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Full Length Article

Impact of Alpha-Tocopherol Seed Priming on Accumulation of Osmolytes and Ion Homeostasis in Sunflower (*Helianthus annuus*) under Salt Stress

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Abstract

Salt stress induces ion toxicity and nutritional disparity drastically affecting physiological functions in glycophytes. Previous studies reported that salt tolerant plant species maintain turgor by accumulating organic osmolytes and inorganic ions especially K^+. This study was conducted to evaluate the role of alpha-tocopherol (α-toc) in accumulation of osmolytes and ion homeostasis in sunflower under salt stress. Seeds of sunflower cultivars FH-572 and FH-621 were immersed for 16 h in four levels [0 (dist. H_2O), 100, 200 and 300 mg L^{-1}] of α-toc before sowing in sand. Salt stress (120 m*M* NaCl) was applied with full strength Hoagland's nutrient solution, 35 days after seed sowing till maturity. Sunflower leaves were sampled 61 days after sowing seeds for analyses. Seed priming with α-toc boosted accumulation of glycine betaine, free amino acids and total soluble sugars to 53.25, 75 and 30% in FH-572 and 75, 31.25 and 25% in FH-621 respectively, under salt stress. Seed priming with α-toc caused significant reduction in Na^+ conc., 5.71 and 25.15% in shoots of the FH-572 and FH-621 respectively in salt stress. Plants raised from α-toc primed seed showed increase in K^+/Na^+ ratio (4.76%) and Ca^{2+} conc. (6.5%) and decrease in Na^+ conc. (8%) in roots of cv. FH-621 in salt stress but these modulations were non-significant. In conclusion, seed priming with 200 mg L^{-1} α-toc was effective in improving salinity tolerance in sunflower at early growth stages. © 2020 Friends Science Publishers

Keywords: Ion homeostasis; α-tocopherol; Sunflower; Osmolytes; Salinity tolerance

Introduction

High salt concentration in the rooting medium induces rapid osmotic stress, ion toxicity and ionic imbalance. In addition, enhanced reactive oxygen species (ROS) production leads to oxidative stress hence, adversely affecting plant growth and production (Krasensky and Jonak 2012). Mechanism of osmolyte accumulation and antioxidant defense are biological markers that restore metabolic homeostasis by maintaining ROS equilibrium *via* signaling (Turkan and Demiral 2009; Pintó-Marijuan and Munné-Bosch 2013). Plants under salt stress sustain their turgor pressure by accumulating compatible solutes or osmolytes (uncharged, polar organic compounds) which do not restrict cell metabolism even at higher concentration (Turkan and Demiral 2009; Gupta and Huang 2014; Al-Farsi *et al.* 2020). In order to balance lower water potential due to ions sequestration in vacuole, the osmolytes like glycinebetaine, free proline, and polyols accumulate in cytoplasm (Farooq *et al.* 2020). However, synthesis/accumulation of organic molecules requires more energy than inorganic ions reducing plant growth under salt stress (Munns and Gilliham 2015). Excessive soluble salts in the rooting medium cause osmotic effect and ion toxicity by rendering plant's root unfit for water extraction from the soil solution (Muhammad and Hussain 2012; Farooq *et al.* 2015) and by accumulating sodium (Na^+) and chloride (Cl^-) ions in shoots, respectively (Tavakkoli *et al.* 2011; Ahanger *et al.* 2014) hence, lead to the disturbed metabolism and oxidative stress (Fayez and Bazaid 2014). Rapid Na^+ influx in plant displaces K^+ (essential for binding ribosomes to tRNA, hence proteins conformation) and Ca^{2+} ions (Hameed *et al.* 2014; Shrivastava and Kumar 2015). Sodium (or Cl^-) ion concentration is toxic when surpasses 30 m*M* (cytosol) and 100-200 m*M* (mitochondria and chloroplast), respectively (Conn and Gilliham 2010; Flowers *et al.* 2015). Nutritional imbalance (reduction in potassium, phosphate, calcium and nitrate availability) as a result of salt (Na^+ and Cl^-) accumulation impair plant productivity (Nasri *et al.* 2015). Salt-tolerant species either exclude Na^+ and Cl^- ions or maintain their low concentration by sequestering them in the vacuole (*e.g.*, in barley) and increase concentration of osmotica (organic or inorganic) to regulate osmotic pressure of the soil and maintain turgor, essential for the growth of plants (Shahbala 2013).

