Influence of foliar-applied triacontanol on growth, gas exchange characteristics, and chlorophyll fluorescence at different growth stages in wheat under saline conditions

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Abstract

A greenhouse experiment was conducted to examine the effect of foliar application of triacontanol (TRIA) on two cultivars (cv. S-24 and MH-97) of wheat (*Triticum aestivum* L.) at different growth stages. Plants were grown in full strength Hoagland's nutrient solution under salt stress (150 mM NaCl) or control (0 mM NaCl) conditions. Three TRIA concentrations (0, 10, and 20 μM) were sprayed over leaves at three different growth stages, *i.e.* vegetative (V), boot (B), and vegetative + boot (VB) stages (two sprays on same plants, *i.e.*, the first at 30-d-old plants and the second 78-d-old plants). Salt stress decreased significantly growth, net photosynthetic rate (P_N), transpiration rate (E), chlorophyll contents (Chl *a* and *b*), and electron transport rate (ETR), while membrane permeability increased in both wheat cultivars. Stomatal conductance (g_s) decreased only in salt-sensitive cv. MH-97 under saline conditions. Foliar application of TRIA at different growth stages enhanced significantly the growth, P_N, g_s, Chl *a* and *b* contents, and ETR, while membrane permeability was reduced in both cultivars under salt stress. Of various growth stages, foliar-applied TRIA was comparatively more effective when it was applied at V and VB stages. Overall, 10 μM TRIA concentration was the most efficient in reducing negative effects of salinity stress in both wheat cultivars. The cv. S-24 showed the better growth and ETR, while cv. MH-97 exhibited higher nonphotochemical quenching.

Additional key words: chlorophyll, photosynthesis, salt stress.

Introduction

Plant hormones increase salinity stress tolerance in plants by regulating various physiological and biochemical processes (Javid *et al*. 2011, Shahbaz *et al*. 2012). Their cruicial roles urge the scientsts to discover new plant growth regulators (PGRs) and elucidation of their roles in regulating different plant processes (Shahbaz *et al*. 2008, Perveen *et al*. 2012). One of a number of relatively new PGRs, triacontanol (TRIA), is reported to affect various plant characteristics, such as increased CO_2 assimilation rate (Haugstad *et al*. 1983), size and number of chloroplasts (Chen *et al*. 2002), and chloroplast membrane viscosity (Ivanov and Angelov 1997). Among these, regulation of photosynthesis is a complex process, which is known to be modulated by TRIA, *e.g.* increased Hill

reaction activity (Verma *et al*. 2011), specific activity of Rubisco (Houtz *et al*. 1985), activity of photosystem (PS) I and II complexes (Moorthy and Kathiresan 1993), upregulation of genes (*rbc*S isogene profile) related to photosynthesis and suppression of stress-related genes in rice (Chen *et al*. 2002).

Soil salinity brings about a variety of changes in plant metabolism including those in hormonal levels, *i.e.* it reduces some growth-promoting hormones (*e.g.*, cytokinins and gibberellic acid, auxins and salicylic acid), while it stimulates stress-related hormones (ABA and jasmonic acid) (Javid *et al*. 2011). Among different approaches, which are known to mitigate the adverse effects of salt stress, foliar application of PGRs is more effective

and it is frequently used in crop plants (Ashraf *et al*. 2008). The foliar application of TRIA at various growth stages has been reported to increase growth and final productivity in various crop species, such as wheat (13–27%), maize (33%), pear millet (20%), groundnuts (15–20%), sorghum (48%), paddy rice (14–27%) (*e.g*., Sivakumar *et al*. 2002), *etc*. However, there are also some reports that show lack of stimulatory effect of TRIA in different plant species, such as lettuce, oat, soybean, and wheat (Marcelle and Chrominski 1978, Perveen *et al*. 2011). Under saline conditions, TRIA is known to increase plant growth by modulating different metabolic processes including photosynthesis, photorespiration, Chl synthesis, cell division, uptake of water and mineral nutrients, and enhanced activities of some key enzymes (Krishnan and Kumari 2008, Perveen *et al*. 2010, 2011, 2012a).

