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Is pre-sowing seed treatment with triacontanol effective in improving some physiological and biochemical attributes of wheat (*Triticum aestivum* L.) under salt stress?

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Summary

In this study, seed-priming of two wheat cultivars S-24 and MH-97 was carried out with three triacontanol (TRIA) levels (0, 10 and 20 μ*M*) for 12 h. Seeds pre-treated with TRIA were allowed to grow for 24 days in full strength Hoagland's nutrient solution in a greenhouse, thereafter two salt treatments (0 and 150 m*M* NaCl) were applied and after 21 days of salt application, changes in growth attributes such as shoot and root dry weights, shoot and root lengths and total leaf area, leaf water relations such as water potential (Ψw), osmotic potential (Ψs), turgor potential (Ψp) and relative water contents (%), membrane permeability (%), total free amino acids, free proline, glycinebetaine and total phenolic contents were determined. Yield attributes such as 100-seed weight, total grain yield, number of grains and number of fertile tillers per plant were recorded at crop maturity. Salinity stress of 150 *mM* NaCl significantly caused reduction in all growth and yield attributes, leaf water relations except leaf turgor potential, while increased membrane permeability (%), leaf free proline, glycinebetaine and total free amino acids in both cultivars. Total phenolics and relative water contents (%) remained unaffected under salt stress. Pre-sowing seed treatment with TRIA did not mitigated the adverse effects of salt stress on wheat and thus could not prove to be effective to promote significantly its growth, yield and other physiological and biochemical attributes (except leaf water potential of cultivar MH-97) under stress and non-stress conditions. Overall, performance of cultivar S-24 was better in growth, leaf water relations and free proline contents as compared to MH-97 under both non-saline and saline conditions.

Introduction

Plant hormones are involved in rapid induction of plant responses to external environmental cues (Pedranzani et al., 2003). Abiotic stresses alter the levels of phytohormones that result in reduced plant growth (Morgan, 1990). However, exogenous application of plant hormones is an attractive approach that has been reported to increase salt tolerance in crop plants by regulating various growth processes (Barakat, 2011; Zeid, 2011; Hussain et al., 2011). Shekoofeh and Shahla (2012) found that treatment with plant hormones enhanced salt stress tolerance in crops by reducing the level of H_2O_2 (oxidative stress), decreasing membrane damages and increasing the accumulation of organic solutes for growth processes. Triacontanol (TRIA) is also known as a plant hormone since 1977 as it efficiently involved in promoting growth and yield of several crop species like rice, wheat, maize, tomato, greengram, hyacinth bean, coffee senna and wild mint (Mamat et al., 1983; Ries et al., 1977; Ries, 1985, 1991; Kumaravelu et al., 2000; Khan et al., 2009; Naeem et al., 2009, 2010, 2011). The stimulatory effects of TRIA on water and nutrients uptake, photosynthesis, enzyme activities, membranes stability and gene regulation have made this plant growth regulator interesting for many researchers working for improving crop productivity under abiotic stress conditions including salt stress (Muthuchelian et al., 1996; Chen et al., 2002, 2003; Kilic et al., 2010, 2011; Perveen et al., 2010, 2011). As an effective growth regulator, TRIA has been used for enhancing shoot and root growth and producing secondary metabolites in different plant species (Reddy et al., 2002; Giridhar et al., 2005; Malabadi et al., 2005; Malabadi and Nataraja, 2007; Parimalan et al., 2009). It inhibits enzymatic and non-enzymatic peroxidative breakdown of membrane lipids (Ramanarayan et al., 2000). For example, seed treatment with TRIA has been reported to increase the activity of a key antioxidant enzyme peroxidase that plays a role in reducing the level of H_2O_2 (a reactive oxygen species) (Perveen et al. 2011).

Soil salinity is one of the major abiotic stresses that contribute to crop yield losses worldwide (Grewal, 2010). Salt stress exerts negative effects on growth, yield and water status of wheat (Akbari Ghogdi et al., 2012). Salt-induced damage to plasma membrane results in increased cell permeability that causes electrolyte leakage (Blum and Ebercon, 1980). However, plants escape osmotic stress by sequestering toxic Na^+ and Cl^- ions into vacuoles and accumulation of some non-toxic compatible solutes such as free amino acids, proline and glycinebetaine in cytoplasm to balance decreased water potential through osmotic adjustment (Di Martino et al., 2003; Heidari, 2012; Geng et al., 2012).

