Full Length Article

Alpha-Tocopherol Induced Modulations in Morpho-Physiological Attributes of Sunflower (*Helianthus annuus*) Grown under Saline Environment

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Abstract

Among abiotic stresses salinity is one of the serious threats for food security worldwide. Sunflower is ranked 5th among crops grown for food security and being moderately salt tolerant is a better choice for growing on saline soil. Application of various compounds including vitamin E might be a beneficial technique for improving growth in plants under saline conditions. A pot experiment was conducted to explore the role of alpha tocopherol seed treatment for inducing salt tolerance in sunflower. Seeds of two sunflower cultivars (FH-572 and FH-621) were soaked for 16 h in four levels of alpha tocopherol i.e. control (distilled water), 100, 200, 300 mg L^{-1} and two levels of NaCl salt (0 and 120 mM) were applied with full strength Hoagland's solution 35 days after seed sowing. Salt stress caused significant decrease in shoot and root fresh weight, leaf relative water content and inhibition in shoot length. Salinity stress increased leaf turgor potential and had non-significant effect on photosynthetic pigments, osmotic potential and gas exchange characteristics. Seed treatment with α-tocopherol considerably increased shoot and root fresh weight, shoot length, net CO_2 assimilation rate, stomatal conductance (g_s) and water use efficiency; however, it showed non-significant effect on photosynthetic pigments, leaf water relation parameters and transpiration rate. Alpha tocopherol, 100 and 300 mg L^{-1} levels were effective in ameliorating the negative impact of salt stress in cv. FH-572 and cv. FH-621, respectively. Results depicts great varietal difference in salinity tolerance and cultivar FH-621 performed better than cv. FH-572 under salinity stress. © 2018 Friends Science Publishers

Keywords: Alpha tocopherol; Sunflower; Salinity; Growth; Photosynthesis

Introduction

Salinity and drought, among abiotic stresses are foremost constraints aggravating 20–50% crop yield worldwide (Abbas *et al*., 2015; Shrivastava and Kumar, 2015). Annual exasperating damages to agribusiness are estimated to exceed beyond 12 billion US dollars due to salt deteriorated soil universally (Shabala, 2013). Out of 1500 Mha of dry land agricultural area, 32 Mha is approximately salt depreciated (FAO, 2008). In Pakistan almost 6 Mha of agricultural land is salt affected and is responsible for productivity deprivation @ 0.02 to 0.04 Mha per annum (Irshad *et al*., 2007; Abbas *et al.*, 2013). Salt stress by reducing water accessibility to plant due to negative osmotic potential, Na$^+$ and Cl$^-$ ion deposition leading to ion toxicity, nutritional disparity and by the cumulative effect of all these factors adversely affects plant morphology and physiology (Ashraf, 1994; Kausar *et al*., 2013). Decline in water uptake by the plant subsequently causes stomatal closure, stunted shoot growth and inhibition in new foliage production and existing leaf area expansion due to restricted cell division and expansion and abscisic acid accumulation (Hsiao and Xu, 2000; Rajendran *et al*., 2009; Gupta and Huang, 2014). Leaf injury and impaired photosynthetic pigments under salinity stress are suitable indicators depicting physiological condition of the plant (Garriga *et al*., 2014). Regulation of water potential and hydraulic conductance is obligatory for plant growth (Chaumont and Tyerman, 2014; Negrão *et al*., 2017). Salt tolerant plants by decreasing root hydraulic conductivity diminish brackish water transport to shoots ultimately reducing water potential (Gama *et al*., 2009; Vysotskaya *et al*., 2010). Crops being intolerant to salt stress, attempts have been made for the improvement in their salinity tolerance by exogenous application of plant growth regulators (Sairam and Tyagi, 2004; Javid *et al*., 2011). Seed priming as a shotgun approach is effective in improving plant germination under saline conditions by triggering diverse metabolic processes (Ashraf and Foolad, 2005; Akram and Ashraf, 2013).

