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CHANGES IN MINERAL COMPOSITION, UPTAKE AND USE EFFICIENCY OF SALT STRESSED WHEAT (*TRITICUM AESTIVUM* L.) PLANTS RAISED FROM SEED TREATED WITH TRIACONTANOL

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Abstract

The role of pre-sowing treatment with triacontanol (TRIA) on two cultivars [MH-97 (moderately salt sensitive) and S-24 (salt tolerant)] of wheat (*Triticum aestivum* L.) was assessed under saline and non-saline conditions. Twenty four day old plants raised from seed pretreated with TRIA for 24 h were subjected to 0 (control) or 150 m*M* NaCl. After 21 days of salt application, changes in shoot and root mineral nutrient concentrations, nutrient uptake and use efficiency, and shoot and root K^+/Na^+ ratios were measured. Salt stress (150 m*M* NaCl) significantly increased the shoot and root Na^+ and Cl^- contents, while decreased that of K^+, and K^+/Na^+ ratios in shoot and root of both cultivars under saline conditions. The uptake of shoot Na^+ increased significantly, while that of the remaining nutrients decreased under saline conditions. Shoot and root nutrient (Na^+, K^+, Ca^{2+}, Cl^-) use efficiency (NUE) decreased significantly in both cultivars under saline conditions. The exogenous application of TRIA as pre-sowing seed treatment significantly increased the shoot Na^+ and K^+ contents, shoot K^+ uptake and shoot Cl^- use efficiency, while its effect was non-significant on the remaining attributes in both cultivars under non-saline or saline conditions. Overall, the performance of S-24 was better than MH-97 in shoot and root K^+ and Ca^{2+} contents and the uptake of these nutrients under both saline and non-saline conditions.

Introduction

Triacontanol (TRIA) is an effective plant growth regulator, which is believed to enhance growth and yield of several agricultural and horticultural crops when applied exogenously (Ries *et al*., 1977; Ries, 1985; 1991; Gatica *et al*., 2008; Naeem *et al*., 2009). Naturally, it occurs in small amounts in cuticular waxes of plant species (Kolattukudy & Walton, 1972). In wheat, it is 3% of the total free alcohols that are found in the leaf waxes (Tulloch & Hoffman, 1974). It stimulates nitrogen-fixation, enzyme activities, photosynthesis, nutrient uptake, mineral nutrition, gene regulation, membrane stability and productivity of many crops (Ries, 1985; Chen *et al.,* 2002; 2003; Malik & Williams, 2005; Ramanarayan *et al*., 2000; Naeem *et al.,* 2009). There are several reports which show that exogenous application of TRIA increases the growth and yield of economically important crop species such as rice *(Oryza sativa* L.), wheat *(Triticurn aesitivum* L.), tomato *(Lycopersicon esculentum* Mill.), green gram [*Vigna radiata* (L.) Wilczek], maize *(Zea mays* L.), and hyacinth bean (*Lablab purpureus* L.) (Ries, 1985; Kumaravelu *et al*., 2000; Khan *et al*., 2006; Naeem *et al*., 2009). In view of a number of reports TRIA alters mineral nutrient composition in plants (Ries & Houtz, 1983; Kumaravelu *et al*., 2000; Chaudhary *et al*., 2006; Khan *et al*., 2006; 2007; Naeem *et al*., 2009).

Salinity is one of the major constraints to crop productivity in arid and semi-arid regions of the world (Ashraf, 1994; Ashraf & Harris, 2004; Al-Karaki, 2006) which causes changes in a variety of metabolic processes including alteration in mineral nutrient uptake (Neel *et al*., 2002; Munns & Tester, 2008; Tavakkoli *et al.,* 2010; Ashraf *et al*., 2010; Yousif *et al.,* 2010). However, the uptake and transport of low Na^+ and maintenance of high K^+/Na^+ in the leaves or shoots is associated with salt tolerance in wheat and some other species (Hussain & Munns, 2005; Munns, 2007; Carpici *et al*., 2010; Pagter *et al*., 2009; Ashraf *et al.,* 2010).

