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Full Length Article

Seed Priming with Alpha Tocopherol Improves Morpho-Physiological Attributes of Okra under Saline Conditions

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Abstract

The α-tocopherol (Vitamin E) is naturally occurring antioxidant in plants, which plays a significant role in environmental stress tolerance. A pot trial was conducted to assess the role of exogenously applied α-tocopherol as seed priming agent in improving the morpho-physiological attributes of okra [*Abelmoschus esculentus* (L.) Moench] under saline conditions. Seeds of two okra varieties (Sabzpari and Noori) were treated with four α-tocopherol levels [0 (distilled water), 100, 200 and 300 mg L^{-1}] before sowing for 16 h and two salinity levels (0 and 100 m*M* NaCl) were established after seed sowing. Salt stress caused significant decrease in shoot and root dry weight, shoot length, photosynthetic pigments, gas exchange characteristics and Ca^{2+} and K^+ ion contents, while increased level of Na^+ ions in shoot, root and fruit tissues was observed in tested okra varieties. Seed priming with 200 mg L^{-1} α-tocopherol increased dry biomass of shoot and root, their lengths, photosynthetic pigments including chlorophyll *a*, *b* and carotenoids, water use efficiency (A/E), transpiration rate (E), net CO_2 assimilation rate (A), stomatal conductance (g_s) and sub-stomatal CO_2 concentration (C_i). Moreover, seed priming with various α-tocopherol levels also increased Ca^{2+} uptake, while; no remarkable change was observed in the concentrations of Na^+ and K^+ ions in shoot, root and fruit tissues of okra. The okra variety Noori performed better than Sabzpari in all parameters. Inclusively, 200 and 300 mg L^{-1} of α-tocopherol were more effective in improving growth by demising brutal effects of salt stress at morpho-physiological levels as seed priming agent. Therefore, use of 200 to 300 mg L^{-1} levels of α-tocopherol as seed priming agent are recommended for growing okra in marginally to moderately saline soils.

Keywords: α-tocopherol; Okra; Salinity; Growth; Ion content

Introduction

Vegetable crops serve as an important beneficial dietary source to fulfill the nutritional requirements worldwide (Abbas *et al*., 2014). Okra [*Abelmoschus esculentus* (L.) Moench] is popular edible crop of the family Malvaceae originated in tropical Africa and widely grown in Mediterranean region (Abbas *et al*., 2014). Fruit and leaves of okra are mainly consumed, which are rich source of iron, calcium, phosphorus, carbohydrates, riboflavin and vitamins (mainly vitamin C and A) along with enormous value of mucilage having medicinal properties (Adewale *et al*., 2017; Esan *et al*., 2017). Seeds of okra are also a great source of edible oil, which contain 48% linoleic acid (Gemede *et al*., 2015). However, salinity is one of major abiotic stresses, that is responsible for reduction in per ha productivity of vegetables (Athar and Ashraf, 2009). This drop-in crops' yield is linked with global food insecurity, especially in developing countries including Pakistan (Bartels and Sunkar, 2005).

Salt stress imposes adverse effects on the whole plant by disturbing its cellular mechanism (Tavakkoli *et al*., 2010). It also hampers the growth and productivity of plants by shunting osmotic, ionic, hormonal and oxidative stresses (Deinlein *et al*., 2014; Hanin *et al*., 2016). Salinity affects the growth of plants by reducing plant height, root and shoot weight, number of leaves and total yield (Dolatabadian *et al*., 2011). Salt stress alters the stomatal function, which results in decreased uptake of CO_2, reduced photosynthesis and inhibited reproductive processes (Khan *et al*., 2015; Hussain *et al*., 2018). Modifications in the levels of K^+, Ca^{2+} and Mn^{2+} occurs due to enhanced Na^+ and Cl^- ions thus, adversely affect the enzymatic activities, cellular turgor and protein biosynthesis (Karimi *et al*., 2005; Chokshi *et al*., 2017).