Vitamins acting as antioxidants are natural growth regulators and in subtle amount have profound influence on biochemical and physiological processes of plants

(El Bassiouny *et al*., 2005). Alpha tocopherol (vit. E) is a lipophilic non-enzymatic antioxidant recognized mainly in 1936, instigating fertility in rats (Munne-Bosch, 2007; Lushchak and Semchuk, 2012). Tocopherols play crucial role in phloem loading and protection of photosystem II from photo inhibition under abiotic stresses (Ivanov and Khorobrykh, 2003; Havaux *et al*., 2005; Matringe *et al*., 2008). Plants deficient in α-tocopherol exhibit deprived germination, poor seedling growth, impair photo-assimilate translocation and accelerate leaf senescence (Sattler *et al*., 2004, 2006). Adverse effects of salt stress on growth of various plant species have been studied (Shahbaz and Ashraf, 2013; Masood and Shahbaz, 2016; Kausar and Shahbaz, 2017). On the contrary, there is little acquaintance about role of alpha tocopherols in improving salinity tolerance in plants.

Sunflower (*Helianthus annuus* L.) is unanimously grown due to its wide range of adaptability and poly unsaturated oil contents (Kaleem *et al*., 2011; Anuradha, 2014). Sunflower after soybean, rapeseed and cotton seed is the 4th largest oil producing crop universally (Rodriguez *et al*., 2005). Sunflower is categorized among moderately salt tolerant crops with 4.8 dS m-1 salinity threshold level (Francois, 1996; Katerji *et al*., 2000). Therefore, sunflower cultivation in saline soils is a good choice. It is further presumed that whether seed priming with α-tocopherol could improve salinity tolerance in sunflower or not. The main objective of this research was to explore the modulations in morpho-physiological aspects of sunflower to pre-sowing seed treatment with α-tocopherol under salt stress.

Materials and Methods

In order to reconnoiter the influence of exogenous application of alpha tocopherol (α-Toco) on sunflower (*Helianthus annuus* L.) as pre-sowing seed treatment under salinity stress two independent pot trials were executed in the successive two years during February to June 2015 and 2016 in the old Botanical Garden at the University of Agriculture, Faisalabad, Pakistan, under ambient environment. Sunflower seeds were obtained from Oilseed Research Section in Ayub Agricultural Research Institute (ARRI), Faisalabad, Pakistan. Completely randomized design was used for each experiment. Two levels of NaCl concentration (0 m*M* and 120 m*M*) in full strength Hoagland's nutrient solution were applied through root growing medium. Hundred healthy seeds of each of two sunflower cultivars (FH-572 and FH-621) were soaked in 100 mL of four alpha tocopherol levels i.e. control (distilled water), 100, 200, 300 mg L-1 separately for 16 h and were blot dried (Kausar and Shahbaz, 2017) before sowing in pots containing sand. Stock solution of α-tocopherol was prepared by dissolving it in 2 mL ethanol then above stated concentrations were maintained by diluting it with distilled water. Initially 10 sunflower seeds were sown in each pot of

10 inches diameter and 11 inches depth but after thinning at three leaves stage six plants in each plastic pot were maintained till data collection. Hoagland's was applied @ 2 liters per pot through rooting medium at 14 days interval till final harvest. Salinity stress was applied 35 days after seed sowing. Initially 60 m*M* NaCl concentration was applied for one week then 120 m*M* NaCl concentration was given after every 14 days along with Hoagland's nutrient solution till the completion of experiment. Data were collected for various morph-physiological features 57 day after seed sowing. Sunflower plants were uprooted 61th day after seed sowing. Shoot and root fresh weight and lengths were recorded the same day. The data for different morph-physiological attributes were pooled and analyzed statistically.

Photosynthetic Pigments

Chlorophyll *a*, *b* and carotenoid content were determined in fully expanded sunflower leaves third from the top, sampled 58 days after seed sowing, using Arnon (1949) and Davis (1976) methods. Leaves (0.5 g) were sliced into pieces and immersed in 10 mL 80% acetone solution in plastic bottles, capped and kept at 0–4oC overnight. Absorbance of the decanted supernatant was recorded at 480, 645 and 663 nm using UV-visible spectrophotometer (Model: U2020 IRMECO).

Leaf Water Potential (-Ψw)

Fully expanded sunflower leaves third from the top of each plant were excised and placed in pressure chamber to record leaf water potential using Scholander *et al*. (1964) stratagem. Leaf water potential was recorded 60th day after seed sowing between 6:30 to 8:30 a.m.