It has been reported that TRIA and its derivatives did not improve germination and growth in durum wheat (Perveen *et al*. 2010, 2011). Similarly, the effect of TRIA has been observed as insignificant on most of growth,

physiological, and biochemical attributes in wheat, when used as a presowing seed treatment (Perveen *et al*. 2010, 2011, 2012a). Although the mechanism of TRIA action is not clearly known, Houtz *et al*. (1985) reported that TRIA stimulates plant growth through increased Rubisco activity. Furthermore, the optimal concentration of TRIA and the age of plants are important factors, which control the growth and yield of various plant species.

Keeping in view all the major roles of TRIA in plants, it was hypothesized that foliar application of TRIA at various growth stages could reduce the negative influence of salt stress on wheat plants and increase its photosynthetic pigments, PSII efficiency, photosynthetic efficiency, and consequently to improve the growth. Thus, the objective of the present investigation was to assess the role of TRIA application at different growth stages on growth, physiological, and biochemical attributes, such as shoot fresh mass, shoot length, gas-exchange characteristics, Chl content, and Chl fluorescence parameters in two wheat cultivars differing in salt tolerance.

Materials and methods

A greenhouse experiment was conducted in the old Botanical Garden, University of Agriculture, Faisalabad under 10/14 h light/dark period, under the irradiance of 800–1,100 μmol(photon) m^{-2} s^{-1} PPFD, day/night temperature cycle of 20°C/6°C, and relative humidity of 54 ± 5%. Two wheat cultivars, S-24 (salt-tolerant) and MH-97 (moderately salt-sensitive), were treated under control (without NaCl) and salt-stressed (150 mM NaCl) conditions with three TRIA concentrations (0, 10, and 20 μM). Ten seeds per pot were sown in each plastic pot containing thoroughly washed sand. When seedlings were 10 d old, thinning was done to reduce the number of plants to six in each pot. The plants were supplemented with full strength Hoagland's nutrient solution of 2 L per pot every week. Salt (*i.e*., NaCl) treatment was initiated, when plants were of 21 d old. The salt concentration was increased stepwise in aliquots of 50 mM every day until the appropriate concentration of 150 mM was attained. The pots were flooded with the treatment solution (Hoagland's nutrient solution + NaCl) to ensure a thorough and uniform distribution of salt within all pots. Salt-stress severity (ECe) was monitored weekly using an electrical conductivity (EC) meter (*InoLab pH/Cond 720, WTW 82362* Weilheim, Germany) to maintain the desired level. Ten concentrations of TRIA (0, 10, 20, 30, 40, 50,

60, 70, 80, and 100 μM) were used first in an initial experiment to optimize TRIA levels. Of these 10 TRIA concentrations, two the most effective ones (10 and 20 μM), together with a control (0 μM), were used for further experiments. The TRIA solutions were prepared in hot distilled water and 0.1% Tween-20 solution. Foliar spray of TRIA was done at the rate of 25 ml pot^{-1} at V (30-d-old plants), B (78-d-old plants), and VB stages (two foliar sprays on same plants *i.e*. the first spray at 30-d-old plants and the second at 78-d-old plants). The design of the experiment was completely randomized with four replicates. When plants were 92 d old, two plants from each replicate were harvested carefully, washed with distilled water, shoot and root fresh mass (FM) along with their lengths were recorded and their means were used.

Gas-exchange characteristics: Using an open-system portable infrared gas analyzer (*LCA-4 ACD, Analytical Development*, Hoddesdon, UK), gas-exchange characteristics such as P_N, E, intracellular CO_2 concentration (C_i) of leaf tissue, g_s, and water-use efficiency (WUE) were measured on the second leaf from top of each plant from 10:00 h to 12:30 h. Following conditions were kept during the measurement:

ambient pressure	98.8 kPa
leaf chamber gas flow rate	251 μmol s^{-1}
leaf surface area	11.25 cm^2
concentration of ambient CO_2	350 μmol mol^{-1}
water vapor pressure in the leaf chamber	6.0–8.9 kPa
temperature of leaf chamber	28.4–32.4°C
relative humidity of the chamber	41.2%
molar flow of air per unit of the leaf area	22.06 mol m^{-2} s^{-1}
photosynthetically active radiation at the leaf surface	700–942 μmol(photon) m^{-2} s^{-1}

Chl content: The method described by Arnon (1949) was used for determination of Chl a and b contents. Fresh leaves (0.5 g each) were cut into small pieces, 10 ml of 80% acetone was added and kept overnight at 0–4°C. The extract was centrifuged at $10,000 \times g$ for 5 min and absorbance (OD) of the supernatant was read at 645 and 663 nm with a UV-visible spectrophotometer (*U2001*, *Hitachi*, Tokyo, Japan).