Although, salinity tolerance is a complex mechanism, agricultural crops adapt various responses to resist salinity at molecular, cellular, and physiological levels with the activation of various compounds such as phytohormones, compatible solutes and antioxidants (Chen *et al*., 2016; Soni *et al*., 2017). Seed priming is a pragmatic approach associated with repairing of damaged DNA and other cellular damages as well as enhanced transpiration rate and ATP synthesis (Balestrazzi *et al*.,

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2011; Weitbrecht *et al*., 2011). Remarkably, primed plants experience a state of activated metabolism to mitigate adverse effects of salinity stress known as "primed state" (Savvides *et al*., 2016). Pre-sowing treatment of plant seeds with vitamins including vitamin C, E and B play vital role to mitigate the salinity induced oxidative stress (Keshavarz and Moghadam, 2017). The α-tocopherol (Vitamin E) is a lipophilic antioxidant (Lushchak and Semchuk, 2012), naturally synthesized in all autotrophs including plants. Chloroplasts and plastoglobuli are the main sites of its synthesis (Vidi *et al*., 2006). It exists in various isomeric forms including α, β, γ and δ, while, α being more active in playing its role in plant physio-biochemical processes (Yang *et al*., 2011; Ji *et al*., 2016). Vegetables and fruits are rich source of vitamin E (Dellapenna, 2005). The α-tocopherol has developed powerful system in plants to demise salinity induced oxidative stress by enhancing the significant production of guaiacol peroxidase, catalase and proline (Farouk, 2011; Semida *et al*., 2014).

Transgenic *Arabidopsis* plants overexpressing tocopherol showed better-quality seed longevity and reduced phospholipid peroxidation (Chen *et al*., 2016). Overall, α-tocopherol accumulates in the seeds during germination process to protect them from adverse environmental factors by quenching reactive oxygen species, protecting photosystems, constraining seed dormancy, dehydration and lipid peroxidation (Oettmeier and Trebst, 2005; Chen *et al*., 2016; Ji *et al*., 2016). The hypothesis of present study was to explore whether α-tocopherol could alleviate the adverse impacts of salt (NaCl) stress on okra. Keeping in view the importance of okra as vegetable crop, its nutritional facts and adverse effects of salinity on this crop, the principal objectives of the current study were to explore whether α-tocopherol could shield okra plants from salinity stress damages, and to identify which variety (Sabzpari or Noori) of okra will perform better under saline and salinity free environments.

Materials and Methods

The current experiment was conducted twice in the Old Botanical Garden at University of Agriculture Faisalabad, Pakistan to assess whether seed priming with α-tocopherol could ameliorate salt stress damages in okra. Seeds of two okra varieties (Sabzpari and Noori) were obtained from Ayub Agricultural Research Institute (AARI), Faisalabad, Pakistan. Experiment was performed in sand filled pots [width (24 cm) and depth (30 cm)] containing 10 kg dry river sand, which was thoroughly washed. Ten seeds were sown in each (plastic) pot, and 6 plants were maintained in each pot after thinning. Two liters full strength Hoagland's nutrient solution (Hoagland and Arnon, 1950) was applied to each pot every week till the termination of experiment. Experiment was laid out in completely randomized design with factorial arrangement. All treatments during both seasons had four replications. Seeds of both okra varieties were soaked for 16 h in four levels of α-tocopherol solution [0 (distilled water), 100, 200 and 300 mg L^{-1}]. Seeds of both varieties were blot dried after soaking period before sowing. Two levels of salinity [0 m*M* NaCl (Full strength Hoagland's nutrient solution) and 100 m*M* NaCl (100 m*M* NaCl + full strength Hoagland's nutrient solution)] were established and maintained during the experiment. The 100 m*M* NaCl level was established by adding 50 m*M* NaCl aliquot with interval of 2 days to reduce the osmotic shock for plants. The Hoagland's nutrient solution (non-saline) and 100 m*M* NaCl along with Hoagland's nutrient solution (saline) were applied @ 2 L per pot with interval of 10 days up to crop maturity. The 100 m*M* NaCl level was achieved @ 5.85 g NaCl/L. Two plants were uprooted carefully from each pot four weeks after salinity treatment. Uprooted plants were washed away, and data were recorded for fresh biomass and shoots and roots length immediately. After recording fresh weights, same plants were kept in oven at 65°C up to their constant weight and their dry biomass was recorded. Additionally, data for following physiological attributes were also recorded.

Photosynthetic Pigments Measurement

For the determination of photosynthetic pigments protocol proposed by Arnon (1949) and Davis (1976) was followed. Fresh leaves (0.5 g) were homogenized with 80% acetone, and optical densities were measured at 663, 645, 505, 453 and 470 nm by using spectrophotometer (IRMECO-U2020).

Leaf Gas Exchange Attributes

For the determination of gas exchange attributes including, *A*, *E*, g_s, C_i, C_i/C_a and *A/E*. LCA-4 ACD portable infrared gas analyzer (IRGA) Analytical Development, Hoddesdon, UK was used. Measurements were recorded during 10:00 a.m. to 1:00 p.m. with IRGA leaf chamber having ambient pressure (P), 98.5 kPa, gas flow rate, (U) 252 μmol s^{-1}, ambient CO_2 concentration 350 µmol mol^{-1}, water vapor pressure, 6.0–9.0 mbar, temperature, 28–32°C, relative humidity, 41.1% and air flow/unit leaf area (Us) 22.05 mol m^{-2} s^{-1}.