Leaf Osmotic Potential (-Ψs)

Same leaf used for determination of water potential was kept in freezer -20oC for one week. Frozen leaf was thawed and crushed with the glass rod for the extraction of leaf sap. Leaf osmotic potential was determined using Wescor pressure Osmometer.

Leaf Turgor Potential (Ψp)

Nobel (1991) method was used to determine leaf turgor potential by subtracting leaf osmotic potential from leaf water potential using equation $\Psi_p = \Psi_w - \Psi_s$ and values were recorded in MPa units.

Leaf Relative Water Content Percentage (LRWC %)

Fully expanded third leaves from the top of each plant were excised 59th day after seed sowing. Leaves fresh weight (FW) were recorded and immersed in distilled water with their cut ends inverted downward in the plastic bottles under

ambient conditions. After 4 h, leaves turgid weight (TW) were recorded after blot drying excess water from leaf surface. Leaves were oven dried at 72°C till constant weight to determine leaves dry weight (DW). Leaf relative water content percentage (LRWC %) was calculated using Turner (1981) formula:

$$LRWC \% = FW - DW / TW - DW \times 100$$

Gas Exchange Attributes

An open system infrared gas analyzer (LCA-4 ACD, Analytical Development, Hoddesdon, UK) was used to record net CO_2 assimilation rate (*A*), transpiration rate (*E*), stomatal conductance (*g*$_s$) and sub-stomatal CO_2 concentration (*C*$_i$). Fully expanded 3rd leaf from the top of every plant was used to record these gas exchange parameters during 11:00 am to 1:00 pm when IRGA showing readings of photosynthetic active radiations (PAR) up to 1796 μmol m^{-2} s^{-1}, ambient pressure of 98.8 kPa, 351 μmol s^{-1} gas flow and temperature in leaf chamber ranges between 32.4 to 36.1°C.

Statistical Analysis

The data of both experiments for various morphological and physiological attributes were combined. Recorded data were statistically analyzed by applying three way analysis of variance (ANOVA) using computer program (Cohort software Berkeley, California) of Snedecor and Cochran (1980) to determine significance of mean square values. The experiment was laid according to CRD statistical design with four repetitions.

Results

Salt stress caused substantial decrease (P ≤ 0.001) in shoot fresh weight in both sunflower cultivars. Sunflower cultivar FH-621 showed higher shoot fresh weight (P ≤ 0.001) compared to cv. FH-572 under saline and non-saline conditions. Pre-sowing seed treatment with α-Toc 100 and 300 mg L^{-1} levels significantly increased shoot fresh weight (P ≤ 0.001) in cv. FH-572 and cv. FH-621, respectively under non-saline condition. Under saline condition, α-Toc level 300 mg L^{-1} showed significant increase in shoot fresh weight in cv. FH-621 whereas α-Toc levels showed non-significant effect on cv. FH-572. Significant interaction (P ≤ 0.01) was found between α-Toc and cultivars (Table 1 and Fig. 1).

Imposition of salt stress (120 m*M*) showed significant reduction (P ≤ 0.001) in root fresh weight. Pre-sowing seed treatment with α-Toc 100 and 300 mg L^{-1} levels significantly increased (P ≤ 0.001) root fresh weight in cv. FH-572 and cv. FH-621, respectively under saline conditions. Significant variation (P ≤ 0.05) between cultivars showed that cv. FH-621 was higher in root fresh weight than cv. FH-572 (Table 1 and Fig. 1).

Salinity stress showed inhibition (P ≤ 0.05) in shoot length in both sunflower cultivars. Pre-sowing seed treatment with α-Toc levels remarkably increased (P ≤ 0.001) shoot length. α-Toc levels 200 mg L^{-1} in cv. FH-572 and 300 mg L^{-1} in cv. FH-621 were effective in improving shoot length under saline condition. Plants of sunflower cv.FH-621 cultivar were much higher (P ≤ 0.001) compared to cv. FH-572 (Table 1 and Fig. 1). Neither imposition of salinity stress nor pre-sowing seed treatment with α-Toc levels had any significant effect on root length in plants of both sunflower cultivars under saline and non-saline conditions (Table 1 and Fig. 1).