Chl a and b contents were calculated using the following formulae:

$$\text{Chl } a = [12.7\,(\text{OD }663) - 2.69\,(\text{OD }645)] \times v/1000 \times w$$
$$\text{Chl } b = [22.9\,(\text{OD }645) - 4.68\,(\text{OD }663)] \times v/1000 \times w$$

where v – volume of the extract [mL]; w – mass of the fresh leaf tissue [g].

Membrane permeability: Fresh leaf (0.5 g) tissue was chopped and placed in 10 ml of distilled water, vortexed for 5 s, and the electrical conductivity (EC_0) was measured. The test tubes containing leaf samples were covered with an aluminium foil, kept at 4°C for 24 h, and then their electrical conductivity (EC_1) was determined. The test tubes containing samples were autoclaved for 1 h, cooled at room temperature and electrical conductivity (EC_2) of dead tissues was measured. The following formula was used to determine a percentage of relative membrane permeability (RMP):

$$RMP\,[\%] = (EC_1 - EC_0/EC_2 - EC_0) \times 100.$$

Chl fluorescence: Before measurements of Chl fluorescence, all leaf samples were kept in dark for 0.5 h by attaching light-exclusion clips to the surface of leaves. Chl fluorescence was determined using an *OS5p Modulator Fluorometer* (*ADC BioScientific Ltd.*, Great Amwell Herts, UK) according to Strasser *et al.* (1995). After 30 min of the dark adaptation, the minimal fluorescence (F_0) was determined by a weak red light (< 0.1 µmol m^{-2} s^{-1}). The frequency of the measuring beam was 6 kHz for all measurements of F_0, whereas that of F_m was 20 kHz during the saturation flash. Then the maximum fluorescence (F_m) was determined using a saturating pulse (8,000 µmol m^{-2} s^{-1}) of 0.8-s duration. Other fluorescence parameters, such as maximum quantum yield of PSII (F_v/F_m), electron transport rate (ETR), photochemical quenching (q_P), and coefficient of nonphotochemical quenching (q_N), were recorded by using 1,500 µmol m^{-2} s^{-1} actinic light. Following formulae were used to calculate following parameter:

$$ETR = Y \times PAR \times 0.84 \times 0.5$$
$$q_P = (F_m' - F)/(F_m' - F_0)$$
$$q_N = (F_m - F_m')/(F_m - F_0),$$

where 0.84 is leaf absorption coefficient and 0.5 is a fraction of absorbed light by PSII antennae.

Statistical analysis: Data from the present study were subjected to analysis of variance (*ANOVA*) using a *COSTAT* computer program. Mean squares from the *ANOVA* were used in tables. The means were compared using the least significant difference (LSD) test according to Snedecor and Cochran (1980).

Results

Shoot and root FM and length decreased significantly in both wheat cultivars under NaCl stress (Table 1, Figs. 1*A*,*B*; 2*A*,*B*). The cv. S-24 was superior to cv. MH-97 in all growth attributes except that root FM was higher in cv. MH-97 under both control and salt-stress conditions. Foliar spray of different TRIA concentrations increased shoot and root FM and their length in both cultivars at various growth stages (V, B, and VB). Such a TRIA-induced increase in the growth was prominent under control conditions in comparison with that under salt stress. However, the effect of various TRIA concentrations on both cultivars was variable under the control and saline conditions. For example, both TRIA concentrations (10 and 20 µM) were effective in increasing shoot FM, while the most effective TRIA concentration for increasing root FM was 10 µM applied at the V stage. TRIA of 20 µM applied at the VB stage increased markedly this attribute in both cultivars under both control and saline conditions. The most effective TRIA concentration for increasing shoot length was 10 µM applied at the B stage and 20 µM applied at the V or VB stages. Similarly, 10 µM TRIA increased root length, when it was applied at the V and B stages.