Mineral Nutrients from Shoots, Roots and Fruits

The protocol proposed by Wolf (1982) was followed to determine mineral elements by acid digestion. To measure them, **s**hoot, root and fruit dried material (0.1 g) was digested in 2.5 mL of concentrated H_2SO_4 overnight at room temperature in digestion flasks. One mL of 35% H_2O_2 was added and tubes were placed in digestion plate at 350°C till the production of fumes, heating process continued for about 30 min and 1 mL of 35% H_2O_2 was again added and recontinued the heating process, and repeated the process, till the mixture became colorless. The mixture was then diluted with distilled water up to 50 mL and filtered. The filtrate was used for the determination of mineral ions by using flame photometer.

Statistical Analysis

By following Snedecor and Cochran (1980) analysis of variance of data for all the parameters with four replicates were calculated under three factor factorial and design of experiment was completely randomized design. The means were compared using least significant difference at 5% (LSD 5%).

Results

Salt stress significantly reduced shoot dry weight and shoot length of okra varieties. Reduction in these attributes was more pronounced in Sabzpari than Noori. Seed treatment with various levels of α-tocopherol did not affect shoot dry weight under saline and non-saline conditions except, 200 mg L^{-1} of α-tocopherol, which slightly improved the shoot dry weight of plants grown under saline and salinity free environments. Although, seed priming with 200 and 300 mg L^{-1} of α-tocopherol caused significant increase in shoot length under stressed and non-stressed conditions (Table 1; Fig. 1).

Salinity caused a significant reduction in root dry weight and root length of okra varieties (Noori and Sabzpari). The two varieties did not differ in term of root dry weight. Seed priming with α-tocopherol at 200 and 300 mg L^{-1} levels was effective in enhancing root dry weight and root length respectively under salt stressed and non-stressed conditions (Table 1; Fig. 1).

The okra varieties significantly differed in net CO_2 assimilation rate (A), transpiration rate (E) and water use efficiency (A/E). However, salt stress significantly reduced these photosynthetic attributes in two varieties, but Noori performed better than Sabzpari in all these parameters under salinity and salinity free conditions. Seed priming with various levels of α-tocopherol significantly improved water use efficiency, transpiration rate and net CO_2 assimilation rate, of tested okra varieties whereas maximum increase was observed at 200 mg L^{-1} in all these attributes (Table 1; Fig. 1).

Salt stress caused a significant decline in stomatal conductance (g_s). The okra varieties markedly differed in terms of g_s. Noori exhibited higher g_s than Sabzpari under stressed and non-stressed regimes. Seed priming with 200 mg L^{-1} α -tocopherol significantly improved the *gs* of tested okra varieties (Table 1; Fig. 1).

Salinity significantly enhanced Sub-stomatal CO_2 concentration (C_i) and relative internal CO_2 concentration (C_i/C_a), whereas okra varieties remained non-significant in this regard similarly, seed priming with α-tocopherol had non-significant effect on C_i under salt stressed and non-stressed conditions. However, seed priming with α-tocopherol exerted significant positive effect on C_i/C_a. Overall, 200 and 300 mg L^{-1} of α-tocopherol proved significant in enhancing C_i/C_a of okra varieties under control and salt stressed conditions (Table 1; Fig. 2).

Application of 100 m*M* NaCl to root growing medium significantly reduced chlorophyll *a* and *b* content. The response of the okra varieties was also significant. Of tested okra varieties, Noori was relatively higher in chlorophyll *a* and *b* content than Sabzpari under saline and non-saline regimes. Seed priming with 200 mg L^{-1} improved chlorophyll *a* and *b* contents under stressed and control plants (Table 1; Fig. 2). Non-significant effect of salt stress was observed on chlorophyll *a*/*b* ratio. Tested okra varieties differed non-significantly in this attribute. Chlorophyll *a*/*b* ratio was lowered in stressed plants as compared to salinity free plants. Seed priming with α-tocopherol caused significantly enhanced chlorophyll *a*/*b* ratio. Overall, 200 mg L^{-1} concentration of α-tocopherol was more effective in increasing chlorophyll *a*/*b* ratio (Table 2; Fig. 2).