Salinity stress and pre-sowing seeds with α-Toc showed non-significant effect on chlorophyll *a* (*Chl. a*), chlorophyll *b*, chlorophyll *a/b* ratio and carotenoids. Significant interaction between cultivars and salinity was observed (P ≤ 0.05) in case of Chl. *a* content. Sunflower cultivar FH-572 showed increase in Chl. *a* content than FH-621 under salt stress (Table 1 and Fig. 2). Sunflower cultivars showed significant variations in carotenoids produced (P≤0.001). Cultivar FH-621 showed considerably higher carotenoid content compared to FH-572 under both saline and non-saline conditions (Table 1 and Fig. 2).

Imposition of salt stress significantly decreased (P ≤ 0.05) leaf water potential. Pre-sowing seed treatment with α-Toc levels showed non-significant effect on leaf water potential. Highly significant interaction between cultivars and salinity (P ≤ 0.001) showed increase in leaf water potential in cv. FH-572 and considerable reduction in leaf water potential in cv. FH-621 under salt stress. Significant interaction exists (P ≤ 0.05) between cultivars and α-Toc (Table 1 and Fig. 3). Both salinity and α-Toc levels did not affect leaf osmotic potential. Sunflower cultivars differed greatly in leaf osmotic potential. Sunflower cv. FH-572 showed significant increase (P ≤ 0.001) in leaf osmotic potential than cv. FH-621 under saline and non-saline conditions. Significant interaction (P ≤ 0.05) was observed between salt stress and α-Toc. Significant interaction also existed among cultivars, salt stress and α-Toc (P ≤ 0.05) showing that α-Toc levels 200 and 300 mg L^{-1} were responsible for increase in leaf osmotic potential in cv. FH-572 under salt stress (Table 1 and Fig. 3). Application of salt stress through root growing medium significantly increased (P ≤ 0.01) leaf turgor potential in sunflower cv. FH-621. Overall increase in leaf turgor potential was more in sunflower cv. FH-572 under both non-saline and saline conditions. Pre-sowing seeds with α-Toc showed non-significant effect on leaf turgor potential. Significant interaction (P ≤ 0.01) between sunflower cultivars and salt stress showed remarkable increase in leaf turgor potential in cv. FH-621 under saline condition (Table 1 and Fig. 3). Imposition of salt stress showed considerable reduction (P ≤ 0.01) in leaf relative water content percentage in both sunflower cultivars. However, exogenous application of α-Toc did not affect leaf relative water content percentage (Table 1 and Fig. 3).

Table 1: Mean squares from analyses of variance of data for growth, photosynthetic pigments, water relation parameters and gas exchange aspects of sunflower plants raised from α-tocopherol primed seeds (16 h) under saline and non-saline regimes

