Pak. J. Bot., 42(5): 3073-3081, 2010.

REGULATION IN GAS EXCHANGE AND QUANTUM YIELD OF PHOTOSYSTEM II (PSII) IN SALT-STRESSED AND NON-STRESSED WHEAT PLANTS RAISED FROM SEED TREATED WITH TRIACONTANOL

SHAGUFTA PERVEEN[1], MUHAMMAD SHAHBAZ[1*] AND MUHAMMAD ASHRAF[1,2]

[1]*Department of Botany, University of Agriculture, Faisalabad, Pakistan*
[2]*Second Affiliation: King Saud University, Riyadh, Saudi Arabia*

Abstract

To assess the effect of exogenous application of triacontanol (TRIA) as a presowing seed treatment on wheat under saline conditions, a greenhouse experiment was performed. Seeds of two wheat cultivars, MH-97 (moderately salt sensitive) and S-24 (salt tolerant) were primed with TRIA for 12 h. Plants raised from TRIA-treated seeds were grown in full strength Hoagland's nutrient solution for 24 days under non-saline conditions, after which time, they were subjected to 0 (control) or 150 m*M* NaCl. After 21 days of salt application, data for different growth, plant pigments and gas exchange characteristics were recorded. Salt stress of the root growing medium markedly decreased shoot and root fresh biomass, net CO_2 assimilation rate (*A*), stomatal conductance, and transpiration rate (*E*), while no significant effect of salinity was observed on chlorophyll pigments (*a, b* and *a/b* ratio), quantum yield of PSII, substomatal CO_2 concentration and water use efficiency (*A/E*). Exogenous application of TRIA as seed priming did not ameliorate the inauspicious effects of salt stress effectively, although it slightly increased photosynthetic rate in both wheat cultivars, transpiration rate in MH-97 and water use efficiency in S-24 under saline conditions.

Introduction

Triacontanol (TRIA) is a 30-carbon primary alcohol which functions as a plant growth promoter (Ries *et al*., 1977). It plays an active role in the up-regulation of many biochemical and physiological processes in plants (Ries & Houtz, 1983; Ivanov & Angelov, 1997; Chen *et al*., 2003). Role of TRIA is well studied not only at whole plants level (Eriksen *et al*., 1981) but also in tissue cultures (Ivanov & Angelov, 1997; Tentos *et al*., 1999; Tantos *et al*., 2001). Exogenous application of TRIA has been reported to enhance some major processes such as growth, chlorophyll contents, chlorophyll fluorescence, photosynthesis, free amino acids, reducing sugars, soluble proteins and crop yield (Eriksen *et al*., 1981; Muthuchelian *et al*., 1995, 1996; Tantos *et al*., 1999; Kumaravelu *et al*., 2000). For example, exogenous application of TRIA showed positive effect on growth, chlorophyll contents, photosystem-II efficiency and gas exchange characteristics in rice seedlings (Kumaravelu *et al*., 2000; Muthuchelian *et al*., 1995), maize (Ries, 1991), wheat (Ries, 1991), etc. Increase in growth could be mainly due to an abrupt TRIA-induced increase in photosynthesis as TRIA has been reported to be involved in the up-regulation of many genes involved in the photosynthetic process (Chen *et al*., 2002, 2003). Triacontanol exogenous application has also been reported to be very effective in reducing the adverse effects of salinity on plants (Krishnan & Kumari, 2008). For example, it increased growth, biomass, chlorophyll and carotenoid contents in salt and water stressed *Erythrina variegata* Lam. seedlings (Muthuchelian *et al*., 1995, 1996).

*Corresponding author's email: shahbazmuaf@yahoo.com

Seed is a vital part of a plant because of its role in the initiation of next generation. Dry seeds remain dormant because of possessing very low moisture contents (5-15%). Seeds need favorable environmental conditions to germinate, but under stressful environments their germination is significantly suppressed. However, a multitude of means are being used world-over for achieving better germination and healthy seedling vigor under stressful conditions (Ashraf & Foolad, 2005; Ashraf *et al*., 2008). Of them, soaking with different types of inorganic and organic solutes as well as plant growth substances is very effective to tailor plants for growing on stressful lands. For example, treating seed with a variety of plant growth regulators (PGRs) can improve yield under stress conditions (Lee *et al*., 1998). Different types of growth regulators have been used as seed priming agents and their positive role observed in different crops e.g., wheat seeds treated with brassinolide (Shahbaz *et al*., 2008; Fariduddin *et al*., 2008), kinetin, cytokinins, polyamines, auxins and gibberellic acid (Iqbal & Ashraf, 2005; Iqbal *et al*., 2006; Iqbal & Ashraf, 2007; Iqbal & Ashraf, 2010), blackgram with triadimefon (Jaleel *et al*., 2009), mungbean with brassinolide (Fariduddin *et al*., 2008), barley with triacontanol (Cavusoglu *et al*., 2007) and radish with gibberelic acid, ethylene, 24-epibrassinolide, triacontanol and polyamines (Cavusoglu *et al*., 2008).