Salt stress significantly suppressed the total chlorophyll content. Okra variety Noori showed high accumulation of chlorophyll than Sabzpari under non-saline as well as saline conditions. Effect of α-tocopherol was also significant on the concentration of total chlorophyll, and of all 200 mg L^{-1} α-tocopherol was effective for improving total chlorophyll content under saline and non-saline regimes (Table 2; Fig. 2).

Salt stress and okra varieties had non-significant effect on β-carotene content. Seed priming with α-tocopherol significantly improved carotenoid content, and 200 mg L^{-1} was more distinct among all α-tocopherol levels (Table 2; Fig. 2). Total carotenoid content decreased significantly under saline stress condition. Varying α-tocopherol levels significantly enhanced total carotenoid content in both okra varieties under non-saline and saline treatments, Noori was superior in leaf total carotenoid content than Sabzpari under saline or non-saline treatments (Table 2; Fig. 2).

Salt stress significantly reduced shoot, root and fruit Ca^{2+} and K^+ contents of the okra varieties. Seed priming with α-tocopherol considerably enhanced Ca^{2+} and K^+ in shoot, root and fruit tissues of Noori than Sabzpari under saline and non-saline regimes. Of all, seed priming treatments 200 and 300 mg L^{-1} proved better in enhancing Ca^{2+} and K^+ contents (Table 2; Fig. 3).

Applied salt level caused non-significant effect on shoot Na^+ ion. However, the trend of Na^+ ion accumulation in the shoot differed significantly as Sabzpari accumulated more Na^+ than Noori. Moreover, the response of tested varieties was also non-significant for the seed priming with α-tocopherol (Table 2; Fig. 3). Root Na^+ ion concentration significantly enhanced in Noori and Sabzpari under salinity stress. Seed priming with α-tocopherol remained non-responsive in terms of Na^+ ion accumulation in roots of okra plants (Table 2; Fig. 3). Root-medium applied salt stress significantly enhanced fruit Na^+ content in okra varieties. The response of two okra varieties was non-significant in term of fruit Na^+ content. Seed priming with α- tocopherol did not affect considerably this ionic attribute (Table 2; Fig. 3).

Table 1: Analysis of variance (p values) for growth and gas exchange attributes of okra treated with different levels of α-tocopherol as pre-sowing seed treatment under control and saline conditions

Source of variation	df	Shoot dry weight	Root dry weight	Shoot length	Root length	*A*	*E*
Varieties (V)	1	0.0075	0.1096	0.0025	0.0000	0.0000	0.0019
Salinity (S)	1	0.0002	0.0000	0.0000	0.0000	0.0000	0.0000
α-tocopherol (α-toc)	3	0.1150	0.0004	0.0213	0.0000	0.0000	0.0221
V × S	1	0.0749	0.8516	0.1791	0.200	0.0215	0.0747
V × α-toc	3	0.9367	0.9491	0.4725	0.7787	0.6073	0.6661
S × α-toc	3	0.4014	0.2710	0.9277	0.4147	0.0000	0.0136
V × S × α-toc	3	0.9934	0.9650	0.2586	0.4277	0.8757	0.8466
Source	df	*A/E*	*gs*	C_i	C_i/C_a	Chl. *a*	Chl. *b*
Varieties (V)	1	0.0029	0.0496	0.1689	0.2847	0.0001	0.0998
Salinity (S)	1	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
α-tocopherol (α-toc)	3	0.0000	0.0143	0.0517	0.0247	0.0000	0.0000
V × S	1	0.6876	0.3645	0.1028	0.1909	0.2860	0.0768
V × α-toc	3	0.7370	0.5949	0.5919	0.3522	0.0000	0.0012
S × α-toc	3	0.0009	0.1776	0.6769	0.3606	0.0019	0.0020
V × S × α-toc	3	0.8107	0.9322	0.8874	0.8783	0.0000	0.0195

df = Degree of freedom; Chl. *a* = Chlorophyll a; Chl. *b* = Chlorophyll b; (A/E) = Water use efficiency (WUE); *A* = Net CO_2 assimilation rate; *E* = Transpiration rate; *gs* = Stomatal conductance; C_i = Sub-stomatal CO_2 concentration; C_i/C_a = Relative internal CO_2 concentration

Table 2: Analysis of variance (P values) for photosynthetic pigments and mineral nutrients of okra treated with different levels of α-tocopherol as pre-sowing seed treatment under control and saline conditions

