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# Exogenously Applied Trinexapac-ethyl Improves Photosynthetic Pigments, Water Relations, Osmoregulation and Antioxidants Defense Mechanism in Wheat under Salt Stress

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The impact of trinexapac-ethyl (TE) on salinity subjected wheat plants was evaluated via pot based experiment. The treatments applied to wheat seedlings included (Ck) control (no NaCl nor TE spray), foliar spray of TE (1.95 ml L<sup>-1</sup>), only NaCl (50 mM) and NaCl+ TE (50 mM+1.95 ml L<sup>-1</sup>). Foliar application of TE was done seven days after imposition of salinity. Growth parameters (root length, shoot length, fresh weight, and dry weight) and photosynthetic pigments content (chlorophyll *a*, *b*, *a+b* and *a/b*), water relation (water potential, osmotic potential, turgor potential and relative water contents) as well as catalase (CAT) activity exhibited marked reduction in comparison to control. In addition, an increment was noted in organic solutes content (proline, soluble protein and soluble sugar) and enzyme activity of superoxide dismutase (SOD), peroxidase (POD), and ascorbate peroxidase (APX) in stressed seedlings over control seedlings. The foliar applied TE mostly enhanced growth of salt stressed seedlings, accompanied by reinforcement in photosynthetic pigments, organic solutes, and enzyme activity (SOD, CAT, POD, and APX) in comparison to stressed seedlings. It is worthy to mention that, TE has potential to enhance salt tolerance of wheat seedlings. Thus, our findings suggest that seedling treated with TE is an effective strategy that can be used to enhance salt tolerance of wheat crop.

**Keywords:** antioxidants, osmo-protectants, salt tolerance, trinexapac-ethyl, water relations

## Introduction

Increasing salt levels in cultivated area limit the crop productivity by imparting drastic effects on plants. Since last few decades, salt stressed conditions have been widely investigated, due to increase in its intensity and expansion of damage. The salt stress affects key physiological processes such as water relations, photosynthesis, osmotic adjustment

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and oxidative metabolism (Farooq et al. 2015). It alters the plant's water relations by lowering soil water availability solution as a result of lowered osmotic potential (Farooq et al. 2015; Liu et al. 2014). The phenomenon of stomatal closure often observed in salt-treated plants, protects tissue dehydration by limiting water losses (Fricke et al. 2004). It also induces the oxidative stress due to accumulation of reactive oxygen species (ROS), which modifies the cellular redox potential, favoring oxidized forms that can cause enzymes inactivation, leading to lipid peroxidation and damage DNA (Liu et al. 2014; Moldovan and Moldovan 2004). Changes in photosynthetic electron transport stimulate production of superoxide radicals which alter thylakoid membranes oxidative status and stomatal movement, leading to slow photosynthetic rate (Guerfel et al. 2009; Grijalva-Contreras et al. 2012). The ROS accumulation is neutralized by antioxidant systems comprised of various scavengers, such as enzymes (superoxide dismutase (SOD), ascorbate peroxidase (APX), peroxidase (POD), and catalase (CAT) and non-enzymatic low molecular metabolites (proline, soluble sugar and carotenoids). Hence, the regulation of these plant antioxidant systems by exogenous supplementation of many substances (plant growth hormones, nutrients, osmolytes etc.) might arbitrate the plant tolerance to salt stress.