Among various strategies to cope the adverse effects of salt stress on agricultural crops, several shot-gun approaches are in vogue these days. Seed-priming (seed-soaking in the solution of some organic/inorganic solutes, plant growth regulators or plant hormones etc.) is one of these approaches that have gained much more importance due to being simple, easy to apply, and with low risk and low cost. Keeping in view the role of TRIA, the objectives of the present study were to assess whether or not pre-sowing seed treatment with TRIA could be effective in reducing the adverse effects of salt stress on growth and yield of wheat crop and whether TRIA could modulate various physiological and biochemical attributes like free amino acids, proline, glycinebetaine, total phenolics, membrane permeability (%) and leaf water relations under saline conditions.

Materials and methods

An experiment was conducted in the wire-house of the Botanical Garden (altitude 213 m, latitude 31°30'N and longitude 73°10'E), to examine the plausible role of TRIA applied as a pre-sowing seed treatment on wheat under salt stress during spring, 2010. The climatic conditions were: mean day and night temperature cycle of 20 and 6°C, 10 and 14 h light and dark period (photoperiod with PPFD 825-1150 μmol m^{-2} s^{-1}), and RH 54 ± 5%. Seed of two spring wheat cultivars namely S-24 (salt tolerant) and MH-97 (moderately salt sensitive), was obtained from the Botany Department, University of Agriculture, Faisalabad, Pakistan and Ayub Agricultural Research Institute, Faisalabad, Pakistan, respectively. Before the start of the experiment, surface sterilization of the seed of both cultivars was done using sodium hypochlorite solution (5%) for 5 min, rinsed with sterilized water and air-dried. Then seeds (one hundred of

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each cultivar) were soaked in varying concentrations of triacontanol solutions (0, 10, and 20 μ*M*) prepared in hot distilled water at 90°C along with 0.1% tween-20 (cited in CHEN et al., 2002). After 12 h of soaking, the seeds were re-dried to original weight with forced air under shade. Ten seeds per pot were allowed to germinate in thoroughly washed sand. After 10 days of seed germination, six plants were maintained by thinning in each pot/replicate. Twenty four day-old plants were treated with saline stress for further 21 days. There were two salt (NaCl) levels, i.e., 0 m*M* (control) and 150 m*M*. Every week Hoagland's nutrient solution (full strength) was applied @ two litters per pot. For attaining the desired level of salt, NaCl in Hoagland's nutrient solution was added in an aliquot of 50 m*M* solution to each pot every day. Every week salt level (150 m*M* NaCl) was applied in Hoagland's nutrient medium until the end of the experiment. The sand moisture content was maintained every day by watering 200 ml H_2O to each pot. There were four replicates of each treatment, and all experimental units arranged in a completely randomized design. From each pot two plants were harvested when plants were 45 days old. After taking fresh weights, plants were oven-dried at 65ºC for one week and their dry biomass recorded (shoot and root dry weights). In addition, data for other growth attributes, e.g., shoot and root length and leaf area per plant, was also recorded. Before harvesting, data for various physiological/ biochemical attributes were recorded.

Total free amino acids

Total free amino acids were determined according to MOORE and STEIN (1957). Fresh leaves (0.5 g) were ground in 10 ml of citrate buffer (pH 5.0) and the mixture was centrifuged at 15000 x *g* for 10 min. The extraction samples were further processed with nin-hydrin solution and the optical density of the solution read at 570 nm using a spectrophotometer (IRMECO U2020).

Total phenolics

The amount of total phenolics was determined following the method of JULKENEN-TITTO (1985). Fresh leaves (0.1 g) were ground properly in 2 ml of 80% acetone, centrifuged at 10,000 x *g*, for 15 min and the supernatant collected in a microfuge tube. To 100 μl of the supernatant 2.0 ml of distilled water, 0.5 ml of Folin-Ciocalteau's phenol reagent, and 2.5 ml of 20% Na_2CO_3 solution were added and raised the final volume to 5 ml by adding distilled H_2O. After 20 min of vortexing, the absorbance was read at 750 nm using a spectrophotometer (IRMECO U2020).