Source of variation	df	Chl. *a*/*b*	Total Chl.	β-carotene	Total carotenoids	Shoot Ca^{2+}	Shoot K^+	Shoot Na^+
Varieties (V)	1	0.1092	0.0000	0.3658	0.0000	0.4077	0.0012	0.0001
Salinity (S)	1	0.2459	0.0000	0.0617	0.0000	0.0010	0.0000	0.3098
α-tocopherol (α-toc)	3	0.0483	0.0000	0.0000	0.0000	0.0000	0.0000	0.2675
V × S	1	0.9916	0.1280	0.0000	0.0005	0.0026	0.8111	0.0005
V × α-toc	3	0.2782	0.0013	0.0001	0.1851	0.0000	0.0000	0.7954
S × α-toc	3	0.1910	0.0004	0.0098	0.4490	0.0054	0.1821	0.0930
V × S × α-toc	3	0.4076	0.0000	0.0009	0.0000	0.0075	0.0000	0.2310
Source	df	Root Ca^{2+}	Root K^+	Root Na^+	Fruit Ca^{2+}	Fruit K^+	Fruit Na^+	
Varieties (V)	1	0.0321	0.1960	0.9567	0.7414	0.0010	.0795	
Salinity (S)	1	0.0002	0.0000	0.0000	0.0004	0.0000	0.0000	
α-tocopherol (α-toc)	3	0.0097	0.8958	0.1016	0.0312	0.0903	0.0899	
V × S	1	0.0255	0.0068	0.1200	0.2240	0.4477	0.8758	
V × α-toc	3	0.0004	0.8416	0.0060	0.0285	0.0007	0.8619	
S × α-toc	3	0.9709	0.0189	0.9267	0.4243	0.0028	0.1292	
V × S × α-toc	3	0.1357	0.0074	0.0777	0.2635	0.0089	0.1840	

df = degree of freedom; Chl. *a*/*b* = Chlorophyll a/b ratio; Total chl. = Total chlorophyll

Discussion

In the current investigation, salinity induced growth suppression (shoot and root dry weights and their lengths) of okra plants was observed. Sodium chloride (NaCl) is a predominant salt inducing salinity stress and its varying levels constrain the plant growth (Kachout *et al*., 2016). However, seed priming with α-tocopherol particularly at 200 mg L^{-1} ameliorated the negative effects of salinity stress on growth of okra plants. This showed that α-tocopherol minimized salinity induced damages in tested okra plants by improving stomatal conductance, water use efficiency and net CO_2 assimilation rate. Among all physiological processes of plants, photosynthesis and transpiration are directly affected by salt stress (Negi *et al*., 2014). In current investigation, salt stress remarkably decreased *A*, *E*, *A*/*E* and g_s: however, C_i and C_i/C_a were significantly enhanced. Reduced stomatal conductance has already been observed in rice under salinity stress by Kausar and Shahbaz (2017). Higher levels of Na^+ and Cl^- dysfunction the stomata and inhibit photosynthesis (Parida and Das, 2005; Khan *et al*., 2015). However, this reduction in gas exchange attributes in okra in the present study was ameliorated by seed priming with α-tocopherol. Mohammed and Tarpley (2011) further reported that exogenously applied α-tocopherol, salicylic acid and glycine betaine enhanced water use efficiency in rice. Increase in photosynthesis and transpiration rates was due to the antioxidative property of α-tocopherol as, it quenches reactive oxygen species and protects the photosystems from oxidation (Bughdadi, 2013).

Excessive amounts of Na^+ and Cl^- ions in root zone of plants severely inhibits reproductive processes by arresting photosynthesis (Flowers *et al*., 2010) and photosynthetic pigments (Khan *et al*., 2016). In current investigation, chlorophyll *a* and *b*, total chlorophyll and total carotenoid contents were significantly decreased due to salinity while, okra plant raised from α-tocopherol primed seeds markedly improved these pigments content. These results suggest the salinity alleviation role of α-tocopherol by shielding photosynthetic pigments from photo-inhibition