Source of variations	df	Shoot fresh weight (g)	Root Fresh weight (g)	Shoot length (cm)	Root length (cm)	Chl. A (mg per g FW)	Chl. B (mg per g FW)
Cultivars (Cvs)	1	18899.4***	42.087*	5736.17***	55.68ns	0.0001ns	0.207ns
Salinity (S)	1	7876.6***	131.389***	1464.02*	49.53ns	0.0007ns	0.0001ns
α-tocopherol (α-Toc)	3	3939.4***	61.42***	3099.45***	34.28ns	0.0003ns	0.044ns
Cvs × S	1	320.4ns	9.687ns	375.87ns	25.38ns	0.001*	0.084ns
Cvs × α-Toc	3	2950.4**	13.533ns	526.73ns	5.080ns	0.0002ns	0.019ns
S × α-Toc	3	104.43ns	15.980	186.38ns	13.09ns	0.0006ns	0.020ns
Cvs × S × α-Toc	3	570.55ns	9.09ns	223.93ns	2.684ns	0.0004ns	0.077ns
Error	48	494.11	9.296	344.69	16.684	0.0002	0.0738
Source of variations	df	Chl. *a/b*	Carotenoids	Water potential	Osmotic potential	Turgor potential	LRWC%
Cultivars (Cvs)	1	0.194ns	2.771***	0.001ns	0.357***	0.403**	75.715ns
Salinity (S)	1	0.003ns	0.243ns	0.131*	0.024ns	0.267**	367.907**
α-tocopherol (α-Toc)	3	0.071	0.269ns	0.004ns	0.021ns	0.022ns	24.568ns
Cvs × S	1	0.159ns	0.090ns	0.316***	0.0008ns	0.285**	45.872ns
Cvs × α-Toc	3	0.040ns	0.048ns	0.055*	0.011ns	0.076ns	21.469ns
S × α-Toc	3	0.031ns	0.061ns	0.018ns	0.054*	0.036ns	31.273ns
Cvs × S × α-Toc	3	0.220ns	0.044ns	0.030ns	0.055*	0.073ns	19.487ns
Error	48	0.110	0.108	0.019	0.0168	0.033	37.790
Source of variations	df	*A*	*E*	(g_s)	C_i	C_i/C_a	WUE (*A/E*)
Cultivars (Cvs)	1	10.48ns	0.365ns	287564.06**	26908.3***	0.216***	0.032ns
Salinity (S)	1	3.450ns	0.274ns	4064.062ns	86.258ns	0.0006ns	0.769ns
α-tocopherol (α-Toc)	3	199.52***	0.558ns	163264.06**	4002.066*	0.032499*	22.47**
Cvs × S	1	23.08ns	0.059ns	68251.563ns	1477.441ns	0.012ns	13.85ns
Cvs × α-Toc	3	1.885ns	0.244ns	110605.73*	1995.041ns	0.016ns	6.344ns
S x α-Toc	3	27.86ns	0.643ns	9230.729ns	2381.779ns	0.019ns	12.32ns
Cvs × S × α-Toc	3	70.352*	0.062ns	23601.562ns	731.595ns	0.006ns	17.35*
Error	48	18.10ns	0.310	30040.104	1168.567	0.009	4.456

*, **, *** Significant at 0.05, 0.01 and 0.001 levels respectively; ns = non-significant; Chl. *a* = Chlorophyll a; Chl. *b* = Chlorophyll b; Chl. *a/b* = Chlorophyll *a/b* ratio; WUE (A/E) = Water use efficiency; *A* = net CO2 assimilation rate; *E* = Transpiration rate; g_s = Stomatal conductance; C_i = Sub-stomatal CO_2 concentration; C_i/C_a = Relative internal CO_2 concentration
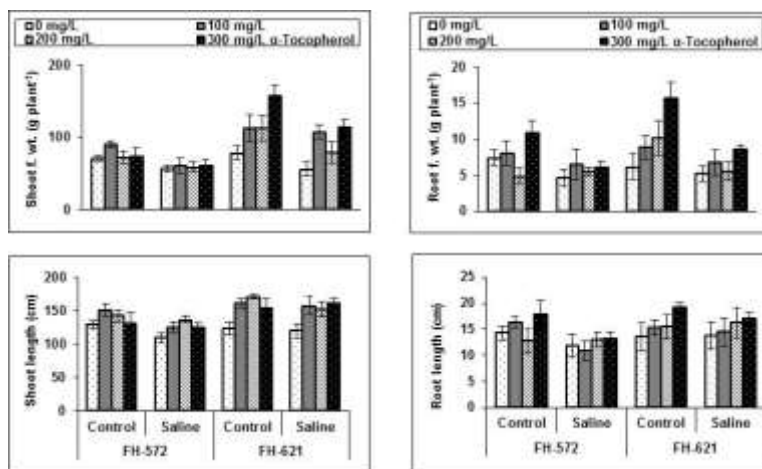
Fig. 1: Growth attributes of sunflower plants raised from α-tocopherol primed seeds (16 h) under saline and non-saline regimes