Trinexapac-ethyl (TE) being anti-gibberellins may possibly facilitate plant performance and development under unfavorable conditions but also increase stress tolerance in crop plants (Xu and Huang 2011). It was narrated that perennial rye grass (*Lolium perenne*) (Jiang and Fry 1998) and Kentucky bluegrass (*Poa pratensis* L.) (Xu and Huang 2011) was able to survive under drought conditions through exogenous applied TE. In another study, it was reported that foliage applied TE significantly improved the performance of creeping bentgrass under high temperature and drought conditions (McCann and Huang 2007). Application of TE significantly enhanced the drought tolerance in plants by improving the water relations, photosynthetic attributes and osmotic adjustment (Bianet al. 2009). TE applied as foliar spray under saline conditions stimulated fresh and dry biomass, leaf pigments (chlorophyll and carotenoids) and lowered proline, sodium and chloride contents in paspalum turfgrass (Sakr 2009). Very first response was TE was observed as limiting mowing frequency of turfgrasses but now it has been established as plant growth mediator by stimulating water use efficiency, heat tolerance and shade tolerance (Wang et al. 2006; Steinke and Stier 2003; Zhang et al. 2003; Ervin et al. 2002). The TE provides a more compact and dense turf with smaller leaf blade that reduced soil evaporation. Reduction in evapo transpiration improves the water availability which may confirm the role of TE as growth enhancer under stressed conditions.

Although the impact of TE on various turfgrasses under drought stress is widely reported, yet there are no published data on its effects on wheat under salt stress. The current study evaluated the potential of TE on morpho-physiological and antioxidative responses of wheat against salt stress, and to identify whether water relations, chlorophyll contents, enzymatic antioxidants activity, proline content, soluble protein and soluble sugar content (SSC) are implicated in salt tolerance of TE-treated wheat.

## Materials and Methods

A pot experiment was planned to determine the potential of TE in improving the salt tolerance in wheat (*Triticum aestivum* L.) seedlings at green house. Wheat variety Glaxay-2013 was used as an experimental material and its source was Ayub Agriculture Research Institute, Faisalabad, Pakistan. The 10 seeds were sown in each pot [15.5 cm × 60 cm (diameter × height)] having 12 kg of well ground and fine soil. The physico-chemical properties of soil are shown in Table 1. In order to maintain the growth of seedling, basal dose of nitrogen (N) at the rate of 100 mg kg<sup>-1</sup> as urea, phosphorus (P<sub>2</sub>O<sub>5</sub>) 90 mg kg<sup>-1</sup> as diammonium phosphate and potassium (K<sub>2</sub>O) 60 mg kg<sup>-1</sup> of soil as potassium sulphate was mixed well into soil. The 5 plants per pot were maintained. Fifteen days old seedlings were grown in each pot without salt treatments and were irrigated with deionized water. NaCl solution (50 mM) was used to create artificial soil salinities which were started 15 days after seedlings emergence. Treatments included in the study were, viz. (Ck) control (no NaCl nor TE spray), TE (sprayed with 1.95 ml L<sup>-1</sup>), only NaCl (50 mM NaCl) and NaCl + TE (50 mM NaCl with 1.95 ml L<sup>-1</sup> TE). Foliar application of TE was done seven days with help of hand sprayer after imposition of salinity. The 10 ml volume of solution was consumed for each pot. The experiment followed completely randomized design (CRD) with three replications.

Table 1. Physio-chemical properties of soil used in the experiment

Soil analysis	Value
<b>Mechanical analysis</b>	
Sand (%)	52
Silt (%)	22
Clay (%)	27
Textural class	Sandy loam
<b>Chemical analysis</b>	
Soil pH	8.5
EC (dSm <sup>-2</sup> )	2.32
Cations exchangeable capacity (dSm <sup>-2</sup> )	2.01
Organic matter (%)	0.78
Calcium carbonate (%)	2.96
Available Si (mg kg <sup>-1</sup> soil)	16
Available Se (mg kg <sup>-1</sup> soil)	12

### Growth characteristics

Fifteen days past salinity treatments, wheat seedling was cut from the soil surface and the roots were cleaned to measure root and shoot lengths and fresh weights. For dry weights of root and shoot the samples were oven-dried at 75 °C till a constant weight. Root shoot ratio was determined on dry weight basis. Root and shoot dry weights were pooled to estimate total dry biomass weight.