Leaf free proline

Estimation of free proline in the leaf tissues was performed following BATES et al. (1973). Toluene was used as a blank and absorbance read at 520 nm on a spectrophotometer. Using a standard curve the concentration of free proline was determined and calculated on the basis of fresh weight as follows:

$$\mu\text{mole proline g}^{-1}\text{ fresh weight} = \frac{\mu\text{g proline ml}^{-1} \times \text{ml of toluene}/115.5}{\text{g of sample}}$$

Glycinebetaine content

Glycinebetaine was determined according to the method of GRIEVE and GRATTAN (1983). Fresh leaf (0.5 g) was homogenized with 10 ml distilled water. Whatman No. 2 filter paper was used for homogenate filtration. The extract (1 ml) was mixed with 1 ml of 2 N H_2SO_4. To 0.5 ml of the above mixture, potassium tri-iodide solution (0.2 ml) was added and then the mixture was cooled in an ice bath for 90 min. To the sample mixture, 2.8 ml of chilled distilled water and 6 ml of 1-2-dichloromethane were added. From two distinct layers, the absorbance of the lower organic layer was read at 365 nm using a UV-visible spectrophotometer (IRMECO U2020).

Relative Membrane Permeability (%)

Fresh leaves (0.5 g) were chopped and placed in 10 ml of distilled water. Then samples were vortexed for 5s and the electrical conductivity (EC_o) determined. The test tubes were kept at 4°C for overnight and the EC_1 measured. Then the samples were autoclaved for 1 h and EC_2 measured after cooling the solution to room temperature. Relative membrane permeability was measured using the following formula:

$$\text{RMP (\%)} = (EC1\text{-}EC_o/EC2\text{-}EC_o) \times 100$$

Leaf water potential: Leaf water potential of the second leaf from top was determined with a Scholander type pressure chamber (Arimad-2-Japan) following the method of SCHOLANDER et al. (1964).

Leaf osmotic potential: Osmotic potential of the leaf used for water potential determination was determined using an osmometer (VAPRO, vapor pressure osmometer, Model 5520, USA).

Turgor potential: Leaf turgor potential was determined as the difference between water potential and osmotic potential values (NOBEL, 1991).

Relative water content (RWC): Relative water content was calculated following the method of JONES and TURNER (1978). Fresh leaf samples were weighed (Fw), soaked in deionized water in the dark for 24 h to re-hydrate and their turgid weight (Tw) recorded. After that, the samples were oven-dried at 80°C for 48 h to get their dry weight (Dw). Relative water content was calculated using the following formula:

$$\text{RWC} = [(\text{Fw} - \text{Tw})/(\text{Fw} - \text{Dw})] \times 100$$

Yield attributes: Yield parameters i.e., grain yield and number of grains per plant, 100-grain weight, and number of fertile tillers, were determined at maturity.

Statistical data analysis: The data for each variable was analyzed by a COSTAT computer package (Cohort Software, Berkeley, CA). The least significance difference test was used to compare mean values (SNEDECOR and COCHRAN, 1980).

Results

Salt stress (150 m*M* NaCl) significantly reduced all the growth and yield attributes such as shoot and root dry weight, shoot and root length, total leaf area, grain yield and number of grains per plant, 100-grain weight and number of fertile tillers per plant of the two wheat cultivars, S-24 and MH-97 (Tab. 1; Fig. 1). Cultivar S-24 was higher in shoot dry weight, and shoot and root lengths as compared to MH-97, while MH-97 was better in root dry weight. In yield attributes both cultivars did not show any significant difference except that number of tillers was more in cultivar MH-97 than S-24 (Tab. 1; Fig. 1). Pre-sowing TRIA treatment increased shoot dry weight and total leaf area in cultivar S-24 under non-saline conditions, while in MH-97 under both saline and non-saline conditions and increase

Tab. 1: Growth and yield attributes, contents of total free amino acids, proline, glycinebetain, total phenolics, membrane permeability (%) and leaf water relations of salt-stressed and non-stressed wheat (*Triticum aestivum* L.) plants raised from seed treated with triacontanol for 12 h.