Triacontanol is a plant growth promoter involved in growth promotion by up-regulating photosynthetic related gene machinery (Chen *et al*., 2002, 2003). Pre-sowing seed treatment with TRIA along with some other growth regulators has been reported to induce salt tolerance in barley and radish seedlings grown under saline conditions (Cavusoglu *et al*., 2007, 2008). However, information on its role in ameliorating the adverse effects of salinity by pre-sowing seed treatment in wheat is not available in the literature. Thus, the major objective of present study was to assess whether or not seed treatment with TRIA is effective in growth enhancement of wheat under saline conditions and whether this growth promotion is related to gas exchange characteristics.

Materials and Methods

Seed of two spring wheat (*Triticum aestivum* L.) cultivars MH-97 (moderately salt sensitive) and S-24 (salt tolerant), was obtained from the Department of Botany, University of Agriculture, Faisalabad, Pakistan and Ayub Agricultural Research Institute, Faisalabad, Pakistan, respectively. An experiment was conducted to assess the effect of exogenous application of TRIA as a seed treatment on wheat under salt stress during spring, 2010 in a wire-house of the Botanical Garden, University of Agriculture, Faisalabad, Pakistan (altitude 213m, latitude 31°30'N and longitude 73°10'E), with a day and night temperature cycle of 20 and 6°C, 10 and 14 light and dark period at 800-1100 µmol m^{-2} s^{-1} PPFD, respectively, and 54 ± 5% relative humidity. 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 50 ml of each of three optimized levels of triacontanol solutions (0, 10, and 20 µ*M*) for 12 h and re-dried to original weight with forced air under shade. Seeds (10 seeds per pot) were allowed to germinate in thoroughly washed sand. Twenty four day-old plants were subjected to saline stress for further 21 days. There were two salt treatments [(control (non-saline) and 150 m*M* NaCl)] supplied with full strength Hoagland's nutrient solution (2 L per 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) 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 plants were harvested after 45 days and data for fresh biomass recorded.

Chlorophyll contents: Chlorophyll *a* and *b* contents were determined according to the method of Arnon (1949). Fresh leaves (0.5 g) were extracted in 80% acetone and centrifuged at 10,000 × *g* for 5 min. Supernatant of extract was used to read absorbance at 645 and 663 nm using a UV-visible spectrophotometer (Hitachi-U2001, Tokyo, Japan).

Chlorophyll fluorescence: Plant efficiency Analyzer (PEA, Handstech Instrument Ltd., King's Lynn, UK) was used to measure the polyphasic rise of fluorescence transients according to Strasser *et al*., (1995). The fluorescence transients were inducted by red light and catered by an array of six light inducing diods (peaks 650 nm), focused on the sample surface to give homogenous light over the exposed area. All the samples were dark adapted for 30 min before to fluorescence measurements.

Gas exchange characteristics: Gas exchange characteristics were measured by using a portable infrared gas analyzer (ACD LCA-4 Analytical Development, Hoddesdon, UK) on second intact leaf from top of each plant. These measurements were made from 10:30 to 12:30 h with the following adjustments/specifications: leaf chamber gas flow rate (U) 251 µmol s^{-1}; ambient pressure 98.8 kPa; leaf surface area 11.25 cm^2; water vapor pressure ranged from 6.0 to 8.9 mbar into the leaf chamber, concentration of ambient CO_2 was 350 µmol mol^{-1}; range of leaf chamber temperature varied from 28.4 to 32.4°C; molar flow of air per unit leaf area (Us) 22.06 mol m^{-2} s^{-1}; RH of the chamber 41.2%; *PAR* (Qleaf) at the leaf surface was up to 942 µmol m^{-2} s^{-1}.

Statistical analysis of data: The data was analyzed using a COSTAT computer package (Cohort Software, Berkeley, CA). To compare the mean values, least significance difference test was applied according to Snedecor & Cochran (1980).