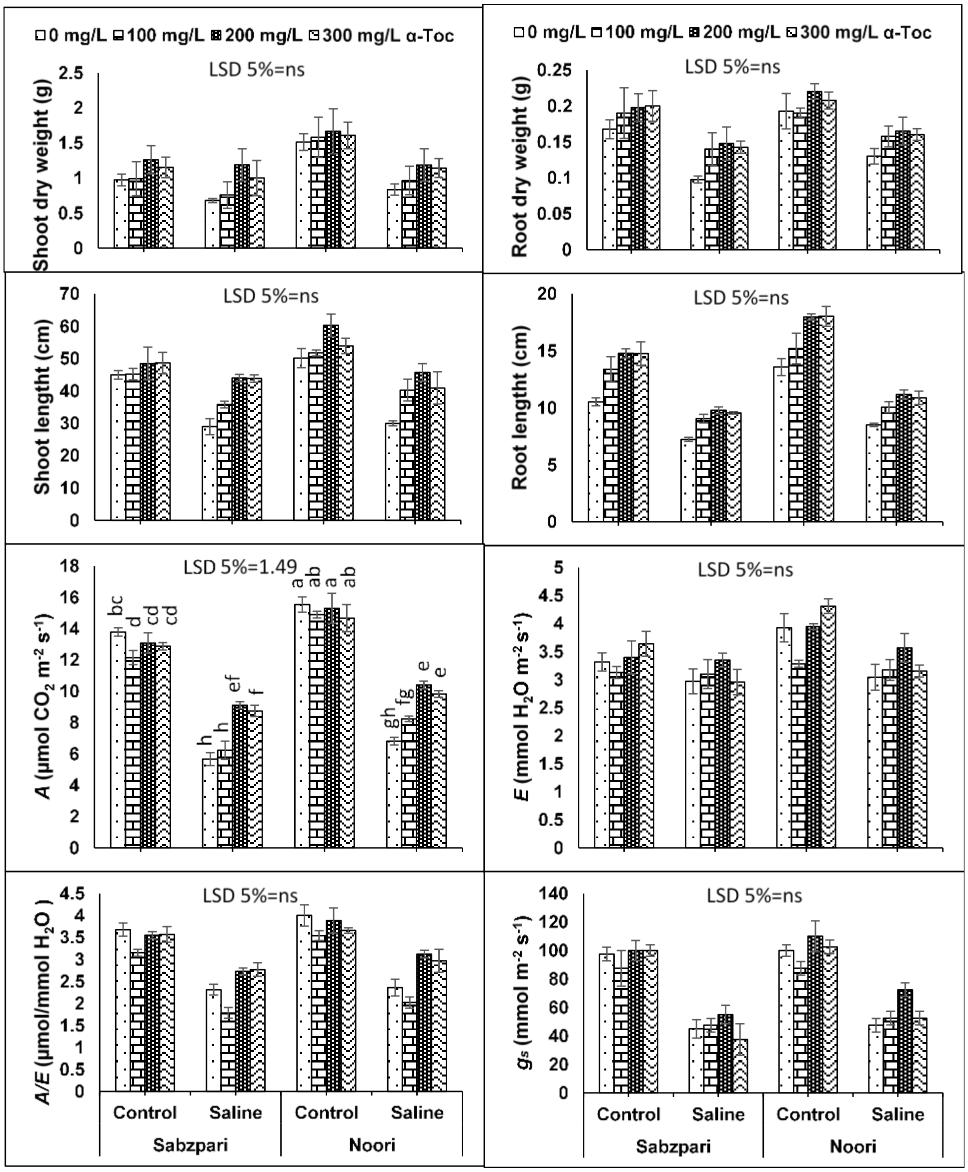

Fig 1: Growth and gas exchange attributes of okra treated with different levels of α-tocopherol as seed priming agent (16 h) under control and saline condition

(Al-Qubaie, 2012; Kumar *et al*., 2012). Salinity-imposed ionic stress leads to metabolic disorders such as inhibited photosynthesis and transpiration (Saleem *et al*., 2011) and reduced enzymatic activities (Tavakkoli *et al*., 2010). Excessive concentrations of toxic ions (Na^+ and Cl^-) caused nutrients imbalance by adversely affecting the uptake of K^+, Ca^{2+}, Mg, Cu and Fe (Farooq *et al*., 2015). In this study, higher concentrations of Na^+ ions had been observed in shoot, root and fruit tissues under salinity stress. Increase in Na^+ ions have also been observed in okra (Saleem *et al*., 2011) and rice (Masood and Shahbaz, 2016). As Na^+ ions increase in plant tissues, osmotic potential increases whereas water potential decreases and reduced water potential is attributed to reduced yield due to suppressed reproductive mechanism (Cha-um *et al*., 2010; Sadiq *et al*., 2017). While, seed priming with α-tocopherol proved successful in minimizing the toxic concentrations of Na^+ ion in root, shoot and fruit tissues of okra plants, suggesting the role of α-tocopherol in osmotolerance by lowering the concentration of NaCl (Farouk, 2011; Sadak and Dawood, 2014). Salt stress significantly decreased leaf, root and fruit tissue Ca^{2+} and K^+ contents of both okra varieties in the current investigation. While, seeds of okra plants raised from α-tocopherol accumulated enhanced concentration of K^+ and Ca^{2+} ion contents in root, shoot and fruit tissues of okra plants. α-tocopherol application induces regulation of physiological processes involved in the increased uptake of K^+ and Ca^{2+} ions (Orabi and Abdelhamid, 2016).