Source of variation	df	Shoot dry wt.	Root dry wt.	Total leaf area	Shoot length	Root length	Grain yield per plant
Cultivars (Cvs)	1	0.183*	0.004**	110.78ns	608.27***	29.34***	5.294ns
Salinity (S)	1	9.001***	0.107***	224486***	1241.38***	108.51***	22.96**
Triacontanol (TRIA)	2	0.033ns	0.001ns	653.28 ns	0.2986ns	1.590ns	0.099ns
Cvs x S	1	0.014ns	0.002*	216.46ns	21.78**	9.507**	0.505ns
Cvs x TRIA	2	0.078ns	0.003**	1773.79ns	13.69**	5.090**	1.291ns
S x TRIA	2	0.089ns	0.0005ns	3133.43*	2.146ns	7.132***	0.836ns
Cvs x S x TRIA	2	0.030ns	0.004**	1040.74ns	6.424ns	4.882**	1.032
Error	24	0.027	0.0004	893.97	2.014	2.014	2.077

Source of variation	df	100-Seed wt.	Number of grains per plant	Number of fertile tillers per plant	Amino acids	Proline	GB
Cultivars (Cvs)	1	0.0003ns	3079ns	7.260**	0.288ns	0.015*	0.0002ns
Salinity (S)	1	3.8025***	5470.8*	5.575**	36.98*	0.070***	0.936***
Triacontanol (TRIA)	2	0.1733ns	27.16ns	0.385ns	2.459ns	0.007ns	0.051ns
Cvs x S	1	0.2336ns	0.012ns	0.340ns	1.708ns	0.008ns	0.006ns
Cvs x TRIA	2	0.0477ns	617.90ns	0.802ns	4.292ns	0.003ns	0.010ns
S x TRIA	2	0.0133ns	716.85ns	2.478*	3.175ns	0.0004ns	0.008ns
Cvs x S x TRIA	2	0.4811ns	208.78ns	0.271ns	3.401ns	0.00006ns	0.018ns
Error	24	0.1625	1144.97	0.561	8.629	0.003	0.023

Source of variation	df	Total phenolics	MP (%)	Water potential	Osmotic potential	Turgor potential	RWC (%)
Cultivars (Cvs)	1	0.622ns	68.555*	0.481***	0.086**	0.160**	72.74*
Salinity (S)	1	5.530ns	257.498***	0.368***	0.256***	0.010ns	38.73ns
Triacontanol (TRIA)	2	0.359ns	12.037ns	0.040*	0.009ns	0.036ns	39.08ns
Cvs x S	1	3.644ns	4.674ns	0.022ns	7.640ns	0.030ns	2.451ns
Cvs x TRIA	2	2.729ns	1.379ns	0.006ns	0.036*	0.055ns	2.387ns
S x TRIA	2	0.492ns	5.393ns	0.003ns	0.010ns	0.021ns	8.812ns
Cvs x S x TRIA	2	4.513ns	21.528ns	0.007ns	0.001ns	0.008ns	2.220ns
Error	24	1.441	11.5576	0.008	0.009	0.017	16.13

*, **, and *** = significant at 0.05, 0.01, and 0.001levels, respectively; ns = non-significant; df = degrees of freedom; GB = glycinebetaine; MP = membrane permeability; RWC = relative water content

in shoot growth was more at 20 μ*M* of TRIA under non-saline conditions (Tab. 1; Fig. 1). Root dry weight increased in cultivar MH-97 under saline conditions, while in S-24 under non-saline conditions by application of TRIA as seed treatment (Tab. 1; Fig. 1). Root length was significantly influenced by TRIA application and 10 μ*M* TRIA was proved to be more effective in enhancing root length under non-saline conditions, while under saline conditions the pre-sowing treatment with TRIA imposed significant effects on root length in S-24 only. Pre-sowing seed treatment with TRIA only increased number of fertile tillers in cultivar MH-97 under non-saline conditions, while its effect was non-significant on all other yield attributes (Tab. 1; Fig. 1).

