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

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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 mM 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 CO₂ 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 CO₂ 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).

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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 gibberelic 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 µM) 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 mM NaCl)] supplied with full strength Hoagland’s nutrient solution (2 L per pot). For attaining the desired level of salt an aliquot of 50 mM solution was added to each pot every day. Salt level (150 mM NaCl) was applied after every week till the end of the experiment. The sand moisture content was maintained daily by adding 200 ml distilled H₂O 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 cm²; water vapor pressure ranged from 6.0 to 8.9 mbar into the leaf chamber, concentration of ambient CO₂ 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 $F_o$ (minimum fluorescence) and $F_m$ (maximum fluorescence) of both wheat cultivars. Salt stress of the growth medium also did not perturb both these attributes. While quantum yield of PSII ($F_v/F_m$) was slightly decreased at 10 µM 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.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>df</th>
<th>Shoot fresh weight</th>
<th>Root fresh weight</th>
<th>Chl. <em>a</em></th>
<th>Chl. <em>b</em></th>
<th>Chl. <em>a/b</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Cultivars (Cvs)</td>
<td>1</td>
<td>1.644ns</td>
<td>0.522***</td>
<td>0.004ns</td>
<td>0.002ns</td>
<td>0.018ns</td>
</tr>
<tr>
<td>Salinity (S)</td>
<td>1</td>
<td>360.7***</td>
<td>1.611***</td>
<td>0.0004ns</td>
<td>0.067ns</td>
<td>0.195ns</td>
</tr>
<tr>
<td>Triacontanol (TRIA)</td>
<td>2</td>
<td>0.487ns</td>
<td>0.072***</td>
<td>0.001ns</td>
<td>0.002ns</td>
<td>0.002ns</td>
</tr>
<tr>
<td>Cvs x S</td>
<td>1</td>
<td>0.003ns</td>
<td>0.256***</td>
<td>0.002ns</td>
<td>0.063ns</td>
<td>0.123ns</td>
</tr>
<tr>
<td>Cvs x TRIA</td>
<td>2</td>
<td>1.314ns</td>
<td>0.017ns</td>
<td>0.0006ns</td>
<td>0.027ns</td>
<td>0.075ns</td>
</tr>
<tr>
<td>S x TRIA</td>
<td>2</td>
<td>1.480ns</td>
<td>0.055**</td>
<td>0.002ns</td>
<td>0.042ns</td>
<td>0.074ns</td>
</tr>
<tr>
<td>Cvs x S x TRIA</td>
<td>2</td>
<td>0.339ns</td>
<td>0.018ns</td>
<td>0.0006ns</td>
<td>0.001ns</td>
<td>0.007ns</td>
</tr>
<tr>
<td>Error</td>
<td>24</td>
<td>0.709</td>
<td>0.006</td>
<td>0.001</td>
<td>0.0170636</td>
<td>0.049</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>df</th>
<th><em>F₀</em></th>
<th><em>Fₘ</em></th>
<th><em>Fᵥ/Fₘ</em></th>
<th><em>A</em></th>
<th><em>E</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Cultivars (Cvs)</td>
<td>1</td>
<td>32.11ns</td>
<td>0.25ns</td>
<td>0.0003ns</td>
<td>0.970ns</td>
<td>0.049ns</td>
</tr>
<tr>
<td>Salinity (S)</td>
<td>1</td>
<td>235.1ns</td>
<td>7482.2ns</td>
<td>0.006ns</td>
<td>9.191**</td>
<td>1.747*</td>
</tr>
<tr>
<td>Triacontanol (TRIA)</td>
<td>2</td>
<td>1477.1ns</td>
<td>58.86ns</td>
<td>0.005ns</td>
<td>0.966ns</td>
<td>0.229ns</td>
</tr>
<tr>
<td>Cvs x S</td>
<td>1</td>
<td>69.44ns</td>
<td>1167.4ns</td>
<td>0.002ns</td>
<td>3.033ns</td>
<td>0.483ns</td>
</tr>
<tr>
<td>Cvs x TRIA</td>
<td>2</td>
<td>1150.5ns</td>
<td>35779.1*</td>
<td>0.003ns</td>
<td>0.077ns</td>
<td>0.056ns</td>
</tr>
<tr>
<td>S x TRIA</td>
<td>2</td>
<td>924.2ns</td>
<td>12539.6ns</td>
<td>0.003ns</td>
<td>0.701ns</td>
<td>0.063ns</td>
</tr>
<tr>
<td>Cvs x S x TRIA</td>
<td>2</td>
<td>47.86ns</td>
<td>22784.ns</td>
<td>0.015*</td>
<td>1.202ns</td>
<td>0.035ns</td>
</tr>
<tr>
<td>Error</td>
<td>24</td>
<td>682.42</td>
<td>8333.4</td>
<td>0.003</td>
<td>0.774</td>
<td>0.352</td>
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</table>

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>df</th>
<th><em>gₛ</em></th>
<th><em>Cᵢ</em></th>
<th><em>Cᵢ/Cₐ</em></th>
<th><em>A/E</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Cultivars (Cvs)</td>
<td>1</td>
<td>1600***</td>
<td>1915.5ns</td>
<td>0.015ns</td>
<td>2.609ns</td>
</tr>
<tr>
<td>Salinity (S)</td>
<td>1</td>
<td>8100***</td>
<td>572.8ns</td>
<td>0.005ns</td>
<td>10.38ns</td>
</tr>
<tr>
<td>Triacontanol (TRIA)</td>
<td>2</td>
<td>325**</td>
<td>302.99ns</td>
<td>0.002ns</td>
<td>0.885ns</td>
</tr>
<tr>
<td>Cvs x S</td>
<td>1</td>
<td>625***</td>
<td>113.3ns</td>
<td>0.0009ns</td>
<td>0.027ns</td>
</tr>
<tr>
<td>Cvs x TRIA</td>
<td>2</td>
<td>1300***</td>
<td>398.6ns</td>
<td>0.003ns</td>
<td>2.609ns</td>
</tr>
<tr>
<td>S x TRIA</td>
<td>2</td>
<td>975***</td>
<td>1291.4ns</td>
<td>0.010ns</td>
<td>0.031ns</td>
</tr>
<tr>
<td>Cvs x S x TRIA</td>
<td>2</td>
<td>175*</td>
<td>82.97ns</td>
<td>0.0007ns</td>
<td>7.205ns</td>
</tr>
<tr>
<td>Error</td>
<td>24</td>
<td>43.75</td>
<td>901.24</td>
<td>0.007</td>
<td>3.397</td>
</tr>
</tbody>
</table>

*, **, and *** = significant at 0.05, 0.01 and 0.001, respectively.

ns = non-significant

df = degrees of freedom; Chl. = chlorophyll; *F₀* = 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 CO₂ assimilation rate; *E* = transpiration rate; *gₛ* = stomatal conductance; *Cᵢ* = sub-stomatal CO₂ concentration; *A/E* = water use efficiency

Net CO₂ 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 *gₛ* to exogenous TRIA was not consistent (Table 1; Fig. 2).

Sub-stomatal CO₂ 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 efficiency 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; \( C_i \) = substomatal CO\(_2\) concentration; \( C_i/C_a \) = relative substomatal 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 CO₂ assimilation rate mediated by stomatal closure, thereby limiting CO₂ 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 CO₂ 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 CO₂ 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 CO₂ 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 CO₂ 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.
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References


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