For determination of relative water contents (RWC), fresh leaves ( $W_f$ ) (0.5 g) were water rinsed till constant weight and weighed ( $W_s$ ). The water saturated leaves were then dried at 80 °C for 24 h to determine dry weight ( $W_d$ ). The formula suggested by (Barrs and Weatherley 1962) was used to determine dry RWC as:

$$\text{RWC (\%)} = (W_f - W_d) / (W_s - W_d) \times 100$$

Water potential ( $\Psi_w$ ) of fresh leaf was determined by using pressure bomb (Santa Barbara, CA, USA). For osmotic potential ( $\Psi_s$ ), same leaf was frozen, thawed, sap expressed, centrifuged (5000 × g) using an osmometer (Digital Osmometer, Wescor, Logan, UT, USA). The difference of  $\Psi_w$  and  $\Psi_s$  determined the Leaf pressure potential ( $\Psi_p$ ).

For determination of leaf chlorophyll contents, grinding of 0.5 g leaf sample was done in 80% acetone to isolate chlorophyll. The absorbance of filtrate was determined through Spectrophotometer (Hitachi-U2001, Tokyo, Japan) at 663 and 645 nm as described by Arnon (1949).

For total soluble proteins, pre-chilled pastor mortar was used to ground fresh plant material (leaves) (0.5 g) with addition of 1 mL extraction buffer (pH 7.2). Before extraction of proteins, cocktail protease inhibitors (1 μM) were added to the buffer. Phosphate buffer saline (PBS) was used, containing 10 mM Na<sub>2</sub>HPO<sub>4</sub>, 2 mM KH<sub>2</sub>PO<sub>4</sub>, 2.7 mM KCl and 1.37 mM NaCl dissolved in distilled water and volume was made up to 1 L. HCl was used to adjust the pH 7.2 of PBS and then autoclaved (Sambrook and Russell 2001). The grounded leaf material was centrifuged at 12,000 × g for 5 min. Supernatant was separated in centrifuge tube for the analysis of soluble proteins, following the Bradford assay (Bradford 1976). The absorbance for the sample supernatant was determined at 595 nm using spectrophotometer (UV 4000 UV-VIS spectrophotometer). Concentration (mg mL<sup>-1</sup>) of total soluble or heat stable fractions of proteins was calculated using a standard curve prepared from bovine serum albumin (BSA). For enzymatic antioxidants determination, 5 ml of 50 mM phosphate buffer (pH 7.8) was used for extraction of fresh leaf sample and centrifuged at 15,000 × g for 20 min, the supernatant was used in further assay for superoxide dismutase (SOD) activity (Giannopolitis and Ries 1977), Catalase (CAT) activity (Chance and Maehly 1955) by recording absorbance at 560 and 240 nm, respectively. The SOD activity was determined by monitoring inhibition of the photochemical reduction of nitroblue tetrazolium (NBT) at 560 nm and expressed as SOD IU min<sup>-1</sup> mg<sup>-1</sup> protein. The reaction mixture included 50 μL enzyme extract and adding 1 ml NBT (50 μM), 500 μL methionine (13 mM), 1 mL riboflavin (1.3 μM), 950 μL (50 mM) phosphate buffer and 500 μL EDTA (75 mM). The reaction was started by keeping reaction solution under 30 W fluorescent lamp illuminations and turning the fluorescent lamp on. Reaction stopped when the lamp turned off 5 min later. The NBT photo reduction produced blue formazane which was used to measure the increase in absorbance at 560 nm. The same reaction mixtures without enzyme extract in dark were used as a blank. The CAT activity was assayed by the decomposition of H<sub>2</sub>O<sub>2</sub> and change in absorbance due to H<sub>2</sub>O<sub>2</sub> was observed after every 30 s for 5 min at 240 nm using a UV-visible spectrophotometer. Reaction mixture for CAT contained 900 μL H<sub>2</sub>O<sub>2</sub> (5.9 mM) and 2 mL phosphate