Various approaches are in vogue to reduce the adverse effects of salinity on crop growth and productivity (Ashraf & Foolad, 2005; 2007; Ashraf, *et al*., 2008). Of various strategies, one is the pre-sowing seed treatment with plant growth regulators which could improve germination, growth, and yield in many crops grown under salt stress (Bradford, 1986; Aldesuquy, 2000; Kaur *et al*., 2002; Ashraf & Foolad, 2005; 2007; Iqbal *et al*., 2006; Iqbal & Ashraf, 2005; 2007; Ashraf *et al*., 2008). TRIA is one of the newly discovered plant hormones which shows growth promoting effects at very low concentrations (Ries *et al*., 1977). For example, TRIA caused significant positive changes in growth, biomass, pigments and solute accumulation in salt stressed *Erythrina variegata* seedlings when applied exogenously (Muthuchelian *et al*., 1996). TRIA also enhanced stress tolerance in common duckweed and stimulated the population growth when supplied in culture media (Kiliç *et al*., 2010). Along with some other growth regulators pre-sowing seed treatment with TRIA was reported to ameliorate salt (NaCl) effect in barley and radish seedlings (Cavusoglu *et al*., 2007; 2008). However, not a single report could be deciphered from the literature showing the effect of pre-sowing seed treatment with TRIA on ameliorating the adverse effects of salt stress on wheat crop.

Thus, the present study was conducted to evaluate the effects of exogenous application of TRIA as pre-sowing seed treatment on mineral ions (Na^+, K^+, Ca^{2+} and Cl^-) accumulation in wheat under saline or non-saline conditions and to investigate whether or not it could alter the uptake or nutrient use efficiency (NUE) in wheat plants under saline conditions.

Materials and Methods

An experiment was conducted in a wire-house of the Botanical Garden, University of Agriculture, Faisalabad, Pakistan, (altitude 213 m, latitude 31°30'N and longitude

73°10'E), to examine the plausible role of exogenous application of TRIA 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 800-1100 µmol m^{-2} s^{-1}, and RH 54 ± 5%. Seed of two spring wheat (*Triticum aestivum* L.) cultivars MH-97 (moderately salt sensitive) and S-24 (salt tolerant), was obtained from the Ayub Agricultural Research Institute, Faisalabad, Pakistan and Department of Botany, University of Agriculture, Faisalabad, Pakistan, respectively. Before the start of the experiment, the seed of both cultivars was surface sterilized with 5% Sodium hypochlorite solution for 5 min, rinsed with sterilized water and air-dried. One hundred seeds of each cultivar were soaked in varying concentrations of triacontanol solutions (0, 10, and 20 µ*M*) prepared in 0.1% tween-20. After 12 h of soaking, the seeds were re-dried to original weight with forced air under shade. Seeds (10 seeds per pot) were allowed to germinate in thoroughly washed sand. After 10 days of seed germination, plants were thinned to six in each pot/replicate. Twenty four day-old plants were subjected to saline stress for further 21 days. There were two salt (NaCl) treatments, i.e., 0 m*M* (control) and 150 m*M*. Two liters of Hoagland's nutrient solution (full strength) were applied weekly to each pot. For attaining the desired level of salt an aliquot of 50 m*M* solution was added to each pot every day. Salt level (150 m*M* NaCl) in full strength Hoagland's nutrient solution was applied after every week till the end of the experiment. The sand moisture content was maintained daily by adding 200 ml distilled H_2O to each pot. The experiment was arranged in a completely randomized design with four replicates. The plants were harvested after 21 days, oven-dried for one week and data for mineral nutrients were recorded.

Mineral ions (Na^+, K^+ and Ca^{2+}): Mineral ions in plant tissues were determined according to Allen *et al*. (1985). Finely ground dried plant shoot or root tissue (0.1 g) was taken in a digestion flask. To this digestion flask, shoot or root tissues were digested in 2 ml of digestion mixture. After completion of digestion process, when the material became colorless, final volume was made up to 50 ml by diluting the digested sample with distilled water and filtered. The filtrate was used for the determination of mineral cations (Na^+, K^+, and Ca^{2+}) using a flame photometer (Jenway, PFP-7, Dunmow, UK).

Determination of Cl^-: The dried ground shoot or root material (0.1 g) was digested in distilled water at 80 °C till the volume reduced to half. After cooling, distilled water was added to each test tube to maintain the volume up to 10 ml again. The Cl^- content was determined with a chloride analyzer (Model 926, Sherwood Scientific Ltd. Cambridge, UK).

Nutrient Uptake: Nutrient uptake was determined using the following formula:
Nutrient uptake (mg) = Nutrient concentration (mg/g) x shoot dry weight (g)

Nutrient Utilization Efficiency (NUE): Nutrient utilization efficiency was calculated using the following formula:

$$\text{NUE } (g^2\ mg^{-1}) = \frac{1}{\text{(Nutrient concentration) (mg/g) in shoot}} \times \text{ Shoot dry matter (g)}$$

Statistical analysis of data: The data for each variable were statistically analyzed using the COSTAT computer package (Cohort Software, Berkeley, CA). The mean values were compared using the least significance difference test according to Snedecor & Cochran (1980).