Saline stress caused a significant decrease in P_N, E, and g_s, while C_i and WUE ($= P_N/E$) remained unaffected in both wheat cultivars under salt stress (Table 1; Figs. 3*A*,*B*; Fig. 4*A*,*B*; Fig. 5*A*). Foliar application of TRIA increased significantly P_N under both saline and control conditions (Fig. 3*A*), g_s under control conditions (Fig. 4*A*), E under salt stress conditions in both wheat cultivars (Fig. 3*B*), while WUE (Fig. 5*A*) was affected under stress conditions only in cv. S-24. Of different TRIA concentrations used, 10 µM was more effective in increasing P_N at all growth stages except for the B stage, where 20 µM TRIA seemed to be more effective in both cultivars. Overall, TRIA application at the VB stage was more effective in stimulation of gas exchange characteristics, especially of P_N in both cultivars under both saline and control conditions, of g_s in cv. MH-97 under salt stress conditions, and of WUE in cv. S-24 at the V stage under control conditions.

Chl a and Chl b contents decreased significantly in both wheat cultivars under salt stress (Table 1, Figs. 5*B*, 6*A*). The cv. MH-97 showed higher Chl a and b contents under control conditions when compared with those of cv. S-24. The foliar application of TRIA (10 µM)

Table 1. Mean squares from analysis of variance (*ANOVA*) of data for growth, gas exchange, and chlorophyll (Chl) content of wheat (*Triticum aestivum* L.) when plants were foliarly sprayed with triacontanol at various growth stages with or without salt stress. Cvs – cultivars; S – salinity; GS – growth stages; TRIA – triacontanol; FM – fresh mass; P_N – net photosynthetic rate; E – transpiration rate; g_s – stomatal conductance; C_i – intracellular CO_2 concentration; WUE – water-use efficiency; MP – membrane permeability. *, **, and *** – significant at 0.05, 0.01, and 0.001, respectively; ns – not significant.