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Experiments have been performed to study the impact of salinity stress on crops and described drastic effects of salt stress on productivity of glycophytes (Wani *et al.* 2013; Rivero *et al.* 2014; Zhang *et al.* 2014; Munns and Gilliham 2015). These studies used diverse methodologies to improve salt tolerance in plants, especially seed priming with various growth regulators (vitamins and hormones). Plant growth regulators are key modulators of various progressions in plants under abiotic stresses (Lalarukh *et al.* 2014). Seed priming is a quick and cost effective method in reducing negative impact of salt stress by improving metabolism, rapid seed germination and consistency in stand establishment at initial stages of different plant species (Farooq *et al.* 2019, 2020).

Alpha-tocopherol acts as an antioxidant (Shao *et al.* 2008), reduces lipid peroxidation (Sattler *et al.* 2006) by detoxifying ROS (Hincha 2008). Maeda *et al.* (2006) reported a crucial role of α-toc in phloem loading. Ludwig (2009) revealed that α-toc strongly effects nutrient remobilization in *Arabidopsis thaliana* lines. In leaves of transgenic alfalfa, increase in α-toc improved protein content and delayed leaf senescence (Jiang *et al.* 2016); whereas overexpression of γ-tocopherol methyltransferase is linked with up-regulation of sugar transport in transgenic plants (Jin and Daniell 2014). In higher plants shifts in α-toc levels in stress response, activate signal transduction pathway (Hyun *et al.* 2011) and regulate carbohydrate metabolism (Li *et al.* 2008).

Sunflower (*Helianthus annuus* L.) is a short duration (90–110 days life cycle) oilseed crop cultivated twice in a year, categorized among moderately salt tolerant crops and can bear 50 m*M* salt stress (Moghanibashi *et al.* 2013; Kumar *et al.* 2014). Therefore, sunflower can be grown in areas where irrigation water is slightly brackish (Riaz *et al.* 2012). However, in presence of (soluble) salts in higher amount in soil can have devastating effects on sunflower production (Wang *et al.* 2017). Previous studies have reported that α-toc, as an antioxidant plays significant role in abiotic stress mitigation, in remobilization of nutrients and modulation of carbohydrate metabolism (Maeda *et al.* 2006; Ludwig 2009; Farouk 2011; Semida *et al.* 2016; Hemida *et al.* 2017). In a previous study, Lalarukh and Shahbaz (2020) observed that plants raised from seeds primed with α-toc increased growth and yield related attributes with increase in enzymatic (catalase and peroxidase), non-enzymatic (total phenolic and ascorbic acid) antioxidants and reduction in lipid peroxidation in sunflower under salt stress. Lalarukh and Shahbaz (2018) reported increase in turgor potential, water use efficiency, net photosynthetic rate and stomatal conductance in leaves of sunflower plants raised from seeds primed with α-toc (vitamin E) along with increase in root and shoot (fresh) weight and shoot length. Inversely, little is known about the impact of α-toc seed priming on accumulation of osmolytes and ion homeostasis under saline condition in sunflower. Therefore, it is assumed that seed priming with α-toc may improve osmolytes accumulation, ion homeostasis and salt stress tolerance in sunflower. Thus, the objective of the present research was to investigate the role of α-toc seed priming in osmolytes accumulation, ion homeostasis and salt stress alleviation in sunflower.

Materials and Methods

Experimental site

During the years 2015 and 2016, pot experiments were executed to study the impact of α-toc seed priming on osmotic adjustment of sunflower under salt stress in ambient environment at the botanic garden, University of Agriculture (31° 30'N latitude, 73° 10'E longitude and 213m altitude), Faisalabad, Pakistan. The seeds of sunflower cultivars (FH 572 and FH 621) were obtained from Ayub Agricultural Research Institute (Oilseed Research Section), Faisalabad, Pakistan.

Experimental treatments

Sunflower achenes (100) were kept immersed in 100 mL solution of 4 concentrations (0, 100, 200 and 300) mg L^{-1} of α-toc each for 16 h. Alpha-tocopherol was dissolved in (2 mL) ethanol (an organic solvent) and then diluted with distilled water up to the required limit. After drying 10 healthy sunflower seeds were sown in sand (10 kg) filled plastic pots (24.5 cm in diameter and 27.94 cm in depth). Plants were supplemented with full strength Hoagland's solution (one liter per pot at the vegetative stage and two liter per pot at the reproductive stage) to fulfill their nutrient demand at two weeks interval. After thinning (at three leave stage), six sunflower plants were kept in each pot. Salt stress (0 m*M* and 120 m*M* NaCl) was applied through rooting medium along with Hoagland's nutrient solution after 35 days of seed sowing till the final harvest (maturity). However, leaves were sampled at reproductive phase for appraisal of organic osmotica and inorganic ions 61 days after seed sowing. Plants were uprooted without any damage to the roots as they were grown in sand culture. Roots were washed and dried in oven for ion analysis.