Application of α-Toc as pre-sowing seed treatment significantly (P ≤ 0.001) enhanced photosynthetic rate in both sunflower cultivars (Table 1 and Fig. 4). Of various α-Toc levels, 100 and 200 mg L^{-1} α-Toc were more effective in increasing net CO_2 assimilation rate in both cultivars. Salinity stress applied through root growing medium did not affect net CO_2 assimilation rate. Significant interaction was observed among cultivars, salinity and α-Toc (P ≤ 0.05). Salt stress and exogenously applied α-Toc levels as pre-sowing seed treatment showed non-significant effect on transpiration rate (*E*). Pre-sowing seeds with α-Toc significantly (P ≤ 0.01) increased stomatal conductance.
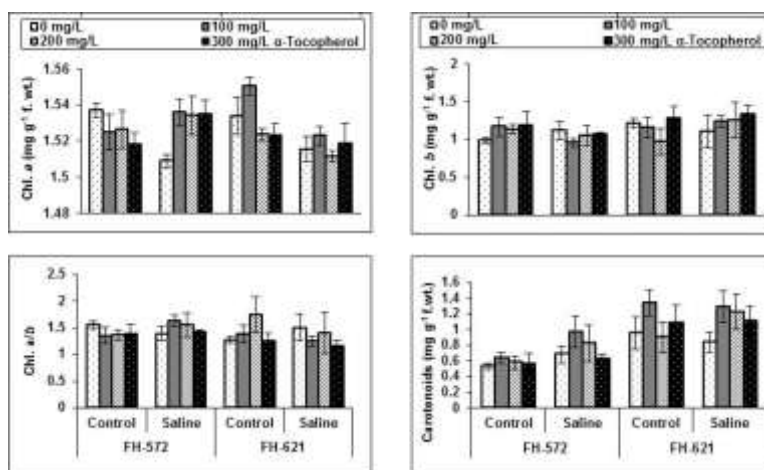
Fig. 2: Photosynthetic pigments of sunflower plants raised from α-tocopherol primed seeds (16 h) under saline and non-saline regimes
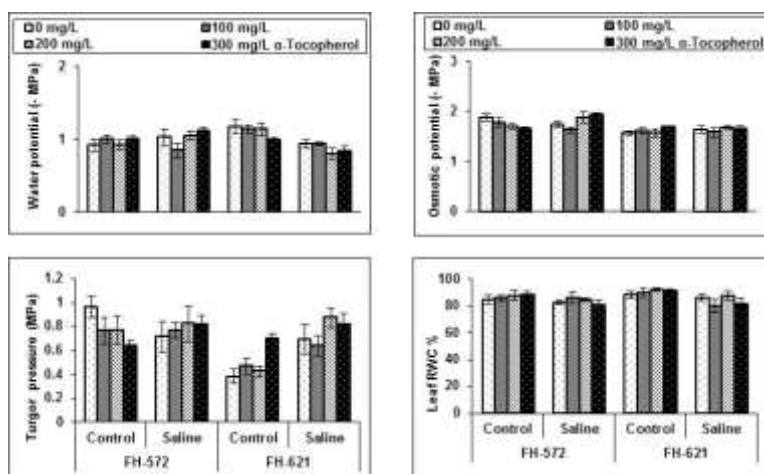
**Fig. 3:** Leaf water relation parameters of sunflower plants raised from α-tocopherol primed seeds (16 h) under saline and non-saline regimes

α-Toc levels 100 and 200 mg L^{-1} were more effective in increasing stomatal conductance in FH-621 and FH-572 under non saline conditions. All three α-Toc levels (100, 200 and 300 mg L^{-1}) in FH-572 and 100 mg L^{-1} in case of FH-621 proved more effective in improving stomatal conductance under saline conditions (Table 1 and Fig. 4). Sunflower cultivar FH-621 showed significantly higher stomatal conductance than FH-572 (P ≤ 0.01). However, salinity did not affect stomatal conductance. Significant interaction was observed between cultivars and α-Toc (P ≤ 0.05). Sub stomatal CO_2 concentration and C_i/C_a ratio was much higher in cv. FH-572 in comparison with cv. FH-621 under both saline and non-saline conditions (P ≤ 0.001). Pre sowing seed with 300 mg L^{-1} α-Toc significantly increased sub stomatal CO_2 concentration and C_i/C_a ratio (P ≤ 0.05) in cv. FH-621 under non-saline conditions (Table 1 and Fig. 4). Pre sowing seed treatment with α-Toc significantly improved water use efficiency (*A/E*). α-Toc level 200 mg L^{-1} significantly increased (P ≤ 0.01) water use efficiency (*A/E*) under non saline conditions in cv. FH-572. Whereas, 200 and 300 mg L^{-1} α-Toc levels were found effective in improving WUE in cv. FH-621 under saline condition. Sunflower cultivars did not differ in water use efficiency. Interaction between cultivars, salinity and α-Toc was significant (P ≤ 0.05) for water use efficiency (Table 1 and Fig. 4).