buffer (50 mM). Reaction was started by adding 100  $\mu\text{L}$  enzyme extract to the reaction mixture. The CAT activity was expressed as  $\mu\text{mol of H}_2\text{O}_2 \text{ min}^{-1} \text{ mg protein}^{-1}$  (Chance and Maehly 1955). The POD activity was determined according to Kara and Mishra (1976). The reaction mixture consisted of 2.5 ml Tris-HCl buffer (0.1 M), 2.5 ml  $\text{H}_2\text{O}_2$  (5 mM), 2.5 ml pyrogallol (10 mM) and 50  $\mu\text{l}$  enzyme extract. The  $\text{H}_2\text{O}_2$  dependent oxidation of pyrogallol was followed by a decrease in the absorbance at 425 nm. Proline was determined as described by Bates et al. (1973). Fresh leaf tissues (0.5 g) from each treatment were homogenized in 10 mL of 3% w/v sulphosalicylic acid, and the homogenate was filtrated. The resulting solution was treated with 2.5% ninhydrine solution and glacial acetic acid. In test tubes, the reaction mixtures were kept in a water bath at 100 °C for 60 min to develop the color. Soon after removal from the water bath, the test tubes were cooled in ice bath and toluene was added to separate chromophores. Optical density was read at 520 nm using UV-VIS spectrophotometer. The concentrations of soluble sugar were determined in this extract as described by Giannakoula et al. (2008).

Fisher's Analysis of Variance technique was used to analyze collected data. Least Significant Difference (LSD) test at 5% probability level was applied to compare the treatment's means (Steel et al. 1997).

## Results

Salt stress implemented adverse effects on wheat seedlings as evident from the reduced growth attributes. The sole application of TE contributed at par response of growth attributes (shoot dry weight, root dry weight and total seedling biomass) with control (Table 2). However, the TE application response in terms of shoot and root length followed control plants. Application of TE on salt stressed wheat seedlings improved shoot dry weight, root dry weight, shoot length, root length and total seedling biomass as compared to salt stressed plants.

Salt stress resulted in reduction of water potential ( $\Psi_w$ ), osmotic potential ( $\Psi_s$ ) and relative water contents of the wheat seedlings, when compared with control. Application of TE without salt stress cause less reduction in  $\Psi_w$  and  $\Psi_s$  as compared to salt stressed seedlings. Supplementation of TE under salt stress also contributed in less reduction of  $\Psi_w$  and  $\Psi_s$  in comparison to salt stressed seedlings (Table 3). Salt application improved turgor potential of wheat seedlings, which was reduced in case of TE application, however when TE was applied to salt stressed seedlings, turgor potential was found to be at par with salt stressed seedlings. TE application under salt stress, improved relative water contents of the wheat seedlings.

Photosynthetic pigments vary significantly in response to salt stress and TE application. Salt stress reduced the chlorophyll *a*, *b* and *a + b*. The solo application of TE showed at par response with control (Table 4). The TE application under salt stress improved chlorophyll *a*, *b* and *a + b* in comparison to saline treatment. The response of chlorophyll *a/b* remained non-significant under various treatments applied.

Saline conditions increased the enzymatic activity of the wheat seedlings. Superoxide dismutase was found to be maximum when saline conditions were supplemented with TE

Table 2. Effect of foliar application of trinexapac-ethyl on growth characteristics of wheat seedlings under salt stress

Treatments	Shoot dry weight (g)	Root dry weight (g)	Shoot length (cm)	Root length (cm)	Total seedlings biomass (g)	Shoot dry weight (g)
Ck	9.17±0.204 a	2.03±0.077 ab	35.36±0.862 a	8.18±0.393 a	11.19±0.274 a	9.17±0.204 a
TE	9.70±0.168 a	2.17±0.081 a	28.10±1.021 b	6.08±0.256 b	11.88±0.234 a	9.70±0.168 a
NaCl	7.33±0.149 c	1.69±0.065 c	24.04±0.813 c	4.33±0.193 c	9.03±0.167 c	7.33±0.149 c
NaCl+TE	8.11±0.148 b	1.84±0.043 bc	26.63±0.512 bc	6.62±0.424 b	9.95±0.170 b	8.11±0.148 b
LSD≤0.05	0.542	0.220	2.63	1.059	0.693	0.542
CV	3.36	6.06	4.90	8.92	3.50	3.36
MS (df=8)	3.38**	0.131*	70.63**	7.54*	4.83**	3.38**

Ck = Control treatment, TE = trinexapac-ethyl, LSD = Least significance difference, CV = Coefficient of variance, MS = Mean square, df = degree of freedom, values represent mean±SE (n = 3). Different small letters indicated that the means are significantly different ( $P \leq 0.05$ ).