Total free amino acids slightly increased under saline conditions in both wheat cultivars i.e. S-24 and MH-97 (Tab. 1; Fig. 1). The pre-sowing TRIA application did not significantly alter total free amino acids contents in both wheat cultivars (Tab. 1; Fig. 1). Root medium salinity markedly increased proline and glycinebetaine contents in both wheat cultivars (Tab. 1; Fig. 2). Cultivar S-24 accumulated higher amount of proline as compared to that of MH-97 especially under saline conditions, while in glycinebetaine the cultivars did not show a significant difference (Tab. 1; Fig. 2). The pre-sowing TRIA application did not significantly alter the proline and glycinebetaine contents in both wheat cultivars (Tab. 1; Fig. 2).

Sodium chloride induced salinity stress did not affect total phenolics in both wheat cultivars (Tab. 1; Fig. 2). The cultivars did not show any difference in total phenolics under both stress and non-stress conditions. The pre-sowing treatment of TRIA was also found to be non-effective in altering total phenolics in both wheat cultivars (Tab. 1; Fig. 2).

Membrane permeability (%) increased significantly in both S-24 and MH-97 wheat cultivars under saline conditions. Cultivar MH-97 was higher in membrane permeability than cv. S-24 under either saline or non-saline conditions (Tab. 1; Fig. 2). The pre-sowing application of TRIA decreased membrane permeability (%) under non-saline conditions in both wheat cultivars (Tab. 1; Fig. 2).

Leaf water relation attributes like Ψw and Ψs significantly decreased in both cultivars under saline conditions (Tab. 1; Fig. 2). Cultivar S-24 was higher in Ψw and Ψs than MH-97 under both prevailing conditions. The pre-sowing treatment of TRIA at 10 μM level significantly increased leaf Ψw, while decreased leaf Ψs of salt-