Results

Salinity stress (150 m*M* NaCl) significantly reduced the plant biomass of the two wheat cultivars namely S-24 and MH-97. However, exogenous application of TRIA as pre-sowing treatment did not ameliorate the salinity effect significantly (as reported earlier in Perveen *et al*., 2010).

Shoot and root Na^+ ions increased significantly under saline conditions in both wheat cultivars (Table 1; Fig. 1). The response of two cultivars was different to salt stress in shoot Na^+ ion accumulation, while root Na^+ accumulation remained unaffected. Cultivar S-24 accumulated more Na^+ in the shoot as compared to MH-97 (Table 1; Fig. 1). The exogenous TRIA application as pre-sowing seed treatment significantly increased the shoot Na^+ contents in both cultivars particularly at 10 µ*M* TRIA under saline conditions, but the wheat cultivars did not differ significantly in their response to TRIA application (Table 1; Fig. 1).

Shoot and root K^+ contents decreased significantly in both cultivars under saline conditions. The pre-sowing TRIA application significantly increased the shoot K^+ contents in both wheat cultivars under non-saline conditions; while under saline conditions only the plants of cv. MH-97 treated with 10 µ*M* TRIA accumulated higher amount of K^+ in shoots. The pre-sowing TRIA application did not affect the root K^+ in both wheat cultivars under non-saline or saline conditions (Table 1; Fig. 1).

The salinity stress did not alter shoot Ca^{2+} significantly in the two wheat cultivars. On the other hand, root Ca^{2+} slightly decreased in both cultivars under saline conditions (Table 1; Fig. 1).The cultivars did not differ significantly in shoot or root Ca^{2+} contents under saline or non-saline conditions (Table 1; Fig. 1). The TRIA application as pre-sowing seed treatment did not alter the shoot or root Ca^{2+} contents significantly in the two wheat cultivars under saline or non-saline conditions (Table 1; Fig. 1). However, under saline conditions root Ca^{2+} slightly increased in MH-97 by pre-sowing TRIA application (Table 1; Fig. 1).

Table 1. Shoot and root mineral composition, uptake and use efficiency of salt-stressed and non-stressed wheat (*Triticum aestivum* L.) plants raised from seed primed with triacontanol for 12 h.

Source of variation	df	Shoot Na^+	Root Na^+	Shoot K^+	Root K^+	Shoot Ca^{2+}	Root Ca^{2+}	Shoot Cl^-	Root Cl^-	Shoot K^+/Na^+ ratio
Cultivars (Cvs)	1	8.507**	0.562ns	103.4***	10.028*	0.0001ns	0.062ns	629.17ns	121.0ns	122.79ns
Salinity (S)	1	706.7***	232.6***	992.2***	1863.4***	0.694ns	3.674*	52022***	23307***	14188***
Triacontanol (TRIA)	2	3.938*	1.507ns	36.19***	4.75ns	0.771ns	0.340ns	185.14ns	71.256ns	123.52ns
Cvs x S	1	7.562**	0.840ns	124.69***	21.778**	0.444ns	0.840ns	269.51ns	3.757ns	57.54ns
Cvs x TRIA	2	2.174ns	0.562ns	0.465ns	0.361ns	0.021ns	0.271ns	215.26ns	2.896ns	88.016ns
S x TRIA	2	3.840*	1.938ns	86.646***	0.361ns	0.049ns	0.340ns	287.59ns	163.59*	114.9ns
Cvs x S x TRIA	2	1.938ns	0.299ns	1.174ns	2.528ns	0.132ns	0.299ns	817.01ns	17.312ns	85.64ns
Error	24	0.924	1.062	1.729	1.930	0.257	0.562	564.62	38.326	172.7
Source of variation	**df**	**Shoot Na^+ uptake**	**Root Na^+ uptake**	**Shoot K^+ uptake**	**Root K^+ uptake**	**Shoot Ca^{2+} uptake**	**Root Ca^{2+} uptake**	**Shoot Cl^- uptake**	**Root Cl^- uptake**	**Root K^+/Na^+ ratio**
Cultivars (Cvs)	1	6.991***	6.240**	86.212ns	0.414ns	4.142*	0.047ns	318.52ns	0.061ns	1.318ns
Salinity (S)	1	50.32***	25.34***	11300.0***	49.66***	133.94***	1.861***	1217.45*	16.76***	223.6***
Triacontanol (TRIA)	2	1.136ns	0.446ns	208.74**	0.191ns	0.938ns	0.025ns	102.54ns	0.016ns	0.236ns
Cvs x S	1	4.369**	0.387ns	115.80*	0.266ns	1.334ns	0.016ns	135.32ns	1.482ns	2.089*
Cvs x TRIA	2	0.202ns	3.681**	72.92ns	1.486**	1.274ns	0.043ns	297.40ns	1.559*	0.605ns
S x TRIA	2	1.790*	1.926*	359.7***	0.186ns	1.706ns	0.026ns	204.3ns	0.197ns	0.053ns
Cvs x S x TRIA	2	0.545ns	1.383ns	35.857ns	1.591**	0.785ns	0.059ns	140.18ns	1.488ns	0.793ns
Error	24	0.364	0.517	25.708	0.216	0.678	0.018	197.51	0.456	0.361
Source of variation	**df**	**Shoot Na^+ UE**	**Root Na^+ UE**	**Shoot K^+ UE**	**Root K^+ UE**	**Shoot Ca^{2+} UE**	**Root Ca^{2+} UE**	**Shoot Cl^- UE**	**Root Cl^- UE**	
Cultivars (Cvs)	1	0.001ns	0.0003*	0.0006***	0.00004***	0.0064ns	0.0004*	0.0003**	0.00008**	
Salinity (S)	1	29.493***	0.0104***	0.0058***	0.0001***	0.6203***	0.0067***	0.0110***	0.0004***	
Triacontanol (TRIA)	2	0.055ns	0.00002ns	0.00006ns	0.000003ns	0.00798ns	0.00001ns	0.0004***	0.00001ns	
Cvs x S	1	0.002ns	0.0002ns	0.0001ns	0.000004ns	0.0007ns	0.0003ns	0.00022**	0.00007**	
Cvs x TRIA	2	0.559ns	0.0003**	0.0001ns	0.00001**	0.0049ns	0.0003*	0.0003***	0.00002*	
S x TRIA	2	0.058ns	0.00002ns	0.00003ns	0.000003ns	0.0077ns	0.00001ns	0.0005***	0.00001ns	
Cvs x S x TRIA	2	0.534ns	0.0004**	0.00002ns	0.00002***	0.0007ns	0.0003**	0.0003***	0.00002*	
Error	24	0.452	0.00005	0.00003	0.000001	0.0029	0.00006	0.00003	0.000007	

