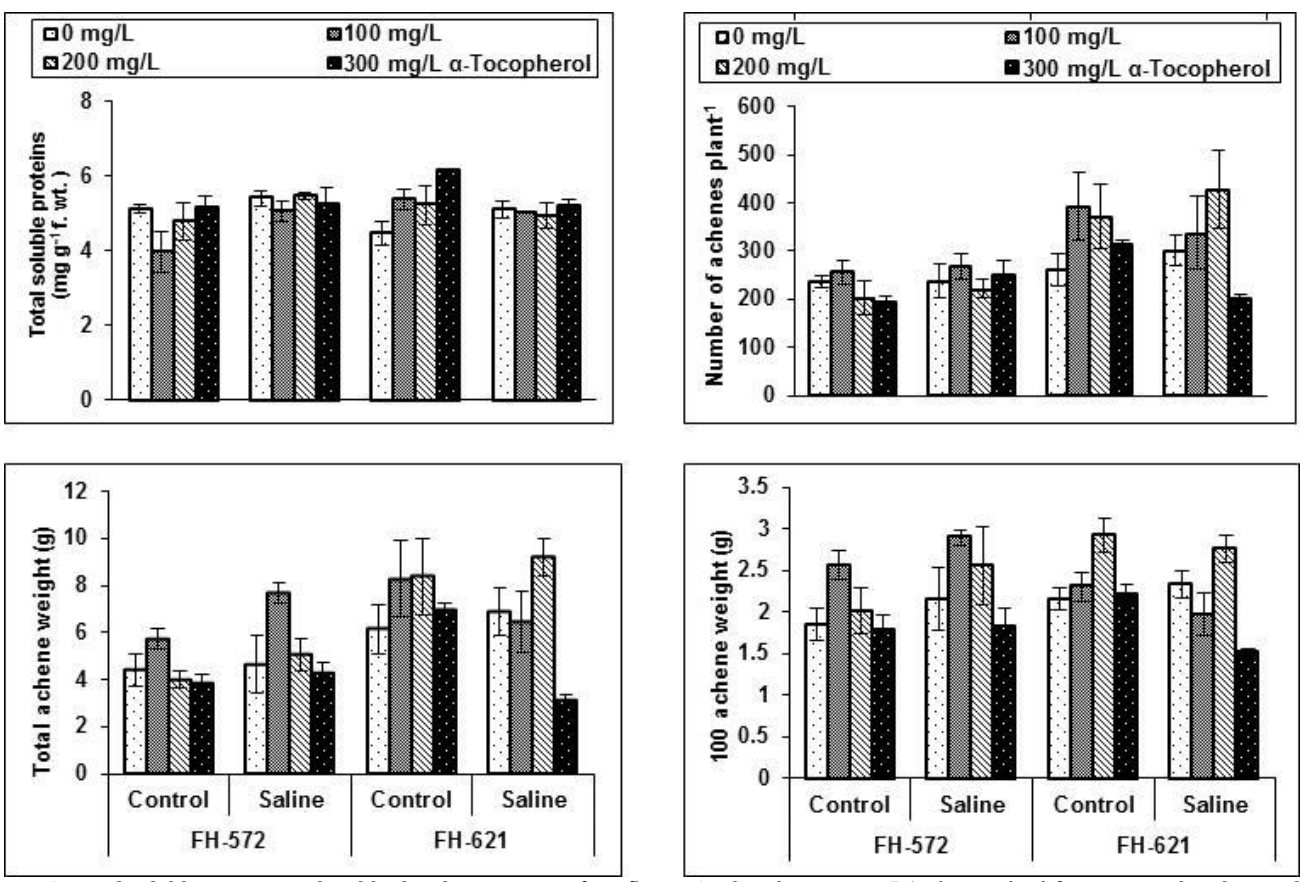

Fig. 3. Total soluble proteins and yield related parameters of sunflower (*Helianthus annuus* L.) plants raised from α-tocopherol treated seeds (16h) under saline and non-saline regimes.