| Source of variation	df	Shoot FM	Root FM	Shoot length	Root length	P_N	E	g_s	C_i	WUE	Chl a	Chl b	MP [%]
Cvs	1	57.74^{*}	2.901^{***}	974.5^{***}	17.36^{**}	0.132^{ns}	0.152^{ns}	336.1^{ns}	129.6^{ns}	1.509^{ns}	0.056^{ns}	0.256^{***}	662.85^{***}
S	1	$2{,}960.7^{***}$	11.00^{***}	$13{,}236.3^{***}$	230.0^{***}	7.058^{***}	1.651^{***}	69.44^{ns}	529.7^{ns}	0.481^{ns}	0.710^{***}	0.917^{***}	909.12^{***}
Cvs × S	1	5.55^{ns}	0.500^{***}	22.26^{ns}	14.69^{*}	0.565^{ns}	0.0003^{ns}	$1{,}344.4^{*}$	148.0^{ns}	0.961^{ns}	0.116^{**}	0.332^{***}	14.46^{ns}
GS	2	0.962^{ns}	0.037^{ns}	13.17^{ns}	0.835^{ns}	0.247^{ns}	0.002^{ns}	34.03^{ns}	345.6^{ns}	0.385^{ns}	0.002^{ns}	0.003^{ns}	4.04^{ns}
Cvs × GS	2	9.755^{ns}	0.035^{ns}	23.74^{ns}	0.106^{ns}	0.581^{ns}	0.102^{ns}	63.19^{ns}	470.0^{ns}	0.338^{ns}	0.034^{ns}	0.007^{ns}	18.86^{ns}
S × GS	2	0.941^{ns}	0.025^{ns}	11.35^{ns}	2.189^{ns}	0.069^{ns}	0.002^{ns}	88.19^{ns}	21.86^{ns}	0.640^{ns}	0.005^{ns}	0.001^{ns}	2.665^{ns}
Cvs × S × GS	2	3.733^{ns}	0.001^{ns}	56.88^{ns}	1.543^{ns}	0.460^{ns}	0.025^{ns}	567.4^{ns}	777.4^{ns}	0.214^{ns}	0.002^{ns}	0.005^{ns}	19.41^{ns}
TRIA	2	56.69^{**}	0.140^{***}	271.2^{***}	11.49^{**}	2.209^{***}	0.050^{ns}	786.1^{*}	295.4^{ns}	0.460^{ns}	0.121^{***}	0.056^{*}	60.46^{*}