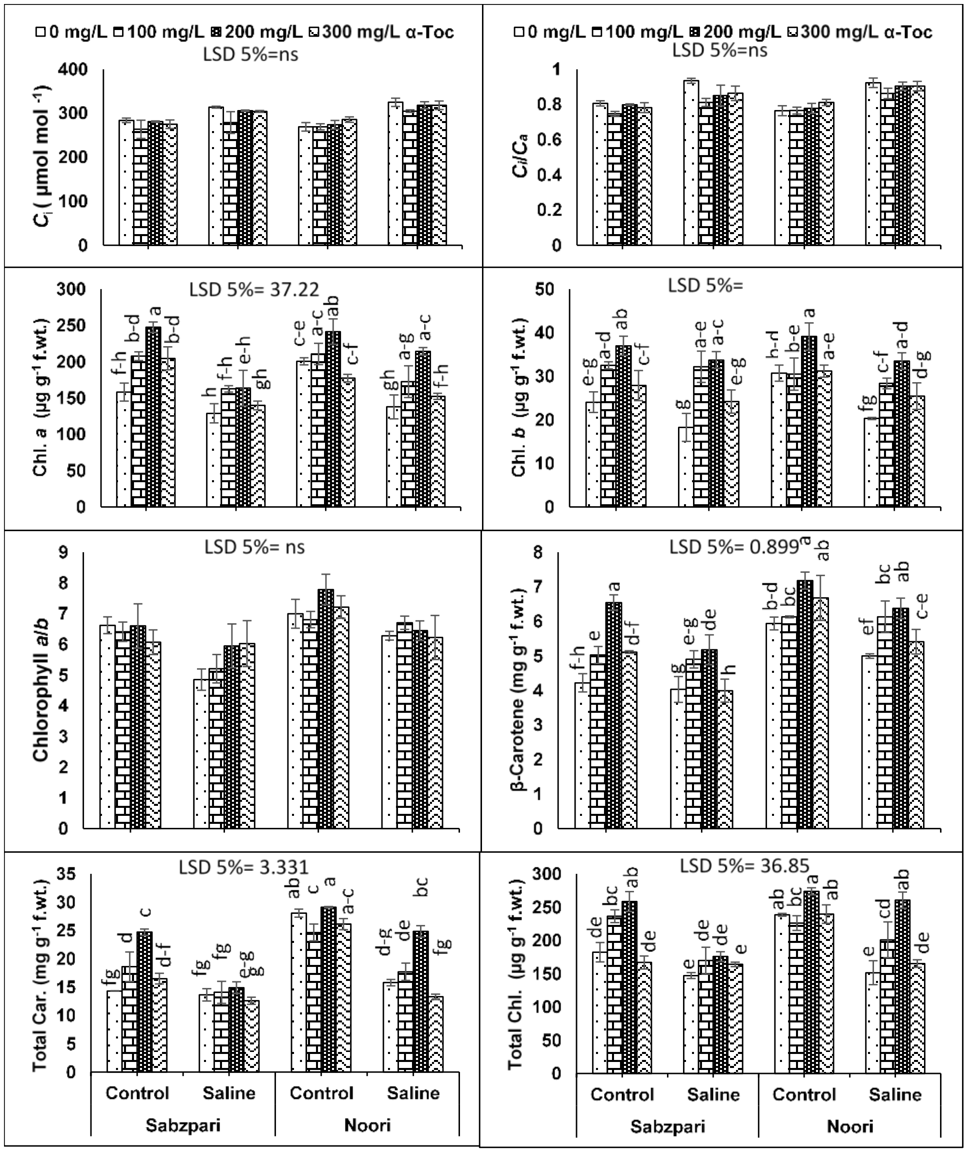

Fig. 2: Gas exchange attributes and photosynthetic pigments content of okra treated with different levels of α-tocopherol as seed priming agent (16 h) under control and saline conditions

The α-tocopherol maintains ionic balance in plants by keeping osmotic homeostasis (Sadak and Dawood, 2014). It is well documented that selective uptake of K^+ than Na^+ is important strategy to demise salinity (Neill *et al*., 2002). Salt tolerant plants accumulates more levels of K^+ than Na^+ to resist salinity induced damages (Zhu, 2003). Higher levels of Na^+ reduce the uptake of Ca^{2+} and K^+ ions inducing ionic imbalances (Akram and Ashraf, 2011). It can be said that a decreased ratio of Na^+ to Ca^{2+} and K^+ avoids the salinity damages to plants under salt stress.