Experimental conditions and design

The design of the experiment was completely randomized design (CRD) with four replications. Sixty four plastic pots were used for this experiment and six plants per pot were maintained till sampling. The experiment was conducted under ambient environmental conditions with 16.5 to 31.8°C temperature, 66 to 39% relative humidity, 67.9 to 11.6 mm rainfall and 5.6 to 10.4 h sunshine, from February to June respectively.

Free proline

Free proline was estimated following Bates *et al*. (1973)

method. Fresh leaf (3rd from the top) 0.5 g was homogenized using mortar and pestle, in (3% w/v) (10 mL) sulphosalicylic acid and then filtered. Acid ninhydrin (2 mL) and glacial acetic acid (2 mL) was added to (2 mL) filtrate and heated for 60 min at 100°C in a water bath. Mixture was cooled by placing test tubes in ice and vortexed after adding toluene (4 mL) for 15 sec. Quantity of free proline was determined by measuring absorbance of upper layer formed in the test tube at 520 nm with the help of spectrophotometer (IRMECO U2020) Germany.

Glycinebetaine

Amount of glycinebetaine produced in leaves was determined by using Grieve and Grattan (1983) method. Freshly sampled 3rd leaf (0.5 g) from the top was ground using distilled H_2O and centrifuged (12000 x *g*) for 10 min. One mL sulphuric acid (2 N) was added to the supernatant (1 mL) extracted in a test-tube. From the above blend (0.5 mL) extract was pipetted out in another test tube and was kept for 90 min in ice after adding periodide solution (0.2 mL). Distilled H_2O (1.4 mL) and 1, 2-dichloromethane (chilled 6 mL) was supplemented in the mixture. Lower layer absorbance was recorded with the help of spectrophotometer (IRMECO U2020) Germany, at 365 nm soon after formation of two distinct layers.

Total free amino acids

Fresh leaves were sampled and homogenized for amino acids determination in phosphate buffer (7.0 pH) following Hamilton and VanSlyke (1943) technique. To 1 mL of extract 10% pyridine (1 mL) and 2 % ninhydrin (1 mL) was added. After heating the mixture on (boiling) water bath for half an hour, distilled water was added to maintain volume up to 50 mL. Optical density of the mixture was recorded at 570 nm using a spectrophotometer. Readings were calibrated with the help of standard curve developed by using amino acid leucine.

Total solvable sugars

Yoshida *et al*. (1976) protocol was used to determine the amount of total (soluble) sugars. In a test tube, 0.1 mL ethanolic aliquot was taken, 3 mL anthrone reagent (freshly prepared) was added, mixed and vortexed. Mixture was heated for 15 min at 95°C, cooled at room temperature and absorbance at 625 nm was recorded using spectrophotometer (IRMECO U2020) Germany.

Ionorganic Ions

Ionic concentration (Na^+, Ca^{2+} and K^+) of shoot and root were determined using Allen *et al*. (1985) procedure. In a digestion flask, 0.1 g oven dried (ground) shoot/root and H_2SO_4 (2 mL) was added and kept for 24 h at room temperature. Flasks were heated to 200°C and H_2O_2 (1 mL) was added to the mixture upon cooling. Volume of the colorless mixture was maintained to 50 mL with distilled water and filtered. Amount of Ca^{2+}, Na^+ and K^+ in roots/shoots was determined with the help of Flame photometer (Sherwood Model 410, Cambridge, U.K.).

Statistical analysis

Snedecor and Cochran (1980) method was used to determine the analysis of variance data for various attributes using COSTAT computer program and mean values were compared. Tukey's test was used for mean separation with 5% level of significance.

Results

Osmolytes accumulation

Seed priming with α-toc showed non-considerable influence on free proline. Sunflower cultivars showed similar (non-significant) response in case of free proline. However, free proline increased ($P \leq 0.05$) considerably in (both) sunflower cultivars under saline condition. Remarkably significant ($P \leq 0.01$) interaction in between salt stress and α-toc revealed that seed priming with 100 and 200 mg L^{-1} α-toc levels amplified free proline production in FH-572 (11.11%) and FH-621 (65.41%) cultivars respectively, in saline condition compared to hydro-primed seeds. Strong interaction ($P \leq 0.05$) was also observed in between cultivars and α-toc (Table 1; Fig. 1).