*, **, and *** = Significant at 0.05, 0.01, and 0.001, respectively; ns = Non-significant; df = Degrees of freedom; UE= Use efficiency

The shoot and root Cl^- contents significantly increased under saline conditions in both wheat cultivars (Table 1; Fig. 1). The cultivars did not differ significantly in Cl^- contents under non saline or saline conditions. The pre-sowing TRIA application did not significantly change the shoot and root Cl^- contents in both cvs under non-saline or saline conditions. However, under saline conditions pre-sowing TRIA application decreased the shoot Cl^- in MH-97 and root Cl^- in both wheat cultivars (Table 1; Fig. 1).

Shoot and root K^+/Na^+ ratios decreased significantly under saline conditions in both wheat cultivars (Table 1; Fig. 1). The cultivars did not differ significantly in shoot K^+/Na^+ ratio, however, in root K^+/Na^+ ratio the cultivars differed significantly under saline conditions and the K^+/Na^+ ratio was higher in MH-97 as compared to that in S-24 (Table 1; Fig. 1). The TRIA application as pre-sowing seed treatment did not alter the shoot and root K^+/Na^+ ratios significantly in the two wheat cultivars under stressed or non-stressed conditions (Table 1; Fig. 1).

Under saline conditions the uptake of all nutrients except the shoot Na^+ significantly decreased in both wheat cultivars (Table 1; Fig. 2). The cultivars did not differ significantly except shoot and root Na^+ and shoot Ca^{2+} uptake under saline or non-saline conditions (Table 1; Fig. 2). For example, S-24 was higher in shoot and root Na^+ and shoot Ca^{2+} uptake than MH-97. The TRIA application as a pre-sowing seed treatment did not change the shoot and root nutrient uptake except that of shoot K^+ in the two wheat cultivars under non-saline conditions (Table 1; Fig. 2). The pre-sowing TRIA application at the level of 20 μM was more effective in increasing K^+ uptake in both cultivars under non-saline conditions, while under saline conditions 10 μM TRIA proved very effective in mitigating the effect of salt stress only in MH-97 (Table 1; Fig. 2).