Table 3. Effect of foliar application of trinexapac-ethyl on water relations of wheat seedlings under salt stress

Treatments	Water potential ( $\Psi_w$ ) (-MPa)	Osmotic potential ( $\Psi_s$ ) (-MPa)	Turgor potential ( $\Psi_t$ ) (MPa)	Relative water contents (%)
Ck	0.47±0.032 c	0.86±0.050 d	0.38±0.017 b	86.31±1.89 a
TE	0.64±0.034 b	1.06±0.051 c	0.41±0.041 b	66.28±1.35 c
NaCl	0.87±0.047 a	1.46±0.044 a	0.59±0.003 a	47.34±3.02 d
NaCl+TE	0.63±0.030 b	1.24±0.059 b	0.60±0.032 a	74.74±2.43 b
LSD≤0.05	0.117	1.164	0.089	7.237
CV	9.47	7.55	9.42	5.60
MS (df=8)	0.078**	0.198**	0.040**	808.6**

Ck = Control treatment, TE = trinexapac-ethyl, LSD = Least significance difference, CV = Coefficient of variance, MS = Mean square, df = degree of freedom, values represent mean±SE (n = 3). Different small letters indicated that the means are significantly different ( $P \leq 0.05$ ).

Table 4. Effect of foliar application of trinexapac-ethyl on photosynthetic pigments of wheat seedlings under salt stress

Treatments	Chlorophyll <i>a</i> (mg g <sup>-1</sup> )	Chlorophyll <i>b</i> (mg g <sup>-1</sup> )	Chlorophyll <i>a + b</i> (mg g <sup>-1</sup> )	Chlorophyll <i>a/b</i> (mg g <sup>-1</sup> )
Ck	2.84±0.086 ab	0.84±0.059 a	3.68±0.039 a	3.41±0.315a
TE	3.10±0.378 a	0.76±0.051 a	3.86±0.414 a	4.03±0.351a
NaCl	1.68±0.165 c	0.47±0.050 b	2.15±0.120 c	3.66±0.711a
NaCl+TE	2.23±0.082 bc	0.67±0.053 a	2.90±0.048 b	3.34±0.356a
LSD≤0.05	0.687	0.171	0.697	ns
CV	14.83	13.19	11.74	21.76
MS (df = 8)	1.215**	0.075**	1.843**	0.294 ns

Ck = Control treatment, TE = trinexapac-ethyl, LSD = Least significance difference, CV = Coefficient of variance, MS = Mean square, df = degree of freedom, values represent mean±SE (n = 3). Different small letters indicated that the means are significantly different ( $P \leq 0.05$ ).

Table 5. Effect of foliar application of trinexapac-ethyl on enzymatic antioxidants of wheat seedlings under salt stress

Treatments	Superoxide dismutase (unit mg <sup>-1</sup> of protein)	Catalase (unit mg <sup>-1</sup> of protein)	Peroxidase (unit mg <sup>-1</sup> of protein)	Ascorbate peroxidase (unit mg <sup>-1</sup> of protein)	Glutathione reductase (unit mg <sup>-1</sup> of protein)
Ck	56.79±6.64 c	2.33±0.363 d	2.01±0.262 d	0.155±0.020 d	0.077±0.010 d
TE	67.72±12.60 c	3.56±0.331 c	3.12±0.238 c	0.240±0.018 c	0.120±0.009 c
NaCl	139.00±19.47 b	5.70±0.439 b	5.64±0.402 b	0.434±0.030 b	0.271±0.015 b
NaCl+TE	236.12±12.86 a	8.05±0.190 a	7.38±0.337 a	0.568±0.025 a	0.284±0.012 a
LSD≤0.05	43.75	1.098	1.019	0.078	0.038
CV	18.61	11.88	11.86	11.86	11.81
MS (df = 8)	20475.2**	18.97**	17.67**	0.104**	0.026**