Plants raised from seeds primed with α-toc exhibited remarkable rise ($P \leq 0.01$) in glycinebetaine (GB). Overall production of GB was greater ($P < 0.001$) in FH-572 than FH-621 cultivar. Salinity had non-significant influence on GB production. Significant ($P \leq 0.01$) interaction among α-toc, salt stress and cultivars indicated that under salt stress, seeds primed with α-toc levels, 300 and 100 mg L^{-1} effectively enhanced production of GB in FH-572 (53.25%) and FH-621 (75%) cultivars, respectively under saline condition compared to hydro-primed seeds (Table 1; Fig. 1).

Seed priming with α-toc considerably increased ($P < 0.001$) total free amino acids. Overall production of total free amino acids was greater ($P < 0.001$) in FH-621 than FH-572 cultivar. Under salt stress, considerable rise ($P \leq 0.05$) in (total) free amino acids in FH-621 cultivar was observed (Table 1; Fig. 1). Substantially higher ($P < 0.001$) interaction between salt stress and cultivars showed rise in total free amino acids in FH-621 and descend in FH-572 under salt stress. Significant ($P \leq 0.05$) interaction in between salt stress and α-toc revealed that seed priming with 200 mg L^{-1} α-toc level enhanced total free amino acids production in FH-572 (75%) and FH-621 (31.25%) cultivars, respectively in saline condition compared to hydro-primed seeds.

Table 1: Mean squares from analyses of variance of data for organic osmolytes in sunflower grown from seeds primed with α-tocopherol (16 h) under salt stress and non-stress conditions

Source of variations	df	Free proline	Glycinebetaine	Free amino acid	Total soluble sugars
Cultivars (Cvs)	1	4.165ns	9592.64***	0.020***	0.007*
Salinity (S)	1	180.79*	397.84ns	0.002*	0.206***
α-tocopherol (α-toc)	3	76.84ns	892.03**	0.007***	0.006*
Cvs × S	1	75.77	551.4ns	0.014***	0.002ns
Cvs × α-toc	3	116.77*	251.4ns	0.0007ns	0.0001ns
S × α-toc	3	185.57**	223.4ns	0.002*	0.007**
Cvs × S × α-toc	3	77.43ns	841.9**	0.001ns	0.001ns
Error	48	35.35	151.989	0.0005	0.002

* = $P \leq 0.05$, ** = $P \leq 0.01$, *** = $P < 0.001$, ns = non-significant, df = degrees of freedom

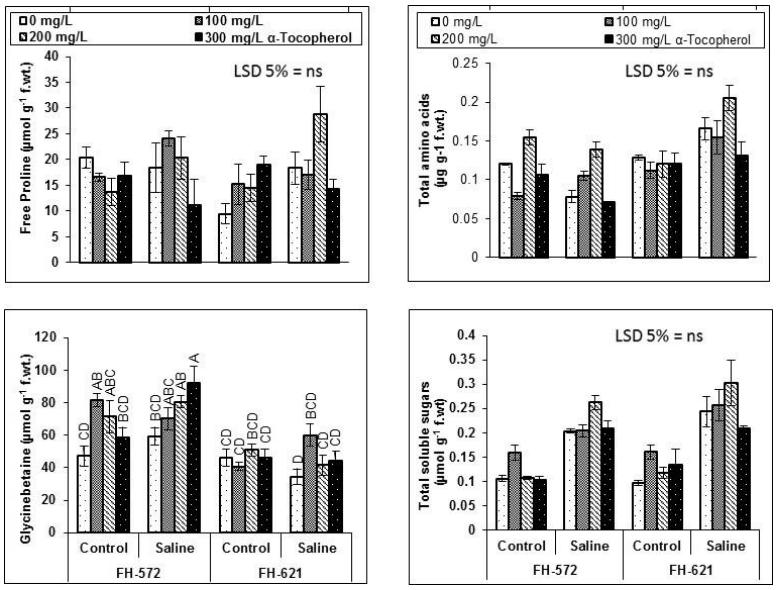

Fig. 1: Osmolytes accumulation in sunflower upraised from seeds treated with α-tocopherol (16 h) under salt stress and non-stress regimes

Plants raised from α-toc primed seeds showed significant increase ($P \leq 0.05$) in (total) soluble sugars. Overall production of (total) soluble sugars was more ($P \leq 0.05$) in FH-621 compared to FH-572 cultivar. Salt stress greatly enhanced ($P < 0.001$) production of (total) soluble sugars in both sunflower cultivars (Table 1; Fig. 1). Considerable interaction amongst α-toc and salt stress ($P \leq 0.01$) indicated that α-toc level, 200 mg L^{-1} played influential role in increasing total soluble sugars under salt stress in FH-572 (30%) and FH-621 (25%) than hydro-primed seeds.