Shoot and root nutrients (Na^+, K^+, Ca^{2+}, Cl^-) use efficiency (NUE) decreased significantly under saline conditions in both cultivars (Table 1; Fig. 3). The cultivars did not differ significantly in shoot Na^+ and Ca^{2+} use efficiency under non-saline or saline conditions (Table 1; Fig. 3). However, in shoot (K^+, Cl^-), and root (Na^+, K^+, Ca^{2+}, Cl^-) use efficiency the cultivars differed significantly (Table 1; Fig. 3). For example, shoot K^+ and Cl^- use efficiency was higher in S-24 as compared to that in MH-97 under stressed and non-stressed conditions. On the other hand, in root the nutrient (Na^+, K^+, Ca^{2+}, Cl^-) use efficiency (NUE) was higher in MH-97 as compared to that in S-24 (Table 1; Fig. 3). The exogenous application of TRIA as a pre-sowing treatment did not alter the nutrient (Na^+, K^+, Ca^{2+}, Cl^-) use efficiency (NUE) significantly except that of shoot Cl^- in both wheat cultivars under non-saline conditions (Table 1; Fig. 3).

Discussion

Pre-sowing seed treatment with some plant growth regulators (PGRs) has gained considerable importance to enhance the crop resistance against salt stress (Ashraf *et al*., 2008; Ashraf & Foolad, 2005; 2007; Iqbal & Ashraf, 2010). In wheat, different types of growth regulators have been tried as seed priming agents, e.g., brassinolide, auxins, gibberellic acid, kinetin, polyamines and cytokinins (Fariduddin *et al*., 2008; Shahbaz *et al*., 2008; Iqbal *et al*., 2006; Iqbal & Ashraf, 2005; 2007; 2010). Triacontanol (TRIA) is also a plant growth regulator that is being widely used to enhance plant growth of different crops (Haugstad *et al*., 1983; Ries, 1985; Misra & Srivastava, 1991; Malik & Williams, 2005; Naeem *et al*., 2009; Aftab *et al*., 2010). Growth enhancing effects of TRIA have been reported to be associated partly with increased nutrient uptake (Misra & Srivastava, 1991), and absorption and translocation of elements (Ramani & Kannan, 1980). It has also been reported in the literature that TRIA can improve growth under salt stress conditions when used as a seed priming agent, e.g., in barley and radish (Cavusoglu *et al*., 2007, 2008).