Cvs × TRIA	2	3.620^{ns}	0.011^{ns}	20.02^{ns}	0.647^{ns}	0.233^{ns}	0.556^{***}	$1{,}702.8^{***}$	97.34^{ns}	1.638^{ns}	0.008^{ns}	0.005^{ns}	27.70^{ns}
S × TRIA	2	35.86^{*}	0.006^{ns}	95.54^{*}	1.782^{ns}	0.129^{ns}	0.302^{**}	$1{,}202.8^{**}$	661.7^{ns}	3.453^{**}	0.016^{ns}	0.009^{ns}	3.49^{ns}
Cvs × S × TRIA	2	0.402^{ns}	0.006^{ns}	10.36^{ns}	1.668^{ns}	0.169^{ns}	0.156^{ns}	552.8^{ns}	561.8^{ns}	0.688^{ns}	0.026^{ns}	0.004^{ns}	13.69^{ns}
GS × TRIA	2	5.518^{ns}	0.036^{ns}	31.77^{ns}	1.845^{ns}	0.689^{ns}	0.071^{ns}	25.69^{ns}	280.0^{ns}	0.139^{ns}	0.080^{ns}	0.021^{ns}	4.24^{ns}
Cvs × GS × TRIA	4	15.51^{ns}	0.006^{ns}	28.88^{ns}	0.142^{ns}	0.163^{ns}	0.094^{ns}	223.6^{ns}	927.2^{ns}	0.893^{ns}	0.007^{ns}	0.009^{ns}	6.59^{ns}
S × GS × TRIA	4	7.920^{ns}	0.019^{ns}	48.80^{ns}	2.725^{ns}	0.199^{ns}	0.029^{ns}	96.52^{ns}	356.0^{ns}	0.405^{ns}	0.056^{ns}	0.010^{ns}	7.09^{ns}
Cvs × S × GS × TRIA	4	14.91^{ns}	0.008^{ns}	50.76^{ns}	0.658^{ns}	0.175^{ns}	0.037^{ns}	169.4^{ns}	580.3^{ns}	0.562^{ns}	0.005^{ns}	0.004^{ns}	3.782^{ns}
Error	108	9.268	0.016	22.07	2.364	0.297	0.051	207.9	440.7	0.637	0.015	0.014	14.74
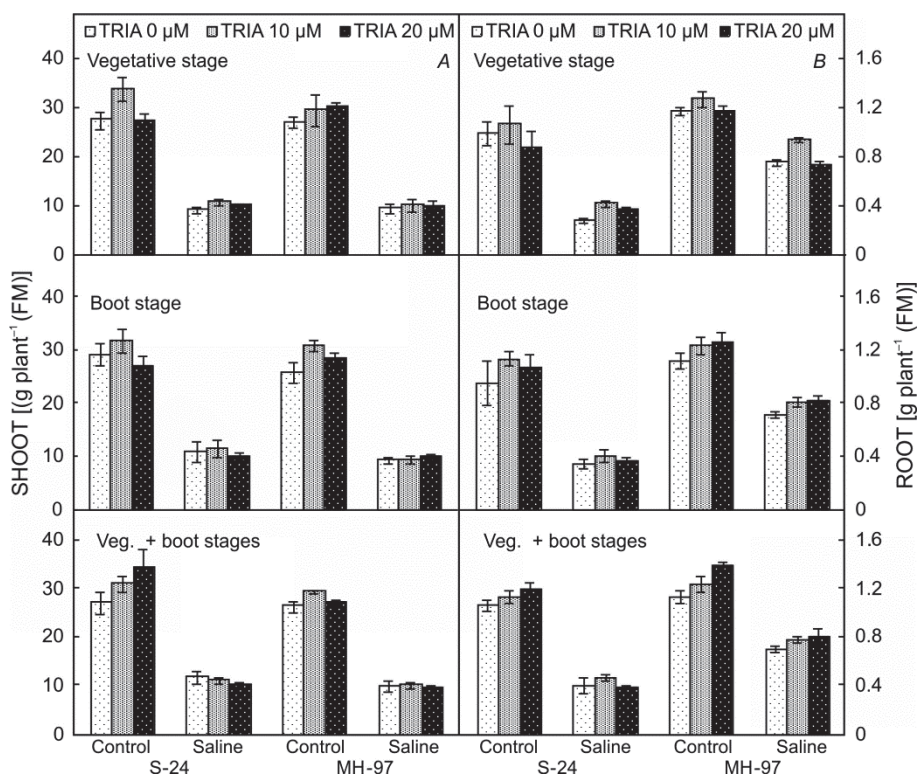
Fig. 1. Shoot fresh mass (*A*) and root fresh mass (*B*) of wheat plants foliarly sprayed with TRIA at different growth stages with or without salt stress (mean ± SE; *n* = 4).
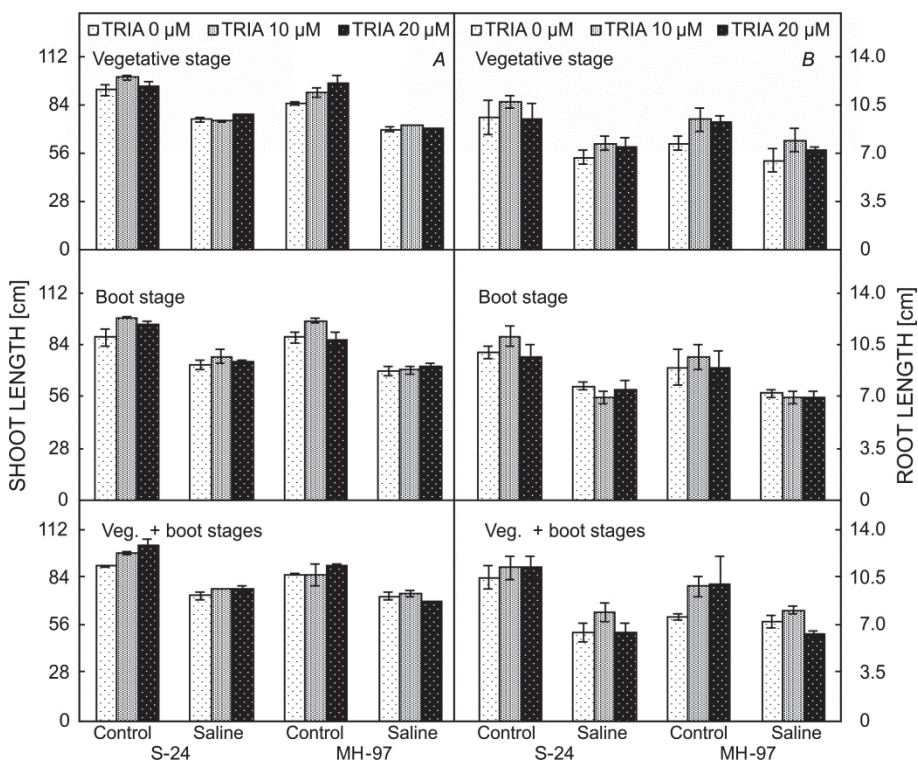