Ion accumulation

Plants raised from α-toc primed seeds exhibited substantial decrease ($P \leq 0.01$) in sodium (Na^+) concentration of shoot. Salinity stress imposition caused significant increase ($P < 0.001$) in shoot Na^+ concentration of FH-572 contrary to FH-621 cultivar which showed reduction in shoot Na^+ concentration compared to non-stressed plants. Overall, decrease in Na^+ concentration of shoot was more pronounced ($P \leq 0.01$) in FH-621 cultivar in salt stress compared to control. Plants raised from α-toc (200 mg L^{-1}) primed seeds exhibited substantial decrease in Na^+ concentration of shoot in FH-572 (5.71%) and FH-621 (25.15%) under saline condition than hydro-primed. Interactions between and among all three factors (salinity, α-toc and cultivars) were highly significant (Table 2; Fig. 2).

The effect of α-toc seed priming on potassium (K^+) concentration of the sunflower shoot was not significant. On the whole, accumulation of K^+ concentration in the shoot of FH-621 was higher ($P < 0.001$) compared to FH-572 cultivar. Potassium concentration increased substantially ($P \leq 0.01$) in the shoots of both sunflower cultivars in salt stress. Significant interaction ($P \leq 0.05$) in between cultivars and salt stress showed more increase in shoot K^+ of FH-572 under salt stress compared to FH-621 cultivar. Substantial ($P \leq 0.05$) interaction also occurs in between α-toc and cultivars. Plants raised from α-toc (level, 300 mg L^{-1}) primed seeds showed more increase in shoot K^+ of FH-572 (67.8%) than FH-621 (1%) cultivar upon seed priming with 300 mg L^{-1} α-toc level, under saline condition than hydro-primed seeds (Table 2; Fig. 2).

Seed priming with α-toc showed non-significant

Table 2: Mean squares from analyses of variance of data for inorganic ions of sunflower grown from seeds primed with α-tocopherol (16 h) under salt stress and non-stress conditions

Source of variations	df	Shoot Na^+	Shoot K^+	Shoot K^+/Na^+	Shoot Ca^{2+}	Root Na^+	Root K^+	Root K^+/Na^+	Root Ca^{2+}
Cultivars (Cvs)	1	150.10**	1980.25***	0.434***	115.562*	42.25ns	210.25***	0.041***	10.560***
Salinity (S)	1	297.50***	900.00**	0.277***	76.562*	6.25ns	182.25***	0.046***	10.560***
α-tocopherol (α-toc)	3	97.56**	33.50ns	0.021ns	11.395ns	40.42ns	18.25ns	0.002ns	0.468ns
Cvs × S	1	637.60***	462.25*	0.003ns	52.562ns	600.25***	6.25ns	0.002ns	0.062ns
Cvs × α-toc	3	94.06**	294.42*	0.065**	15.562ns	33.75ns	2.92ns	0.002ns	0.718ns
S × α-toc	3	253.60***	85.17ns	0.041*	9.562ns	55.75*	8.92ns	0.004*	0.573ns
Cvs × S × α-toc	3	162.90***	117.42ns	0.039*	43.229ns	57.75*	22.25*	0.003ns	1.135ns
Error	48	17.65	75.79	0.011	16.729	14.58	6.58	0.001	0.554

* = $P \leq 0.05$, ** = $P \leq 0.01$, *** = $P < 0.001$, ns = non-significant, df = degrees of freedom