Results

Data for shoot and root fresh weights of salt stressed and non-stressed plants of two wheat cultivars raised from seed primed with triacontanol (TRIA) presented in Fig. 1 show that root medium salinity markedly reduced both shoot and root fresh weights of both cultivars. Exogenous application of TRIA as a seed treatment did not ameliorate the adverse effects of salt on shoot fresh weight, while a slight decrease in root fresh weight was noted by exogenous application of TRIA under non-saline conditions, but such a negative effect was not observed under saline conditions (Table 1).

Chlorophyll *a*, *b* and *a/b* ratio did not vary significantly under saline conditions or due to pre-soaking seed treatment with TRIA. Although chlorophyll *b* slightly increased and *a/b* decreased in MH-97 under saline conditions, but this increase or decrease was not significant. Similarly, chlorophyll *b* increased in both cultivars slightly by exogenous application of TRIA under saline conditions (Table 1; Fig. 1).

Seed treatment with triacontanol did not affect *Fo* (minimum fluorescence) and *Fm* (maximum fluorescence) of both wheat cultivars. Salt stress of the growth medium also did not perturb both these attributes. While quantum yield of PSII (*Fv/Fm*) was slightly decreased at 10 µ*M* TRIA under saline conditions, but only in MH-97. Effect of salinity or various levels of TRIA did not increase or decrease quantum yield of PSII of MH-97 particularly under non-stress conditions and that in S-24 under both saline and non-saline conditions.

Table 1. Growth attributes, chlorophyll contents and gas exchange characteristics of salt-stressed and non-stressed wheat (*Triticum aestivum* L.) plants raised from seed treated with Triacontanol (TRIA) for 12 h.

Source of variation	df	Shoot fresh weight	Root fresh weight	Chl. *a*	Chl. *b*	Chl. *a/b*
Cultivars (Cvs)	1	1.644ns	0.522***	0.004ns	0.002ns	0.018ns
Salinity (S)	1	360.7***	1.611***	0.0004ns	0.067ns	0.195ns
Triacontanol (TRIA)	2	0.487ns	0.072***	0.001ns	0.002ns	0.002ns
Cvs x S	1	0.003ns	0.256***	0.002ns	0.063ns	0.123ns
Cvs x TRIA	2	1.314ns	0.017ns	0.0006ns	0.027ns	0.075ns
S x TRIA	2	1.480ns	0.055**	0.002ns	0.042ns	0.074ns
Cvs x S x TRIA	2	0.339ns	0.018ns	0.0006ns	0.001ns	0.007ns
Error	24	0.709	0.006	0.001	0.0170636	0.049
Source of variation	**df**	***Fo***	***Fm***	***Fv/Fm***	***A***	***E***
Cultivars (Cvs)	1	32.11ns	0.25ns	0.0003ns	0.970ns	0.049ns
Salinity (S)	1	235.1ns	7482.2ns	0.006ns	9.191**	1.747*
Triacontanol (TRIA)	2	1477.1ns	58.86ns	0.005ns	0.966ns	0.229ns
Cvs x S	1	69.44ns	1167.4ns	0.002ns	3.033ns	0.483ns
Cvs x TRIA	2	1150.5ns	35779.1*	0.003ns	0.077ns	0.056ns
S x TRIA	2	924.2ns	12539.6ns	0.003ns	0.701ns	0.063ns
Cvs x S x TRIA	2	47.86ns	22784.ns	0.015*	1.202ns	0.035ns
Error	24	682.42	8333.4	0.003	0.774	0.352
Source of variation	**df**	g_s	C_i	C_i/C_a	***A/E***	
Cultivars (Cvs)	1	1600***	1915.5ns	0.015ns	2.609ns	
Salinity (S)	1	8100***	572.8ns	0.005ns	10.38ns	
Triacontanol (TRIA)	2	325**	302.99ns	0.002ns	0.885ns	
Cvs x S	1	625***	11.33ns	0.00009ns	0.027ns	
Cvs x TRIA	2	1300***	398.6ns	0.003ns	2.609ns	
S x TRIA	2	975***	1291.4ns	0.010ns	0.031ns	
Cvs x S x TRIA	2	175*	82.97ns	0.0007ns	7.205ns	
Error	24	43.75	901.24	0.007	3.397	

*, **, and *** = significant at 0.05, 0.01 and 0.001, respectively.
ns = non-significant

df = degrees of freedom; Chl. = chlorophyll; *Fo* = minimum fluorescence with all PSII reaction centers open; *Fm* = maximum fluorescence with all PSII reaction centers open; *Fv/Fm* = maximal quantum yield of PSII; A = net CO_2 assimilation rate; *E* = transpiration rate; *gs* = stomatal conductance; *Ci* = sub-stomatal CO_2 concentration; A/E = water use efficiency

Net CO_2 assimilation rate (*A*) and transpiration rate (*E*) decreased significantly in both cultivars under the saline medium. Exogenous application of TRIA as seed treatment particularly at 10 µ*M* had a promising effect in ameliorating the adverse effects of salt stress on both wheat cultivars.