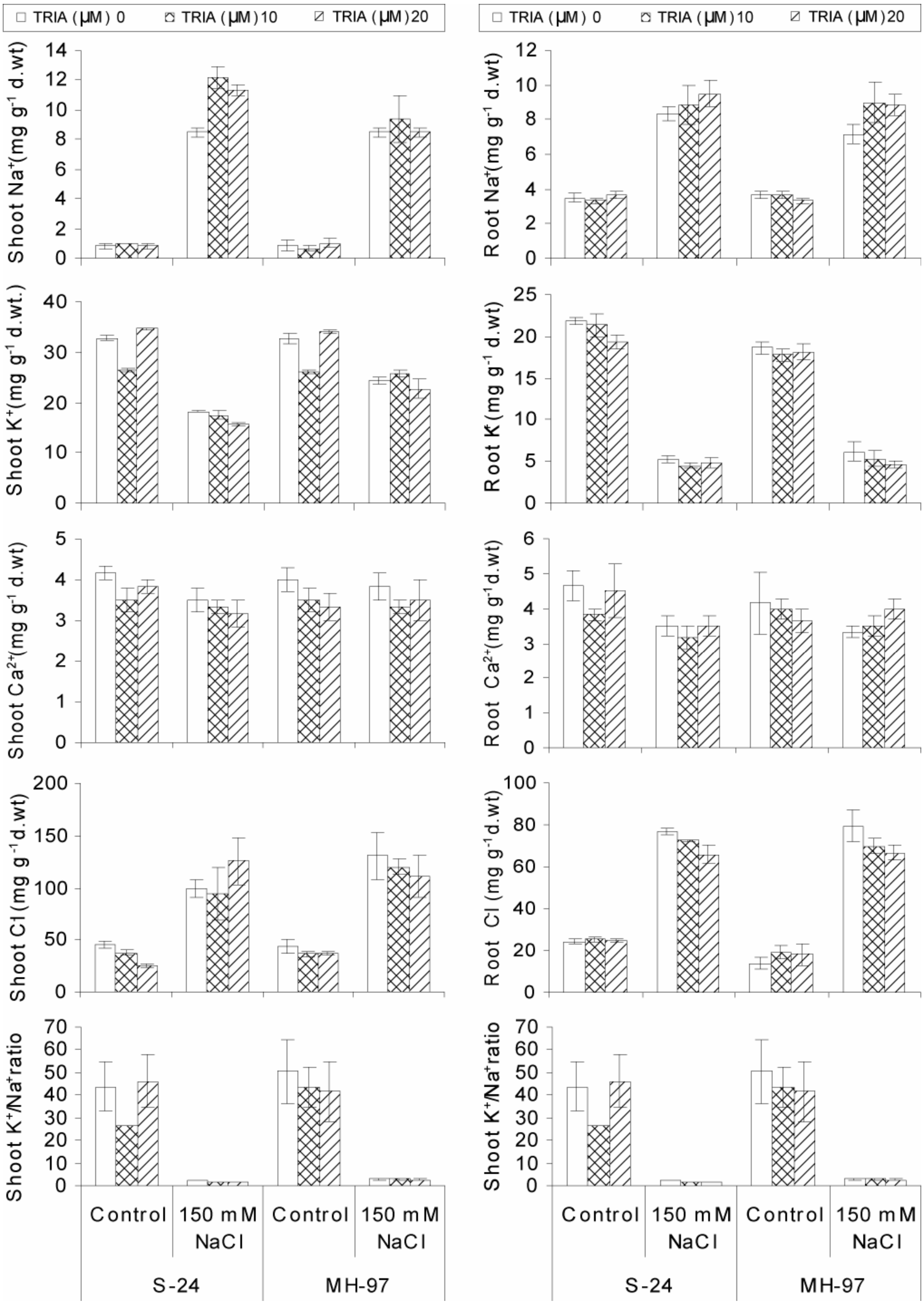

Fig. 1. Shoot and root mineral nutrients of salt-stressed and non-stressed wheat (*Triticum aestivum* L.) plants raised from seed primed with triacontanol for 12 h.