Conclusion

It is concluded that okra variety Noori performed better than Sabzpari under salt stress and α-tocopherol seed priming @ 200 mg L^{-1} was the most effective level which played a vital role in alleviation of salinity induced damages in okra through enhancement of growth, stomatal conductance, photosynthesis and inorganic ions (K^+ and Ca^{2+}).

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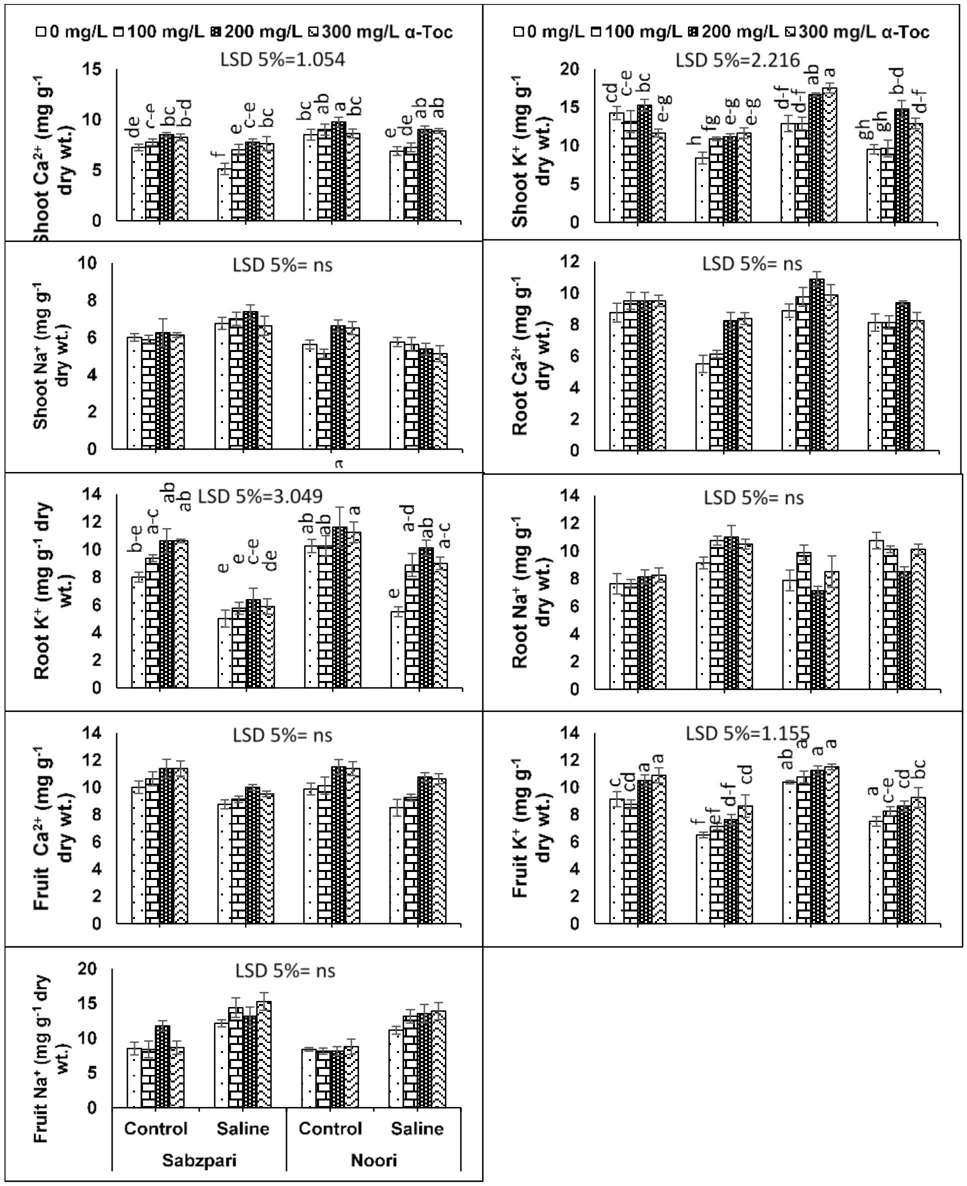

Fig. 3: Mineral Nutrients of okra treated with different levels of α-tocopherol as seed priming agent (16 h) under control and saline conditions

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