Stomatal conductance of both wheat cultivars decreased significantly under saline conditions. However, this decrease was low in S-24 as compared to that in MH-97. Application of TRIA promoted stomatal conductance only under non-saline conditions, while under saline conditions, the response of both cultivars in terms of g_s to exogenous TRIA was not consistent (Table 1; Fig. 2).

Sub-stomatal CO_2 concentration and *Ci/Ca* ratio were also similar under both saline and non-saline conditions. Performance of both salt tolerant (S-24) and moderately salt sensitive (MH-97) cultivars was similar in terms of these two gas exchange parameters. Both attributes were not affected by exogenous TRIA. Furthermore, rooting medium salinity and exogenously applied TRIA did not alter the water use efficiency of both cultivars.

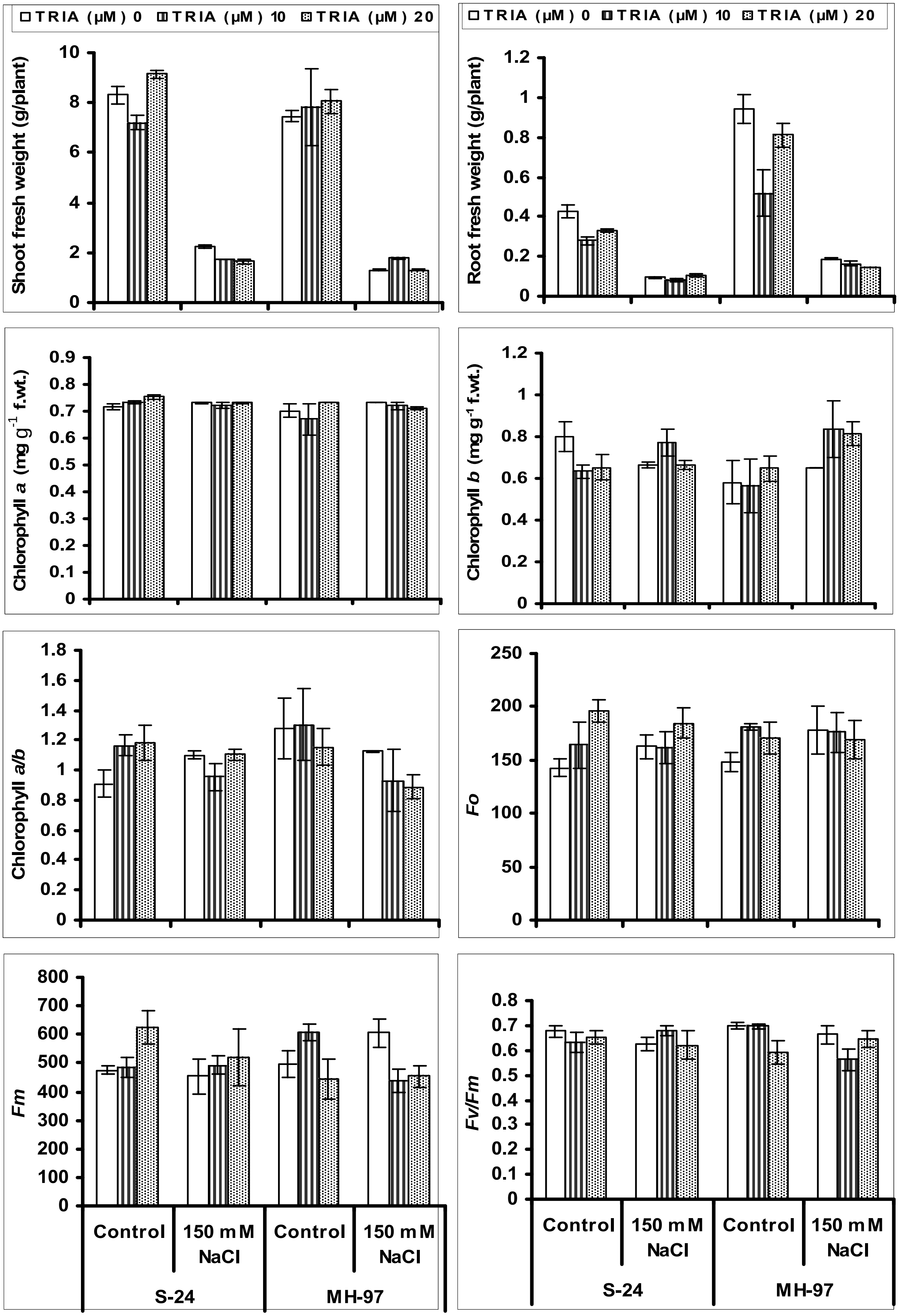

Fig. 1. Growth attributes, chlorophyll contents and photosystem-II effeciency of salt-stressed and non-stressed wheat (*Triticum aestivum* L.) plants raised from seed treated with triacontanol for 12 h.

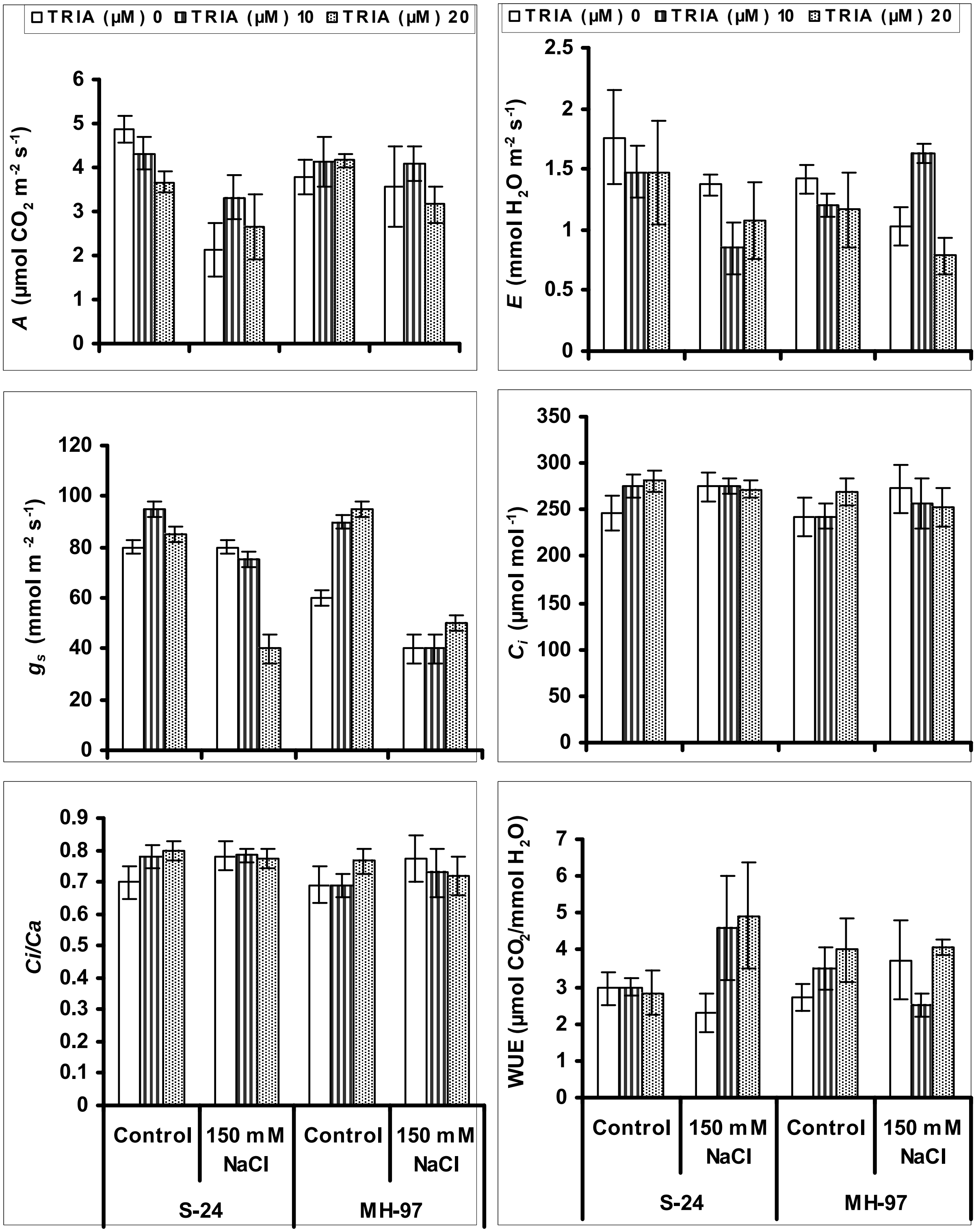

Fig. 2. Gas exchange characteristics of of salt-stressed and non-stressed wheat (*Triticum aestivum* L.) plants raised from seed primed with triacontanol for 12 h.