Ck = Control treatment, TE = trinexapac-ethyl, LSD = Least significance difference, CV = Coefficient of variance, MS = Mean square, df = degree of freedom, values represent mean±SE (n = 3). Different small letters indicated that the means are significantly different ( $P \leq 0.05$ ).

with a percentage increase of 70%, when compared with saline conditions and 316%, when compared with control (without salt treatment). The percentage increase of catalase activity of wheat seedlings with sole application of TE was observed to be 53%, with saline treatment only as 145% and with TE application with salt stress as 245%, when compared with control (Table 5). However, the percentage increase of 41% was observed in catalase activity in response to NaCl + TE, when compared with saline conditions. TE application with and without saline conditions resulted in improved peroxidase activity of wheat seedlings with percentage increase of 55% and 67%, when compared with control. The percentage increase of 31 was observed when NaCl + TE was compared with saline conditions. APX activity was observed to increase by a percentage of 55 and 266, when TE was applied alone and under saline conditions, respectively. In comparison to saline conditions, 31% increase in APX activity was observed in response to NaCl + TE treatment. Increment in glutathione reductase (GR) activity was also noted to be 56% and 269% in TE application and NaCl + TE application, respectively (Table 5). The percentage increase of 6% was observed in treatment NaCl + TE, when compared with saline conditions.

Proline contents were incremented by 27%, 59% and 115% with application of TE, NaCl and NaCl + TE, respectively, when compared with control. The increment was 35%, when application of NaCl + TE was compared with saline (Table 6). Soluble protein contents were found to be reduced under saline conditions but increased with application of TE alone and TE under saline conditions. This increase was 32% and 111%, respectively as compared to control conditions. Under saline conditions, the increase of soluble proteins was observed to be 148.14%, with application of TE. Saline conditions improved soluble sugars by 114%, TE alone and under saline conditions enhanced soluble sugars by 53% and 219%, in comparison to control (Table 6). The increment was 49%, when TE application under saline conditions was compared with saline treatment.

Table 6. Effect of foliar application of trinexapac-ethyl on osmo-protectants of wheat seedlings under salt stress

Treatments	Proline ( $\mu\text{mol g}^{-1}$ FW)	Soluble protein ( $\text{mg g}^{-1}$ FW)	Soluble sugar ( $\text{mg g}^{-1}$ FW)
Ck	5.47 $\pm$ 0.38 c	1.59 $\pm$ 0.34 b	1.91 $\pm$ 0.49 c
TE	6.93 $\pm$ 0.49 c	2.10 $\pm$ 0.16 b	2.93 $\pm$ 0.35 bc
NaCl	8.71 $\pm$ 0.44 b	1.35 $\pm$ 0.28 b	4.08 $\pm$ 0.18 b
NaCl+TE	11.78 $\pm$ 0.67 a	3.35 $\pm$ 0.15 a	6.09 $\pm$ 0.40 a
LSD $\leq$ 0.05	1.64	0.80	1.20
CV	10.61	20.46	17.07
MS (df = 8)	22.14**	2.36*	9.60**

Ck = Control treatment, TE = trinexapac-ethyl, LSD = Least significance difference, CV = Coefficient of variance, MS = Mean square, df = degree of freedom, FW = Fresh weight, values represent mean $\pm$ SE (n = 3). Different small letters indicated that the means are significantly different ( $P \leq 0.05$ ).