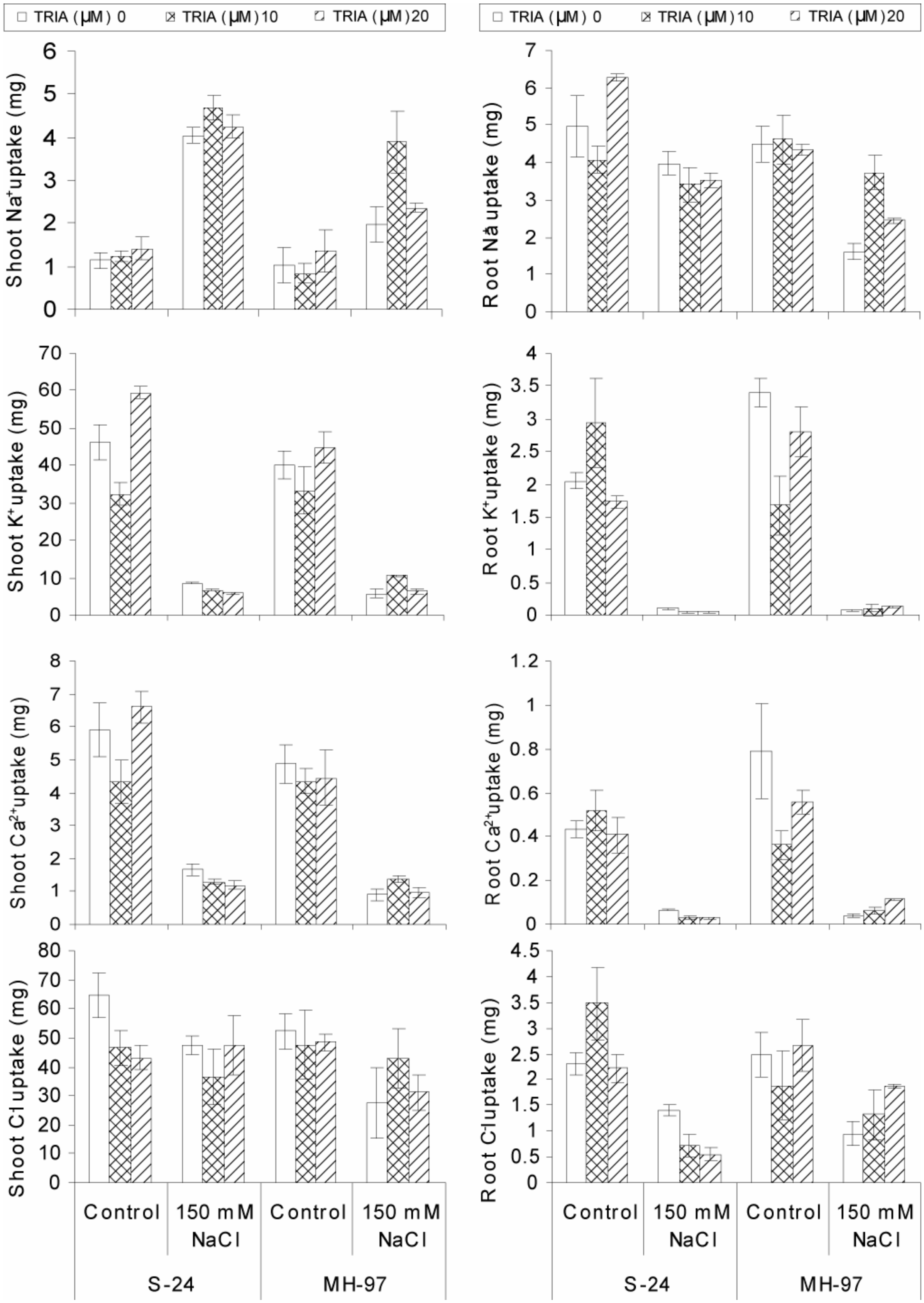

Fig. 2. Shoot and root mineral nutrient uptake of salt-stressed and non-stressed wheat (*Triticum aestivum* L.) plants raised from seed primed with triacontanol for 12 h.

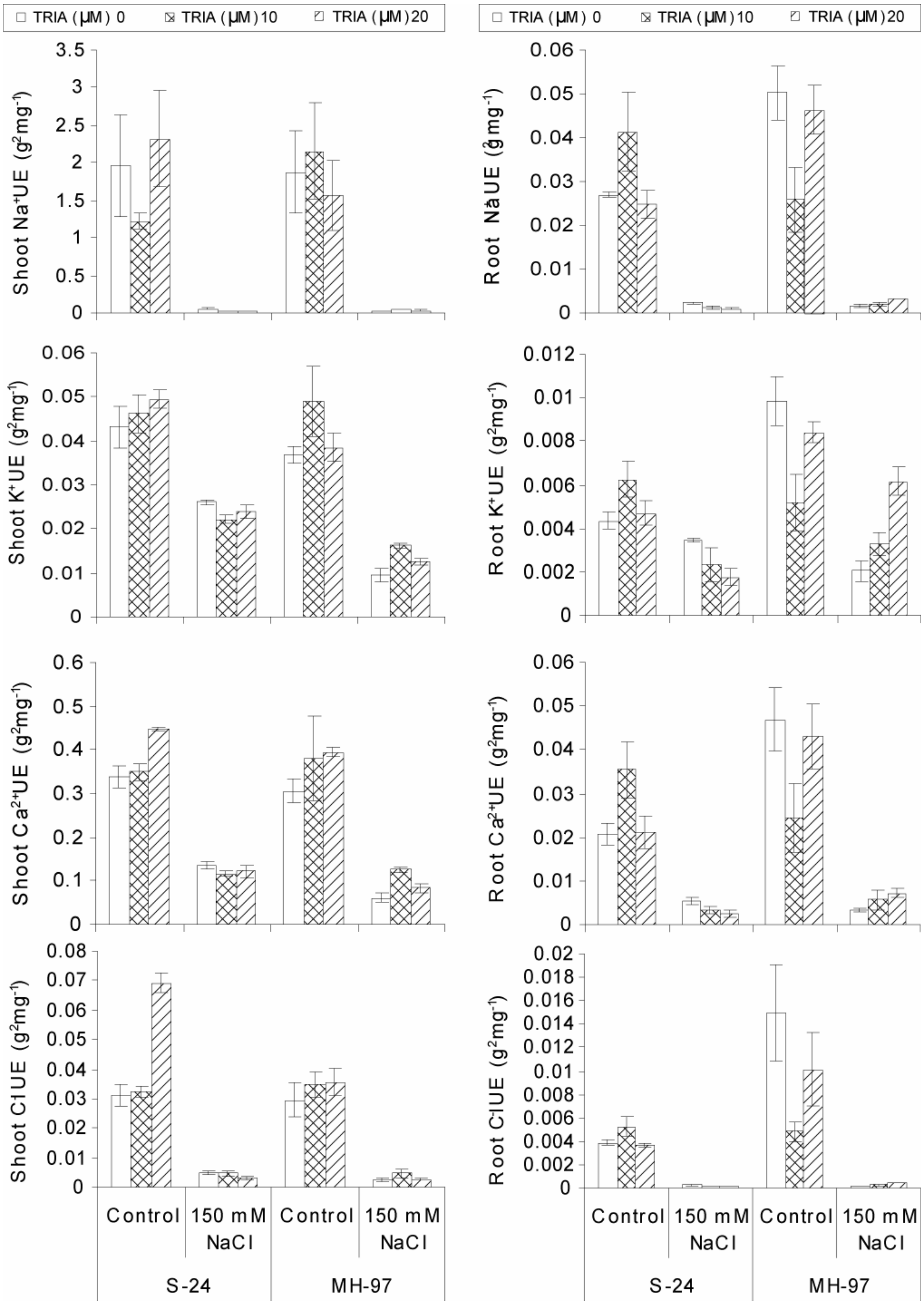

Fig. 3. Shoot and root mineral nutient use efficiency (NUE) of salt-stressed and non-stressed wheat (*Triticum aestivum* L.) plants raised from seed primed with triacontanol for 12 h.