A = net CO_2 assimilation rate; *E* = transpiration rate; g_s = stomatal conductance; C_i = sub-stomatal CO_2 concentration; C_i/C_a = relative sub-stomatal CO_2 concentration; WUE = water use efficiency

Discussion

Triacontanol (TRIA) is a plant growth promoter which increases plant growth by activating a variety of growth processes (Chen *et al*., 2002, 2003). Mostly the role of exogenous application of TRIA as foliar spray in promoting growth and yield was assessed on different crops such as rice (Muthuchelian *et al*., 1995; Kumaravelu *et al*., 2000), maize (Ries, 1991), wheat (Ries, 1991), etc. In present study, TRIA was applied on two wheat cultivars as seed-treatment and its effect on growth and some physiological parameters was examined under saline and non-saline conditions. Salt stress markedly reduced the fresh biomass of both shoot and root in both cultivars as has earlier been reported in different studies (Ashraf *et al*., 2008; Grewal, 2010). One of the possible reasons for reduced biomass could be due to the reduction in net CO_2 assimilation rate mediated by stomatal closure, thereby limiting CO_2 diffusion into the chloroplast (Degl'Innocenti *et al*., 2009).

Triacontanol has been reported as an effective plant growth regulator by many investigators (Tantos *et al*., 1999, 2001; Reddy *et al*., 2002; Fraternale *et al*., 2003; Malabadi *et al*., 2005) because it can enhance metabolism and growth processes of plants by influencing the enzymes involved in carbohydrate metabolism (Ries *et al*., 1977; Ries & Houtz, 1983). However, in contrast, the effect of TRIA in the present study was found to be non-significant under saline or non-saline conditions when applied as pre-sowing seed treatment.

Foliar application of TRIA has been reported to increase chlorophyll contents in different crops such as pearl millet (Sivakumar *et al*., 2006), soybean (Krishnan & Kumari, 2008), and rice (Chen *et al*., 2002, 2003). However, when TRIA was applied as seed treatment did not alter the chlorophyll pigments of both wheat cultivars. Furthermore, no significant improvement in quantum yield of PSII was observed in the present study when TRIA was applied as pre-soaking seed treatment under saline or non-saline conditions, though in other studies TRIA when applied as a foliar spray was found to improve the quantum yield of PSII in rice (Muthuchelian *et al*., 1995; Kumaravelu *et al*., 2000; Chen *et al*., 2003) and tomato (Borowski *et al*., 2000).

Exogenous application of TRIA as seed treatment improved the net CO_2 assimilation rate and transpiration rate when applied @ 10 μM under saline conditions and stomatal conductance under non-saline conditions. Positive effects of foliar-applied TRIA on net CO_2 assimilation rate, transpiration rate and stomatal conductance have already been observed on different crops e.g., rice (Chen *et al*., 2003), maize (Ries, 1991), wheat (Ries, 1991) and soybean (Krishnan & Kumari, 2008). The mechanisms involved in effects of TRIA on photosynthesis are not clearly known yet. However, TRIA-induced increase in the activity of rubisco has been suggested as one of the possible mechanisms reported by (Houtz *et al*., 1985) and this possibility was also supported by increased expression of *rbc* by TRIA in rice seedlings (Chen *et al*., 2002).

In conclusion, rooting medium salt stress adversely affected shoot and root fresh biomass, net CO_2 assimilation rate, transpiration rate and stomatal conductance, but exogenous application of TRIA as seed treatment did not improve plant growth and gas exchange characteristics. Although, 10 μM TRIA improved net CO_2 assimilation rate under saline conditions and stomatal conductance under non-saline conditions, but this level and others did not enhance the shoot fresh biomass of both cultivars.