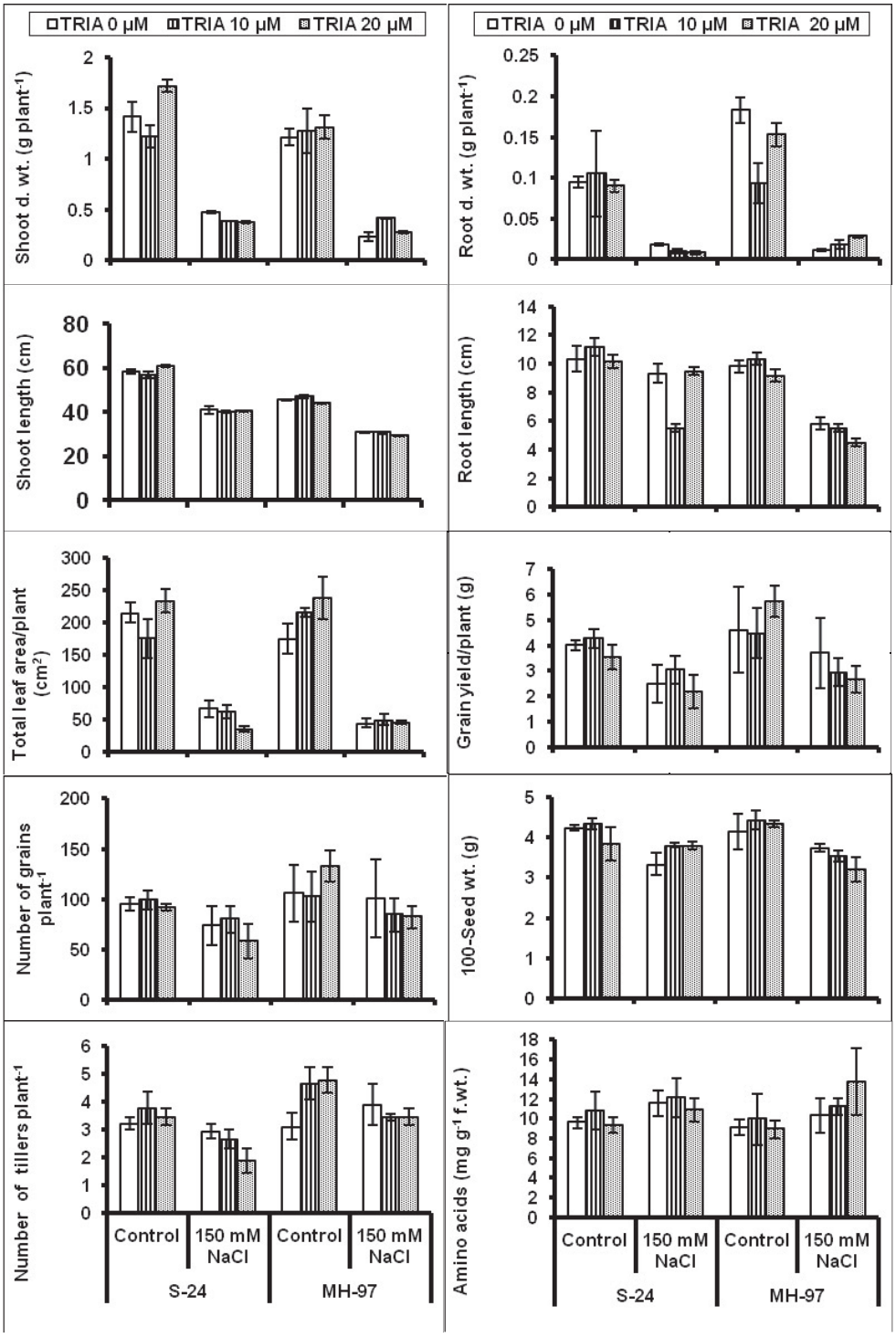

Fig. 1: Growth and yield attributes and total free amino acids of salt-stressed and non-stressed wheat (*Triticum aestivum* L.) when 24-day old plants subjected for 21 days to saline or non-saline conditions (seed priming for 12 h).

sensitive wheat cultivar MH-97 (Tab. 1; Fig. 2). Salinity stress did not alter the leaf turgor potential significantly; however, the two cultivars differed significantly in leaf turgor potential as cultivar S-24 was higher in this attribute than MH-97 under both normal and salt stress conditions. The pre-sowing application of TRIA did not significantly affect the leaf turgor potential; however, in MH-97 the leaf turgor potential value was higher at the level of 10 µ*M* under non-saline or saline conditions (Tab. 1; Fig. 2). The relative water content (%) remained unchanged in both wheat cultivars both under salinity stress and TRIA treatment. However, water contents were higher in S-24 than those in MH-97 both under prevailing conditions (Tab. 1; Fig. 2).

Statistical data analysis: The data for each variable was analyzed by a COSTAT computer package (Cohort Software, Berkeley, CA). The least significance difference test was used to compare mean values (SNEDECOR and COCHRAN, 1980).