Ries *et al*., (1993) reported that TRIA application in crops like cucumber, tomato and maize elicit a signal through a secondary messenger L(+)-adenosine, that moves rapidly from cuticle to plasma membrane of leaf and increases the concentrations of apoplastic ions (Ca^{2+}, Mg^{2+} and K^+), which regulate metabolic activity and consequently growth in plants. In view of some reports it is evident that exogenous application of PGRs can induce cell division and cell wall extensibility due to stimulation in intrinsic genetic potential of the plant (Taiz & Zeiger, 2006; Khan *et al*., 2006). In our study, salinity stress of 150 m*M* significantly enhanced the shoot and root Na^+ and Cl^- contents, while decreased K^+ contents and K^+/Na^+ ratios of both shoot and root in both cultivars (Fig. 1 Table 1). However, the effect of pre-sowing application of TRIA was non-significant on all nutrients except shoot Na^+ and K^+ contents (Table 1; Fig. 1). Krishnan & Kumari (2008) reported that exogenous application of TRIA increased the K^+ and Ca^{2+} contents of soybean leaves under salt stress. However, another report by Nandini & Subhendu (2002) showed that K^+ and Ca^{2+} contents of the hormone-(indole-3 acetic acid, gibberellic acid and kinetin) treated mung bean plants were similar to those of control plants under salt stress.

Krishnan & Kumari (2008) also reported that exogenous application of TRIA enhanced the nutrient uptake of salt stressed soybean plants, which was contradictory with our findings that salinity stress significantly decreased the uptake of all nutrients (except shoot Na^+), and the nutrient (Na^+, K^+, Ca^{2+}, Cl^-) use efficiency in both cultivars. TRIA application as a pre-sowing seed treatment only enhanced the shoot K^+ uptake under non-saline conditions, while it did not prove to be effective in enhancing the use efficiency of these nutrients except that of shoot Cl^- in both cultivars under non-saline conditions (Table 1; Fig. 1). Under normal growth conditions, TRIA application has been found to be effective in altering the pattern of accumulation of nutrients (K^+ and Ca^{2+}) and their uptake in plants (Ries & Houtz, 1983; Kumaravelu *et al.,* 2000; Khan *et al*., 2006; 2007; Naeem *et al*., 2009). Under different stresses and in different crop species, the effectiveness of various priming agents varies (Iqbal & Ashraf, 2010). For example, in poppy TRIA application had differential effects on the uptake and distribution of micronutrients (Srivastava & Sharma, 1990), while in roots of barley seedlings plasma membrane-enriched Ca^{2+} and Mg^{2+} dependent ATPase activity rapidly increased (Lesniak *et al*., 1986). The increased uptake of nutrients due to TRIA application (Miniraj & Shanmugavelu, 1987) might result in increased dry weight, contents of soluble protein, starch, sugars, total free amino acids and photosynthetic pigments, and consequently growth in different crop species (Kumaravelu *et al*., 2000; Naeem *et al*., 2009; Aftab *et al*., 2010).

In conclusion, salinity stress significantly increased the shoot and root Na^+ and Cl^- contents, while decreased those of K^+ as well as K^+/Na^+ ratios of both shoot and roots of both cultivars under saline conditions. Shoot Na^+ uptake significantly increased, while the uptake of remaining nutrients decreased under saline conditions. Shoot and root nutrients (Na^+, K^+, Ca^{2+}, Cl^-) use efficiency (NUE) decreased significantly under saline conditions in both cultivars. Pre-sowing TRIA application significantly increased the shoot Na^+ and K^+ contents, shoot K^+ uptake and shoot Cl^- use efficiency in both wheat cultivars. TRIA at 10 µ*M* level was more effective to improve root nutrient (K^+, Cl^-) uptake and use efficiency in S-24 when compared to MH-97. Cultivar S-24 was better in shoot and root Na^+, K^+, Ca^{2+} contents and their uptake as compared to MH-97.

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