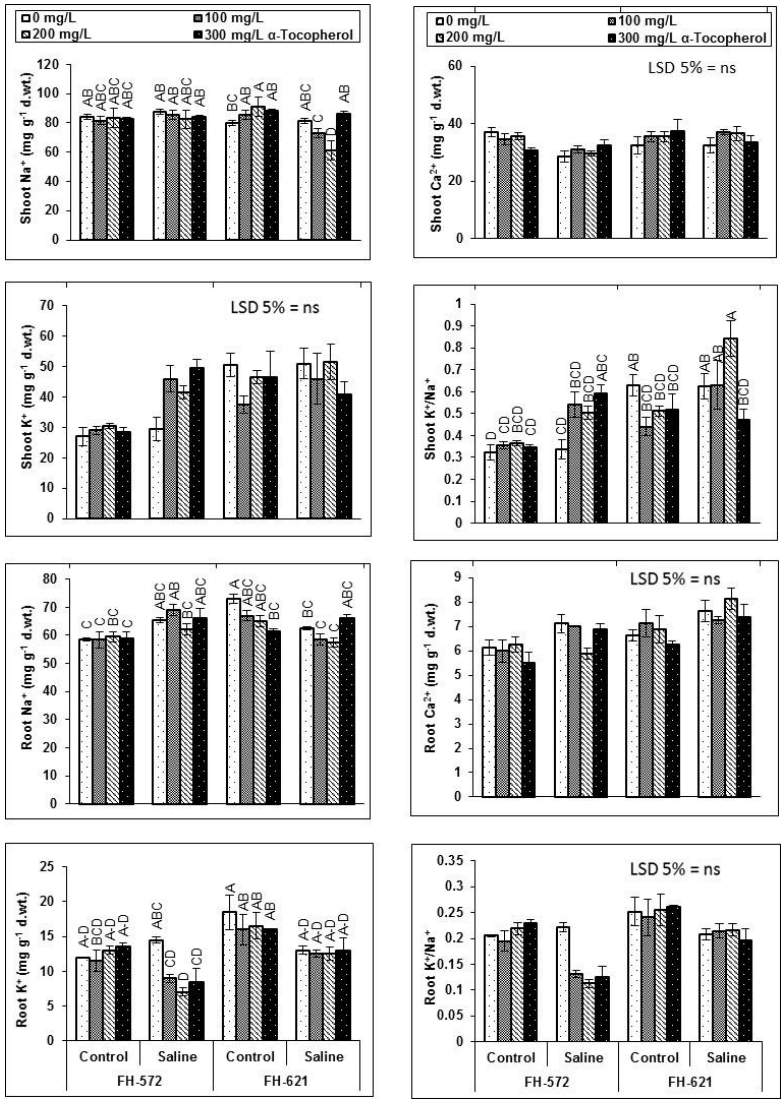

Fig. 2: Ionic concentration in sunflower upraised from seeds primed with α-tocopherol (16 h) under salt stress and non-stress regimes

influence on potassium to sodium (K^+/Na^+) ratio in shoots of sunflower. Overall, K^+/Na^+ ratio was considerably higher ($P < 0.001$) in shoots of FH-621 cultivar compared to FH-572. Salt stress substantially increased ($P < 0.001$) K^+/Na^+ ratio in both sunflower cultivars (Table 2; Fig. 2). Interaction between α-toc and cultivars ($P \leq 0.01$) showed that in salt stress, seed priming with 300 mg L^{-1} α-toc caused substantial increase in K^+/Na^+ ratio in shoots of FH-572 (73.53%) whereas, priming with 200 mg L^{-1} α-toc increased K^+/Na^+ ratio in shoots of FH-621 cultivar to only 0.47% than hydro-primed seeds. Significant interactions between α-toc and salinity ($P \leq 0.05$) and

amongst all three factors ($P \leq 0.05$) (α-toc, salt stress and cultivars) were also observed.

Seed priming with α-toc had no significant effect on calcium (Ca^{2+}) concentration in sunflower shoot. On the whole, the amount of Ca^{2+} concentration in the shoot of FH-621 cultivar was higher ($P \leq 0.05$) compared to FH-572 cultivar. Imposition of salt stress significantly reduced Ca^{2+} concentration ($P \leq 0.05$) in the shoot of FH-572 cultivar whereas; it triggers the amount of Ca^{2+} concentration in the shoot of cv. FH-621 (Table 2; Fig. 2).

All three factors, seed priming with α-toc, cultivars and salt stress had non-significant effect on Na^+ concentration of the root (Table 2; Fig. 2). However, substantial interaction ($P < 0.001$) between salt stress and cultivars showed increase and considerable reduction in Na^+ concentration of root in FH-572 and FH-621 cultivars respectively, in salt stress. Significant interaction ($P \leq 0.05$) in between salinity and α-toc and amongst all three factors (α-toc, salt stress and cultivars) ($P \leq 0.05$) were observed. Plants raised from α-toc (200 mg L^{-1}) primed seeds exhibited substantial decrease in Na^+ concentration of root in FH-572 (5.34%) and FH-621 (8%) under saline condition than hydro-primed.