Total soluble protein and yield: Response of total soluble protein (TSP) remained non-significant to α-Toc seed treatment and salt stress (Table 1; Fig. 3). Interaction within salt (NaCl) stress and (vit. E) α-Toc was significant ($p \leq 0.05$) showing increase and reduction in total soluble proteins of FH-[572 and 621] cultivars respectively, under stress. α-Toc seed treatment and salinity had no (significant) influence on total number (No.) of achene plant^{-1} (Table 1; Fig. 3). However, cv. FH-621 produced more achenes plant^{-1} ($p \leq 0.001$) than FH-572 cultivar. Total achene weight was remarkably higher in FH-[621 than 572] cultivar ($p \leq 0.001$) (Table 1; Fig. 3). Whereas, total achene weight did not (significantly) respond to NaCl stress. However, seed priming with (vit. E) α-Toc (significantly) increased total achene weight ($p \leq 0.01$). Strong interaction ($p \leq 0.05$) in salt stress and cultivars showed increase in total achene weight in both cultivars in saline condition. However, significant interaction ($p \leq 0.05$) in α-Toc seed treatment and cultivars showed that (100 and 200) mg L^{-1} of α-Toc concentrations were responsible for improving total achene weight in cv. FH-572 and cv. FH-621 respectively. Imposition of salt stress had non-significant influence on 100 achene weight (Table 1; Fig. 3). α-Toc seed treatment caused substantial increase ($p \leq 0.001$) in 100 achene weight. Strong interaction ($p \leq 0.05$) in salinity and cultivars (cvs.) showed upsurge and drop in 100 achene weight in cv. FH-572 and FH-621 cultivar respectively under NaCl stress. Remarkably higher interaction ($p \leq 0.01$) within α-Toc and cultivars showed that (100 and 200) mg L^{-1} of α-Toc concentrations played influential role in maximizing 100 achene weight in FH-[572 and 621] cultivars respectively, under stressed and non-stressed environment.

Sadak *et al*., (2010) and Rady *et al*., (2011) revealed that in sunflower, salt stress caused decrease in TSP. Conversely, in this study salinity and α-Toc seed priming did not (significantly) influenced TSP. In our study, NaCl stress did not (significantly) effect yield related parameters contrary to the previous researches on mung bean, flax and onion (Orabi & Abdelhamid, 2016; Sadak & Dawood, 2014; Semida *et al*., 2016) but in accordance with the observation of Kumar *et al*., (2014) on sunflower reporting sunflower (being moderately salt tolerant) can bear moderate salt stress. In this research, increase in yield of sunflower (total achene weight and 100 achene weight) in response to α-Toc pre-sowing seed treatment might be due to enhanced rate of photosynthesis and higher photosynthates production (Lalarukh & Shahbaz, 2018). Alpha-tocopherol induced increase in yield has been reported in *Calendula officinalis*, faba beans and wheat (Soltani *et al*., 2012; Orabi & Abdelhamid, 2016; Dawood *et al*., 2014). Accordingly, researchers accredited increase in yield to alpha-tocopherol's effect on more sugars and proteins (photosynthates) production and delayed leaf senescence.

Conclusion

Alpha-tocopherol (vitamin E) pre-sowing seed treatment alleviated the adverse effects of salt stress and exhibited increase in (shoot and root) dry weights and yield (total achene weight and 100 achene weight) of sunflower possibly by regulating antioxidant defense system and reducing lipid peroxidation. Seed treatment with alpha-tocopherol increased activities of catalase and peroxidase (enzymatic antioxidants), ascorbic acid and total phenolics (non-enzymatic antioxidants). Production of MDA (a product of lipid peroxidation) was much lower in plants grown from seeds treated with alpha-tocopherol. Sunflower cv. FH-621 showed enhanced tolerance to salt stress and alpha-tocopherol level, 200 mg L^{-1} was effective in improving yield (33.41% total achene weight and 16.46% hundred achene weight) and 300 mg L^{-1} in increasing shoot (139.89%) and root (77%) dry weight compared to untreated plants in this cultivar. Therefore, farming of sunflower cv. FH-621 is recommended in saline soils and pre-sowing seed treatment with alpha-tocopherol could protect the sunflower plants from damaging effects of salt stress.

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