Fig. 2. Shoot length (*A*) and root length (*B*) of wheat plants foliarly sprayed with TRIA at different growth stages with or without salt stress (mean ± SE; *n* = 4).

increased markedly Chl *a* and *b* contents in both cultivars. Although the foliar application of TRIA increased Chl *a* and *b* contents in all growth stages, when applied at the VB stages, Chl *b* increased in cv. MH-97 under control conditions.

RMP increased significantly in both wheat cultivars under saline conditions. However, a different response was found in both cultivars in terms of RMP (Table 1). The cv. MH-97 showed higher RMP compared with cv. S-24 under both stress and control conditions (Fig. 6*B*). The foliar application of TRIA reduced significantly RMP in both wheat cultivars at all growth
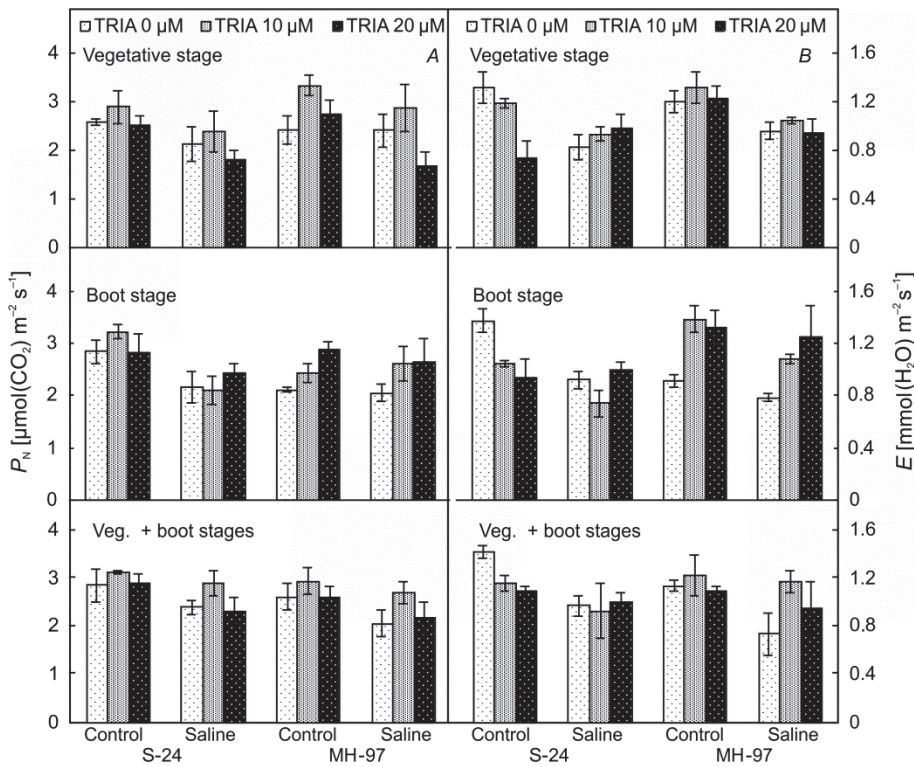
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Fig. 3. Net photosynthetic rate (P_N) (*A*) and transpiration rate (*E*) (*B*) of wheat plants foliarly sprayed with TRIA at different growth stages with or without salt stress (mean ± SE; $n = 4$).
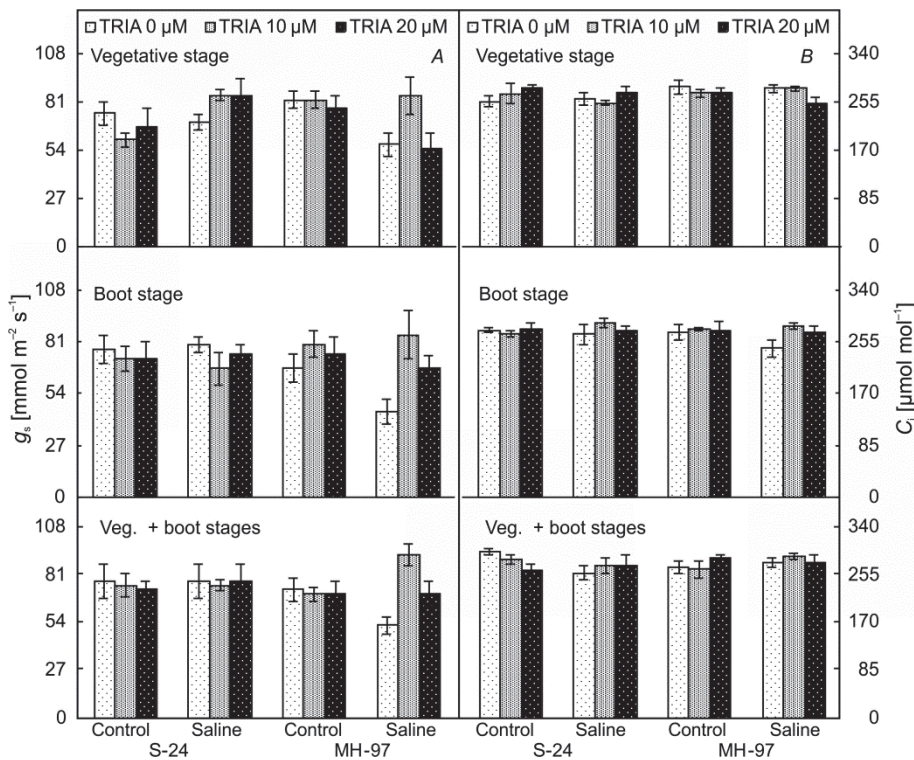