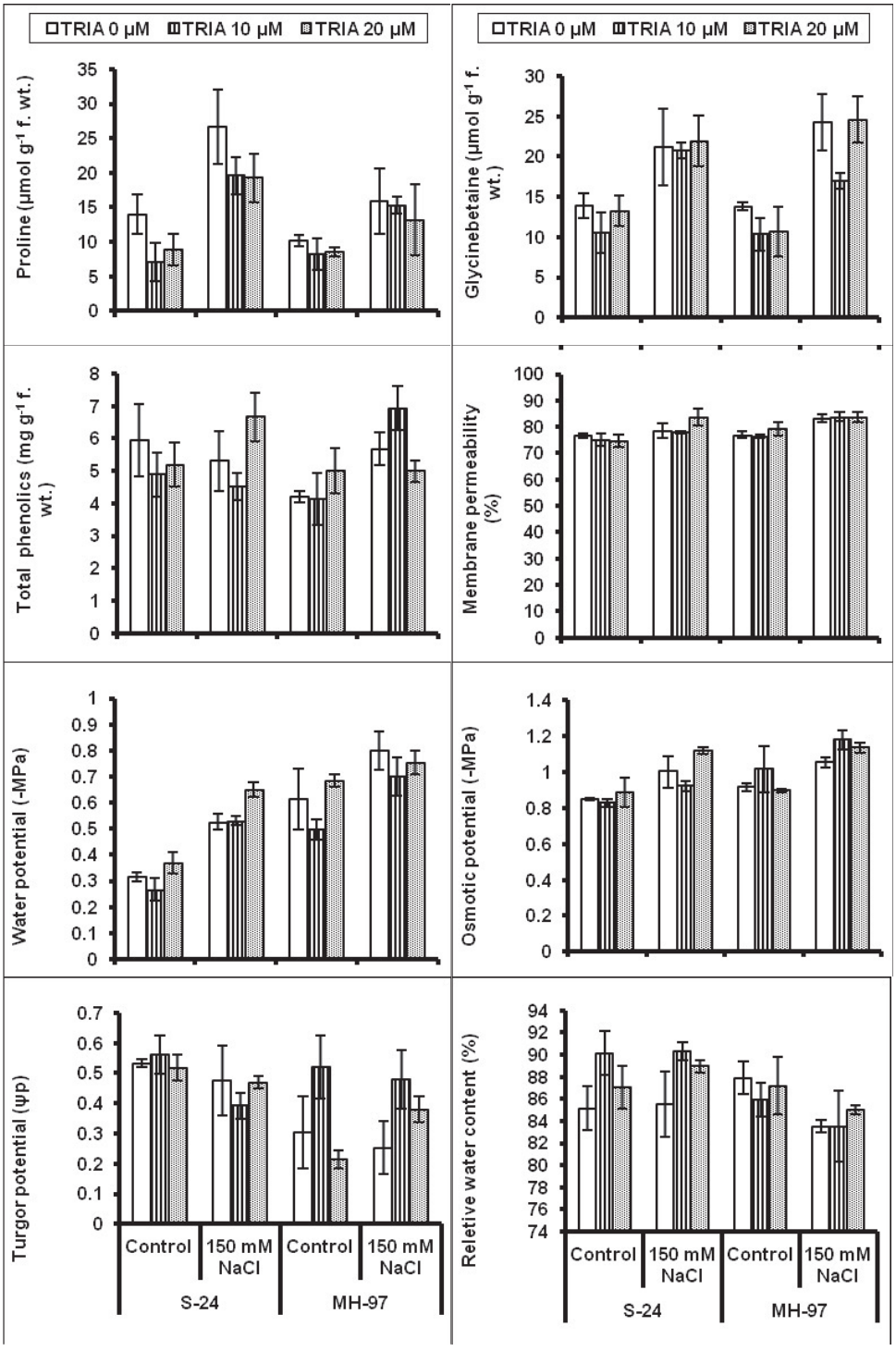

Fig. 2: Contents of free proline, glycinebetaine, total phenolics, membrane permeability (%) and leaf water relations of wheat (*Triticum aestivum* L.) when 24-day old plants subjected for 21 days to saline or non-saline conditions (seed priming for 12 h).

Discussion

Due to their role in regulating various physiological and biochemical processes, new plant growth regulators are being extensively used these days in improving abiotic stress tolerance in crop plants (PELEG and BLUMWALD, 2011). Plant growth regulators are also being used as seed priming agents to reduce the adverse effects of salt stress in different studies (IQBAL and ASHRAF, 2007). Triacontanol is a growth promoter that naturally occurs in plant epicuticular waxes (RIES et al., 1977). It is reported that exogenous application of TRIA stimulates the induction of a secondary messenger (9-β-L (+)-adenosine) which moves rapidly throughout the plant body and regulates various physiological and biochemical processes and thereby increasing plant growth and yield (RIES and HOUTZ, 1983; RIES, 1985, 1991; RIES et al., 1990, 1993).

In our study, salinity stress (150 m*M* NaCl) exerted a negative

effect on all growth and yield attributes like shoot and root dry weight, total leaf area, shoot and root lengths, grain yield and number of grains per plant, 100-grain weight, and fertile tillers of both wheat cultivars, S-24 and MH-97. This is analogous to what has been observed in a number of earlier studies (Shahbaz et al., 2008, 2011; Akram et al., 2011; Chaabane et al., 2011). In our findings, although improvement in some growth attributes (root dry weight under saline and total leaf area under non-saline conditions in MH-97, while shoot and root length and number of fertile tillers under both stress and non-stress conditions in both wheat cultivars) was observed due to TRIA application. However, overall effect of pre-sowing seed treatment with TRIA proved non-significant on all growth and yield attributes. These results are in agreement with some other studies on various crop species. For example, Bittenbender et al. (1978) found a non-significant effect of TRIA on photosynthesis and final yield of rice seedling under dark. In another report by Bole and Dubetz (1978) TRIA did not improved yield and yield components of water stressed wheat plants when applied as a soil supplement. Charlton et al. (1980) reported that Leeds durum wheat seeds treated with TRIA and its derivatives did not promote germination and growth. In tissue culture studies, germination and seedling growth of various weed and horticultural crops (lettuce, oat, soybean and wheat etc.) remained unaffected by TRIA application (Marcelle and Chrominski, 1978; Hoagland, 1980; Eriksen et al., 1982). Non-significant effect of TRIA as pre-sowing seed treatment on fresh biomass of wheat plants has also been reported earlier (Perveen et al., 2010).