Discussion

Although imposition of salt stress inhibited growth, decreasing shoot and root fresh weight and shoot length in both sunflower cultivars however, in current study seed priming with α-tocopherol (α-Toc) was exceedingly effective in improving shoot and root fresh weight and shoot length of both sunflower cultivars under saline and non-saline conditions (Fig. 1).
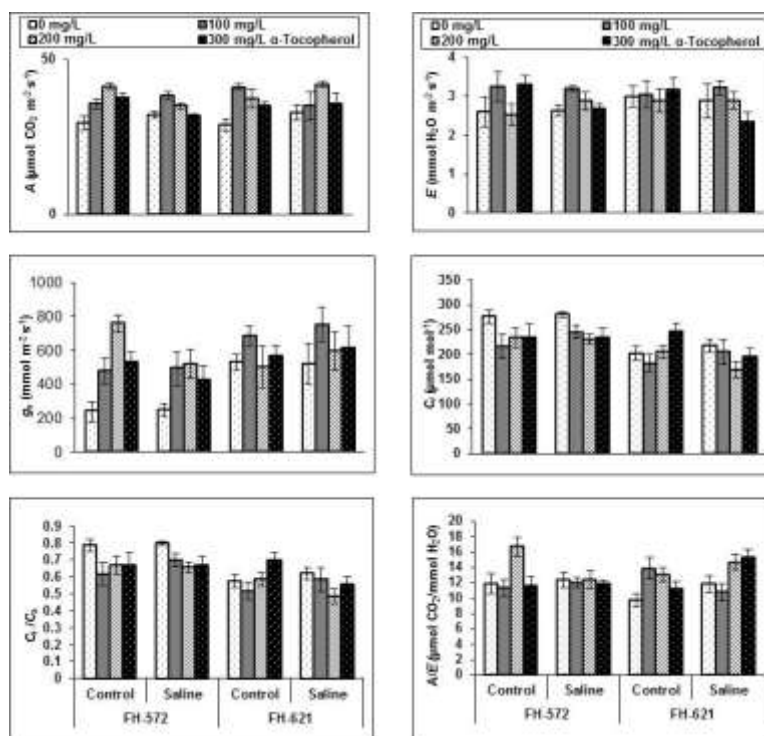
Fig 4: Gas exchange attributes of sunflower plants raised from α-tocopherol primed seeds (16 h) under saline and non-saline regimes

Likewise improvement in growth attributes induced by α-tocopherol exogenous application were reported in wheat (Kumar *et al*., 2013), Shoe flower (El-Quesni *et al*., 2009), sunflower (Al-Qubaie, 2012) and *Viciafaba* (Orabi and Abdelhamid, 2016) under salinity stress. Semida *et al*. (2014) reported that 0.50 m*M* α-tocopherol foliar application significantly improved growth in *Viciafaba* under saline conditions. Similarly Soltani *et al*. (2012) while using 0, 50 and 100 ppm levels of α-tocopherol as foliar spray stated that 100 ppm α-tocopherol was more effective in increasing fresh and dry weights of above ground parts of *Calendula officinalis*. whereas, it had non-significant effect on root fresh and dry weight. In our case, growth enhancement in sunflower plants raised from α-tocopherol primed seeds might be due to improvement in stomatal conductance and net CO_2 assimilation rate and increased water use efficiency.

In the present study neither salt stress nor seed priming with α-Toc showed any significant effect on chlorophyll *a*, *b*, *a/b* ratio and carotenoids. However, sunflower cultivar FH-621 produced more carotenoids than cv. FH-572 under both saline and non-saline conditions. Koyro (2006) described that increased carotenoid/chlorophyll ratio decreased harms of photo inhibition by reducing electron flow through photosystems. Semchuk *et al*. (2011) further reported that antioxidant activity and carotenoid content increases in *Arabidopsis thaliana* under stressful conditions. Carotenoids not only act as light harvesting pigment it also

function as an antioxidant and protect chloroplast by detoxifying reactive oxygen species especially singlet oxygen, modulate gene expression under salt stress thus enabled plants to acclimatize (Havaux, 2014). In this study cv. FH-621 showed acclimation response to salt stress by increasing carotenoid content.