Seed priming with α-toc had no considerable effect on potassium (K^+) concentration of the root. On the whole, amount of K^+ retained in roots of FH-621 was significantly higher ($P < 0.001$) compared to FH-572 cultivar. Salt stress significantly reduced ($P < 0.001$) K^+ concentration in roots of both sunflower cultivars. Significant ($P \leq 0.05$) interaction amongst α-toc, salt stress and cultivars was observed. Plants raised from α-toc (100 mg L^{-1}) primed seeds exhibited minimum decrease in K^+ concentration of root in FH-572 (38%) whereas, FH-621 showed no decrease in K^+ concentration of root in FH-621 upon seed priming with 300 mg L^{-1} α-toc, under saline condition than hydro-primed seeds (Table 2; Fig. 2).

Plants grown from seeds primed with α-toc had non-significant influence on potassium/sodium (K^+/Na^+) ratio of roots in sunflower. Overall increase in K^+/Na^+ ratio of roots in FH-621 was much higher ($P < 0.001$) compared to FH-572 cultivar. Sunflower cultivars showed substantial reduction ($P < 0.001$) in K^+/Na^+ ratio of roots in salt stress. However, significant ($P \leq 0.05$) interaction between α-toc and salt stress showed that seed priming with 200 mg L^{-1} α-toc increased K^+/Na^+ ratio in roots of FH-621 to 4.76% whereas all three levels (100, 200 and 300 mg L^{-1}) of α-toc showed decrease in K^+/Na^+ ratio in roots of FH-572 than hydro-primed seeds under saline condition (Table 2; Fig. 2).

Seed priming with α-toc had no remarkable influence on calcium (Ca^{2+}) concentration of the roots in sunflower. Overall increase in the Ca^{2+} concentration of the roots was more pronounced ($P < 0.001$) in FH-621 compared to FH-572 cultivar. Salt stress considerably increased ($P < 0.001$) Ca^{2+} concentration in the roots of both sunflower cultivars (Table 2; Fig. 2).

Discussion

In the present investigation, increase in the amount of free proline in both sunflower cultivars in salt stress was similar to the previous findings on sunflower, *Vicia faba* and cotton (Rady *et al.* 2011; Orabi and Abdelhamid 2016; Hussien *et al.* 2015). Accumulation of free proline under abiotic stress is a common aspect in plants and protects the plants from adversities of salt stress (Saxena *et al.* 2013; Bose *et al.* 2014). Proline improves salinity tolerance in plants by accelerating (enzymatic) antioxidants activities (Hoque *et al.* 2008), photosynthetic rate (Ben-Ahmed *et al.* 2010), maintaining plant water relation (Deivanai *et al.* 2011) and detoxifying ROS (Matysik *et al.* 2002). Proline is an osmolyte which protects complex II in (mitochondrial) electron transport chain and also PS I and II from hydroxyl radical and singlet oxygen (Szabados and Savoure 2010). Results from previous studies on sunflower (Rady *et al.* 2011), *Vicia faba* (Semida *et al.* 2014) and onion (Semida *et al.* 2016) have shown increase in free proline in response to α-toc exogenous application however, in the present study plants grown from seeds treated with α-toc had no influence on the tissue accumulation of free proline.

Results from the present study revealed an increase in GB amount in both sunflower cultivars grown from α-toc primed seeds. Glycine betaine is an osmolyte which helps in salt stress mitigation by stabilizing proteins and shields photosynthetic machinery from ROS injury (Makela *et al.* 2000; Cha-Um and Kirdmanee 2010; Yildiztugay *et al*. 2013). Accumulation of free proline and GB help in maintaining turgor pressure essential for plants elongation by regulating osmotic potential under salt stress (Hajlaoui *et al.* 2010; Munns and Gilliham 2015). Fitzgerald *et al*. (2009) reported that GB protects membranes, photosynthetic apparatus and PS-II (oxygen evolving complex) even in small concentration. Hassine *et al*. (2008) revealed that GB and free proline levels increased in (salt tolerant) *Atriplex halimus* L. upon exposure to 160 m*M* salt stress. Plants grown from seeds primed with α-toc showed remarkable increase in (total) free amino acids. Stress induced increase in free amino acids was more distinct in FH-621 cultivar. Sadak *et al*. (2010) and Rady *et al*. (2011) reported the same in sunflower. Accumulation of amino acids, lower osmotic potential and also act as osmoprotants in plants.