In our experiment, total phenolic contents remained unaffected, while total free amino acids increased significantly in both wheat cultivars under saline conditions. Similarly, Tammam et al. (2008) reported an increase in free amino acid content in wheat under saline conditions. Salama et al. (1994) reported that under salinity stress, crop plants accumulate free amino acids which help maintain the osmotic balance by decreasing the cellular osmotic potential in plants under saline conditions. In this study, pre-sowing application of TRIA did not alter the total free amino acids or total phenolics significantly in both wheat cultivars under control or saline conditions. Contrarily, application of TRIA significantly enhanced the accumulation of free amino acids and phenols in green gram under normal growth conditions (Kumaravelu et al., 2000).

Proline plays a significant role as an endogenous osmotic regulator in wheat, and its accumulation in plant tissues indicates plant ability to tolerate salt stress (Munns et al., 2006). In our experiment, salt stress increased total free proline and glycinebetaine in both wheat cultivars. S-24 accumulated higher proline compared to that in MH-97. As a negative correlation was found between leaf free proline or glycinbetaine contents, and leaf water potential (Ψw), which became more negative due to increased osmotic potential, so it could be suggested that these two osmotica play a central role in osmotic adjustment under salt stress (Shao et al., 2006; Moghaieb et al., 2004). Pre-sowing TRIA application proved ineffective in changing the contents of proline and glycinebetaine under normal or saline conditions. Borowski and Blamowski (2009) reported the ameliorating effect of TRIA on reducing the chilling stress in *Ocimum basilicum* L. plants by decreasing free proline content, while increasing leaf area and activity of catalase. Contrarily, Krishnan and Kumari (2008) reported that proline content decreased in TRIA-treated soybean plants under salt stress.

There are several reports which show that TRIA reduces the level of membrane damages due to its action as an antioxidant compound to inhibit peroxidation of membrane lipids (Ramanarayan et al., 2000; Grzegorezyk et al., 2006; Khan et al., 2009). In the present study, application of TRIA as seed treatment decreased membrane permeability (%) of the two wheat cultivars only under normal conditions.

Salinity has been known to disturb the water relations of plants as a consequence of decreased leaf osmotic potential in external soil solution (Munns, 2005). In our study, leaf Ψw and Ψs decreased in the two wheat cultivars under saline regime. However, leaf turgor potential and relative water contents (RWC) remained unaffected under salinity stress. Early responses to salinity included decrease in water potential and RWC (Gucci et al., 1997; Alarcon et al., 1993). Pre-sowing TRIA application (10 μM) increased the leaf water potential, while decreased osmotic potential of cv. MH-97 both under control and salt stress. These findings are in agreement with those of Krishnan and Kumari (2008) who reported that TRIA could improve leaf water relations by decreasing leaf osmotic potential in soybean under salinity stress. Non-significant effect of TRIA on most of growth and yield attributes, free amino acids, free proline, glycinebetaine, total phenolics, leaf turgor potential and relative water contents (%) under stress or non-stress conditions could be attributed due to its lack of effective penetration through seed coat as earlier reported by Hoagland (1980) or due to the formation of some inhibitory compounds by trace amounts of other aliphatic alcohols, esters or chemicals (Laughlin et al., 1983; Jones et al., 1979; Ries et al., 1983, 1984).

From the present study, it could be concluded that salinity stress significantly decreased shoot and root dry weight, total leaf area, shoot and root lengths, leaf water relations except turgor pressure, and yield attributes, i.e., grain yield per plant, 100-seed weight, number of grains and number of fertile tillers per plant, while increased membrane permeability (%), contents of total free amino acids, leaf proline and glycinebetaine in both wheat cultivars. Furthermore, treatment of wheat seeds with TRIA did not reduce the harmful effects of salinity stress on wheat plants and exerted no stimulatory or inhibitory effects on growth, yield and physiological and biochemical attributes in this study.

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