Acknowledgments

The work presented in this manuscript is a part of PhD research work being conducted by Ph.D. scholar Miss Shagufta Perveen PIN No. 074-3756-Bm4-061 whose study is funded by the Higher Education Commission through Indigenous PhD Scheme.

References

Arnon, D.T. 1949. Copper enzyme in isolated chloroplasts polyphenoloxidase in *Beta vulgaris*. *Plant Physiol*., 24: 1-15.

Ashraf, M. and M.R. Foolad. 2005. Pre-sowing seed treatment- A shotgun approach to improve germination, plant growth, and crop yield under saline and non-saline conditions. *Adv. Agron*., 88: 223-270.

Ashraf, M., H.R. Athar, P.J.C. Harris and T.R. Kwon. 2008. Some prospective strategies for improving crop salt tolerance. *Adv. Agron*., 97: 45-110.

Borowski, E., Z.K. Blamoski and W. Mickalek. 2000. Effects of Tomatex/Triacontanol on chlorophyll fluorescence and tomato (*Lycopersicon esculentum* Mill) yields. *Acta Physiol. Plant*., 22: 271-274.

Cavusoglu, K., S. Kilic and K. Kabar. 2007. Effects of triacontanol pretreatment on seed germination, seedling growth and leaf anatomy under saline (NaCl) conditions. *Sdu. Fen. Edebiyat Fakültesi Fen Dergisi (E-Dergi)*, 2(2): 136-145.

Cavusoglu, K., S. Kilic and K. Kabar. 2008. Effects of some plant growth regulators on leaf anatomy of radish seedlings grown under saline conditions. *J. Appl. Biol. Sci*., 2(2): 47-50.

Chen, X., H. Yuan, R. Chen, L. Zhu and G. He. 2003. Biochemical and photochemical changes in response to triacontanol in rice (*Oryza sativa* L.). *Plant Growth Regul*., 40: 249-256.

Chen, X., H. Yuan, R. Chen, L. Zhu, B. Du, Q. Weng and G. He. 2002. Isolation and characterization of triacontanol-regulated genes in rice (*Oryza sativa* L.): possible role of triacontanol as plant growth stimulator. *Plant Cell Physiol*., 43: 869-876.

Degl'Innocenti, E., C. Hafsi, L. Guidi and F. Navari-Izzo. 2009. The effect of salinity on photosynthetic activity in potassium-deficient barley species. *J. Plant Physiol*., 166: 1968-1981.

Eriksen, A.B., G. Sellden, D. Skogen and S. Nilson. 1981. Comparative analysis of the effect of triacontanol on photosynthesis, photorespiration and growth of tomato (C3-plants) and maize (C4-plants). *Planta*, 152: 44-49.

Fariduddin, Q., S.A. Hasan, B. Ali, S. Hayat and A. Ahmad. 2008. Effect of modes of application of 28-homobrassinolide on mung bean. *Turk. J. Biol*., 32: 17-21.

Fraternale, D., L. Giamperi, D. Ricci, M.B.L. Rocchi, L. Guidi, F. Epifano and M.C. Marcotullio. 2003. The effect of triacontanol on micropropagation and on secretory system of *Thymus mastichina*. *Plant Cell Tissue Organ Cult*., 74: 87-97.

Grewal, H.S. 2010. Water uptake, water use efficiency, plant growth and ionic balance of wheat, barley, canola, and chickpea plants on a sodic vertosol with variable subsoil NaCl salinity. *Agri. Water Manage*., 97: 148-156.

Houtz, R.L., S.K. Ries and N.E. Tolbert. 1985. Effect of triacontanol on *Chlamydomonas*. I. Stimulation of growth and photosynthetic CO_2 assimilation. *Plant Physiol*., 79: 357-64.

Iqbal, M. and M. Ashraf. 2010. Gibberellic acid mediated induction of salt tolerance in wheat plants: growth, ionic partitioning, photosynthesis, yield and hormonal homeostasis. *J. Env. Exp. Bot*., NO. 002.

Iqbal, M. and M. Ashraf. 2005. Changes in growth, photosynthetic capacity and ionic relations in spring wheat (*Triticum aestivum* L.) due to pre-sowing seed treatment with polyamines. *Plant Growth Regul*., 46: 19-30.

Iqbal, M. and M. Ashraf. 2007. Seed treatment with auxins modulates growth and ion partitioning in salt-stressed wheat plants. *J. Integ. Plant Biol*., 49(7): 1045-1057.