In our study, sunflower cv. FH-621 showed significant reduction in leaf water potential and increase in leaf turgor potential than cv. FH-572 under salt stress. Overall increase in leaf osmotic potential was more in sunflower cultivar FH-572 under non-saline and saline conditions. Increase in net CO_2 assimilation rate and stability of the proteins involved in photosynthetic process might be due to increased osmotic potential and water potential (Ashfaque *et al*., 2014). Osmotic stress generated by higher accumulation of soluble salts reduces Plant's efficiency of water uptake from soil thus decreasing its turgor pressure. In this research increased turgor potential in sunflower cultivar FH-621 under saline conditions might be due to accumulation of free proline, total free amino acids, lesser accumulation of Na^+ content in root and shoot and greater K^+/Na^+ ratio in shoot. Accumulation of free proline and glycine betaine might be responsible for increased osmotic potential value of sunflower cultivar FH-572 under salt stress enabling them to adjust osmotically. Silveira *et al*. (2009) Chen and Jiang (2010) Hajlaoui *et al*. (2010) in previous studies reported that plants under stressful conditions show osmotic adjustment by increasing the amount of GB, free proline,

amino acids and K^+. However, results of this research showed non-significant effect of α-Toc seed pre sowing on leaf water potential, osmotic potential, turgor potential and leaf relative water content. In the present study, salinity stress significantly reduced leaf relative water content. Previous studies on sunflower, barley and onion also confirmed reduction in leaf relative water content percentage in response to salinity stress (Hariadi *et al.*, 2011; Agami, 2014; Semida *et al.*, 2016).

Physiological processes mostly prone to salinity stress are cell expansion and photosynthesis. Contrary to the preceding studies in our study salt stress showed non-significant effect on net CO_2 assimilation rate (A), stomatal conductance (g_s), transpiration rate (E), water use efficiency, substomatal CO_2 concentration and C_i/C_a ratio. However plants raised from α-Toc primed seeds substantially improved net CO_2 assimilation efficiency, stomatal conductance and water use efficiency. This enhancement in assimilation efficiency might be due to α-Toc antioxidant activity which by neutralizing harmful lipid free radicles especially singlet oxygen protect photosynthetic apparatus specifically PS II from oxidative damage (Lushchak and Semchuk, 2012; Bagheri and Saheri, 2013; Bughdadi, 2013; Mène-Saffrané and Pellaud, 2017). Mohammed and Tarpley (2011) informed that exogenous application of α-Toc, salicyclic acid and glycine betaine substantially improved water use efficiency in rice. In *Avicennia marina* moderate salinity stress (50–75% marine water) improved stomatal conductance and photosynthetic rate (Nguyen *et al.*, 2015) however, water use efficiency showed no response. A previous study on rice showed considerable reduction in net CO_2 assimilation rate, transpiration rate and stomatal conductance in salt sensitive cultivar however tolerant lines immediately closed stomata in salt stress and after acclimation showed improvement in stomatal conductance (Moradi and Ismail, 2007).

It is concluded that seed treatment with 100 and 300 mg L^{-1} α-tocopherol levels increased shoot fresh weight to 6.7% and 105.9% and root fresh weight to 40.59% and 64.76% in in cv. FH-572 and cv. FH-621, respectively, under saline conditions compared to untreated plants. Moreover, seed treatment with 200 and 300 mg L^{-1} α-tocopherol levels increased shoot length to 24.88% and 34.43% under saline conditions in cv. FH-572 and cv. FH-621 respectively, than non-treated plants. Therefore, overall 100 and 300 mg L^{-1} levels of alpha tocopherol were effective in alleviated the devastating effect of salinity stress on sunflower cultivars FH-572 and FH-621, respectively by substantially improving root and shoot fresh weight, shoot length, net CO_2 assimilation rate (marker of stress tolerance), stomatal conductance and water use efficiency compared to control (hydro-primed seeds). Both sunflower cultivars differed momentously in their acclimation response to salt stress. Overall performance of cv. FH-621 was better than cv. FH-572 under salt stress.

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