Although both sunflower cultivars showed increase in total (soluble) sugars under salt stress but seed priming with α-toc (vitamin E) was found quite effective in producing more sugars especially in sunflower cv. FH-621 under salt stress, hence increased salt tolerance in sunflower. Likewise, Sadak *et al*. (2010) observed increase in total carbohydrates upon exogenous application of α-toc and reported that sugars and protein accumulation delayed leaf senescence in sunflower. Sadak and Dawood (2014) reported α-toc and ascorbic acid mediated increase in total soluble sugars and proteins.

In the present study, sunflower plants raised from α-toc (200 mg L^{-1}) primed seeds have shown considerable reduction in Na^+ concentration of shoot in cv. FH-621. However, differential response of both sunflower cultivars *i.e*., decrease and increase in Na^+ concentration of shoots in cvs. FH-621 and FH-572 respectively under salt stress have shown that cv. FH-621 is a salt tolerant variety. Haleem and Mohammed (2007) and Cuin *et al*. (2009) reported that Na^+ ion not only competes antagonistically with K^+ ion but also reduces its uptake and Ca^{2+} ion concentration in the plants. Previous studies have shown that increased Na^+ accumulation reduced the uptake of essential nutrients (especially K^+) in roots and shoots of sunflower and mungbean (Shahbaz *et al.* 2011; Kanwal *et al.* 2013). Inhibition in cell elongation and division, metabolic dysfunction, membrane disruption and inhibition of enzymes activities are all attributed to increased Na^+ toxicity in salt sensitive plant species (Kassem 2006). Results from the present investigation have shown the accumulation of K^+ ions in shoots of both sunflower cultivars but was more pronounced in cv. FH-572 under salt stress. Ashraf and Tufail (1995) have reported that tolerant accessions of sunflower in comparison with salt sensitive ones deposit more K^+, K^+/Na^+ ratio and less Cl^- ion in leaves under salt stress. However, seeds priming with α-toc had no remarkable impact on K^+ and Ca^{2+} ions concentration and K^+/Na^+ ratio in the shoots, in the present study.

In the current investigation, shoot K^+/Na^+ ratio though increased in both sunflower cultivars but was considerably more significant in cv. FH-621 in salt stress. More K^+ ions accumulation protect the plants from Na^+ ion toxicity and maintain water potential lower to accomplish osmotic adjustment. Previously it is shown that salt tolerant genotypes retained higher K^+/Na^+ ratio while sequestering Na^+ ions in the vacuole (Rahnama *et al.* 2011). Results from this research revealed considerable increase and decrease in Ca^{2+} concentration of shoot in cvs. FH-621 and FH-572 respectively, under salt stress. Calcium, being a sec messenger, plays significant role in stress related signal transduction pathways. A previous study on sunflower has revealed that seed soaking with α-toc and nicotinamide enhanced K^+, Mg^{2+} and Ca^{2+} and reduced Na^+ accumulation (Rady *et al.* 2011). In the current study, neither α-toc seed priming nor salt stress had any substantial influence on Na^+ concentration of root. However, K^+ ions and K^+/Na^+ ratio decreased in roots of sunflower under saline condition. Increase in Ca^{2+} accumulation in roots of both sunflower cultivars under salt stress revealed its importance as sec messenger in stress related signal transduction pathways and also as membrane stabilizer. Similar to our study a previous study has reported increase in Ca^{2+} accumulation in the root of mungbean under salt stress (Kanwal *et al.* 2013). However, in the present study seed priming with α-toc showed no considerable impact on root ionic accumulation of sunflower.

Conclusion

Among organic solutes seed priming with α-toc improved accumulation of glycinebetaine, amino acids and soluble sugars in the leaves of sunflower by regulating proteins and carbohydrates metabolism. Seed priming with α-toc had non-significant influence on ion homeostasis however, it caused significant reduction in Na^+ ion concentration of the shoot by the mechanism needed further investigation. Sunflower cv. FH-572 showed overall more accumulation of glycinebetaine but FH-621 cultivar a potentially high yielding variety by its inherent ability of maintaining overall, high K^+, K^+/Na^+ ratio and Ca^{2+} ions in shoot and root was proved to be more salt tolerant. In most of the studied parameters seed priming with medium concentration of α-toc (200 mg L^{-1}) was found effective in alleviating the negative impact of salt stress.

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Author Contributions

I.L performed research, collected data and wrote manuscript whereas, M.S. checked and supervized the whole work. Authors approved the final manuscript version.

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