Iqbal, M., M. Ashraf and A. Jamil. 2006. Seed enhancement with cytokinins: changes in growth and grain yield in salt stressed wheat plants. *Plant Growth Regul*., 50: 29-39.

Ivanov, A.G. and M.N. Angelov. 1997. Photosynthesis response to triacontanol correlates with increased dynamics of mesophyll protoplast and chloroplast membranes. *Plant Growth Regul*., 21: 145–152.

Jaleel, C.A., M. Iqbal, and R. Panneerselvam. 2009. Triadimefon protects blackgram (*Vigna mungo* (L.) Hepper) plants from Sodium chloride stress. *Plant Stress*, 3(1): 13-16.

Krishnan, R.R. and B.D.R. Kumari. 2008. Effect of n-triacontanol on the growth of salt stressed soyabean plants. *J. Biosci*., 19(2): 53-6

Kumaravelu, G., V.D. Livingstone and M.P. Ramanujam. 2000. Triacontanol-induced changes in the growth, photosynthetic pigments, cell metabolites, flowering and yield of green gram. *Biol. Plant*., 43: 287-290.

Lee, I.S., O.K. Kim, K.S. Cho and J.S. Park. 1998. Studies on the enzyme activities and heavy metals of forest soil. *J. Ecol*., 21: 695-702.

Malabadi, R.B., G.S. Mulgund and K. Nataraga. 2005. Plant regeneration via somatic embryogenesis in *Pinus kesiya* Royle ex. Gord., influenced by triacontanol. *Acta Physiol. Plant*., 27(4): 531-537.

Muthuchelian, K., C. Murugan, R. Harigovindan, N. Nedunchezhian and G. Kulandaivelu. 1995. Effect of triacontanol in flooded *Erithrina variegata* seedlings. 1. Changes in growth, photosynthetic pigments and biomass productivity. *Photosynthetica*, 31: 269-275.

Muthuchelian, K., C. Murugan, R. Harigovindan, N. Nedunchezhian and G. Kulandaivelu. 1996. Ameliorating effect of triacontanol on salt stressed *Erythrina variegata* seedlings. Changes in growth, biomass, pigments and solute accumulation. *Biol. Plant*., 38: 133-136.

Reddy, B.O., P. Giridhar and G.A. Ravishankar. 2002. The effect of triacontanol on micropropagation of *Capsicum frutescens* and *Decalepis hamiltonii* W€A. *Plant Cell Tissue Organ cult*., 71: 253-8.

Ries, S.K. 1991. Triacontanol and its second messenger 9-β-L (+)-adenosine as plant growth substances. *Plant Physiol*., 95: 986-989.

Ries, S.K. and R. Houtz. 1983. TRIA as a plant growth regulator. *Hort. Sci*.,18: 654-662.

Ries, S.K. 1985. Regulation of plant growth with triacontanol. CRC. *Crit. Rev. Plant Sci*., 2: 239-285.

Ries, S.K. and V.F. Wert. 1982. Rapid effects of triacontanol *In vivo* and *In vitro*. *Plant Growth Regul*., 1: 117-127.

Ries, S.K., V.F. Wert, C.C. Sweeley and R.A. Leavitt. 1977. Triacontanol: a new naturally occurring plant growth regulator. *Science*, 195: 1339-1341.

Shahbaz, M., M. Ashraf and H.R. Athar. 2008. Does exogenous application of 24-epibrassinolide ameliorate salt induced growth inhibition in wheat (*Triticum aestivum* L.)? *Plant Growth Regul*., 55(1): 51-64.

Sivakumar, R., G. Pathmanaban and S. Nithila. 2006. Effect of foliar application of growth regulators on physiological and biochemical attributes, grain yield and quality in Pearl millet. ISMN, 47.

Snedecor, G.W. and G.W. Cochran. 1980. *Statistical Methods*. 7th edition. The Lowa State University Press. Ames, Lowa.

Strasser, R.J., A. Srivastava and Govindjee. 1995. Polyphasic chlorophyll 'a' fluorescence transients in plants and cyanobacteria. *Photochem. Photobiol*., 61: 32-42.

Tantos, A., A. Meszaros and J. Kissimon. 1999. The effect of triacontanol on micropropagation of balm, *Melissa officinalis* L. *Plant* Cell *Rep*., 19: 88-91.

Tantos, A., A. Meszaros, T. Farkas, J. Szalai and G. Horvath. 2001. Triacontanol-supported micropropagation of woody plants. *Plant Cell Rep*., 20: 16-21.

(Received for publication 8 January 2010)