## Discussion

Plant growth regulators have the ability to alter the plant physiological activities. Trinexapac-ethyl (TE) is typically a growth inhibitor which counteracts the conversion of inactive gibberellic acid to its metabolically active form (Xu and Huang 2011; Matysiak 2006). The TE applied under salt stressed conditions in wheat stimulated the growth parameters. The physiological modulation reduces the plant shoot and root length under normal conditions, as depicted by the results of current study. The response of TE application under salt stress however modified the plant behavior, which resulted in improved growth attributes (Sakr 2009). The current investigation also led to improved chlorophyll contents and its components as well as water relations of TE treated salt stressed wheat seedlings. The improved growth parameters can be attributed to improved water relations and photosynthetic activity of the salt stressed plants, supplemented with TE (Jiang and Fry 1998). The TE application did not increase the shoot length considerably but maintained the total chlorophyll contents and its components. The dark green appearance of these TE treated wheat seedlings was due to increased chlorophyll contents and mesophyll cell density, as supported by Ervin and Koski (2001); Heckman et al. (2005) and McCullough et al. (2006). Slow degradation of chlorophyll contents under salt stress enhances the photosynthetic efficiency of the plants thus increase their stress tolerance (Arghavani et al. 2012).

Salt stress negatively impacted the water relations of wheat seedlings. The TE application under salt stress improved these parameters, by maintaining higher relative water contents and turgor potential. Maintenance of high RWC is an indication of improved survival rate of plant under salt stressed conditions. Decreased water potential and solute potential as a result of TE application under stress can be a reason of improved relative cellular hydration level (Etemadi et al. 2015). Reduced solute potential of TE applied salt stressed wheat seedlings can be attributed to slow growth rate of plants, which is accomplished by less utilization of assimilates (Munns 1988; Fan et al. 2009). It can also be related to stress tolerance induction by TE application through slow growth rate and improved osmotic adjustment (Stier and Rogers 2001). Osmotic adjustment is a physiological phenomenon associated with accumulation of solutes and inorganic ions which leads to improved survival rate of plants under stress conditions (Elansarya and Salem 2015; Koch et al. 2017). Proline contents, soluble protein and soluble sugars can be regarded as osmolites used for osmotic adjustment. Such solute accumulation reduced the solute potential of the plants. The stressed conditions increased the proline and soluble sugar contents, which was more improved by TE application. The increased soluble sugar contents of TE treated plants enables the more availability of free sugars for osmotic adjustment (Bian et al. 2009).

Production of ROS is a prominent phenomenon of abiotic stresses including salt stress. The ROS have the ability to disrupt cellular membranes, damage DNA and modify the cell metabolism (Foyer et al. 1994). Plants have inherent ability to scavenge ROS through antioxidants including enzymes and organic molecules. The enzymes convert the highly active oxygen species into harmless water and oxygen (Fu and Huang 2001). The studied

enzymatic activity of SOD, POD, CAT, APX and GR increased following TE application under salt stress. The phenomenon of increased antioxidant activity due to TE application is not fully understood but it can be linked to lower MDA contents (Chen et al. 2009). Less MDA contents refer to less oxidative damage to cellular membranes. The TE may serve to improve the cellular thermo-stability (Heckman et al. 2002) which led to less susceptibility of oxidative stress damage. The prominent effects of TE under salt stress are depicted by growth promotion, enhanced chlorophyll contents, improved water relations and antioxidant enzyme activity coupled with accumulation of osmolites furnishes its use to induce salt stress (Baldwin et al. 2006).

### Conclusion

From the results it is concluded that TE application decrease damages to wheat seedlings under salt stress probably via improving growth attributes, water relations, chlorophyll contents, antioxidant activity and osmo-protectants content. As salt stress increased, enzyme activity decreased, but ROS injury to the cells increased. Wheat seedlings were found to have a higher activities of SOD, CAT, POD and APX activities, more water relations attributes, chlorophyll contents, and osmo-protectants content during the salt-stress period when treated with TE. Thus, our findings suggest that a seedling treated with TE is an effective strategy that can be used to enhance salt tolerance of wheat crop.

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