Fig. 4. Stomatal conductance (g_s) (*A*) and intracellular CO_2 concentration (C_i) (*B*) of wheat plants foliarly sprayed with TRIA at different growth stages with or without salt stress (mean ± SE; $n = 4$).

stages (Fig. 6*B*). A consistent decrease in RMP with the increasing concentration of foliar-applied TRIA was observed in cv. S-24 under both stress and control conditions at different growth stages, while in cv. MH-97, only the TRIA application at the B stage was found to be effective in decreasing RMP in salt-stressed plants.

The efficiency of PSII (F_v/F_m) did not change, however, ETR decreased in both wheat cultivars under salt stress (Table 2). The cv. S-24 showed higher values of ETR than those of cv. MH-97 (Fig. 7*A*,*B*). The foliar application of TRIA enhanced markedly F_v/F_m in both cultivars, while ETR was stimulated only in MH-97
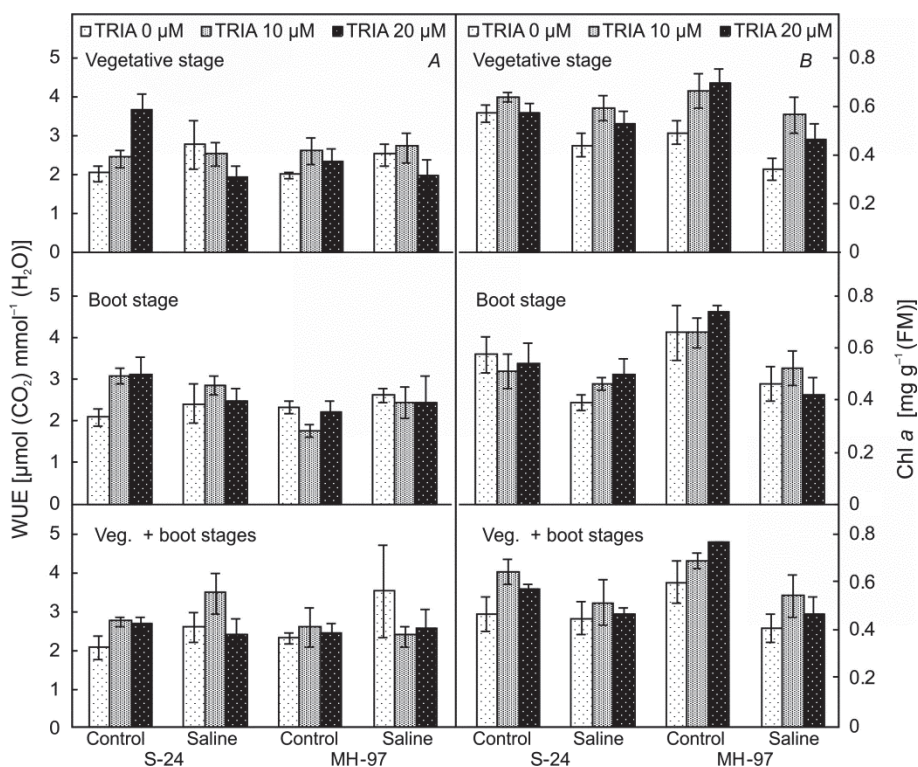
Fig. 5. Water-use efficiency (WUE) (*A*) and chlorophyll *a* (*B*) of wheat plants foliarly sprayed with TRIA at different growth stages with or without salt stress (mean ± SE; *n* = 4).
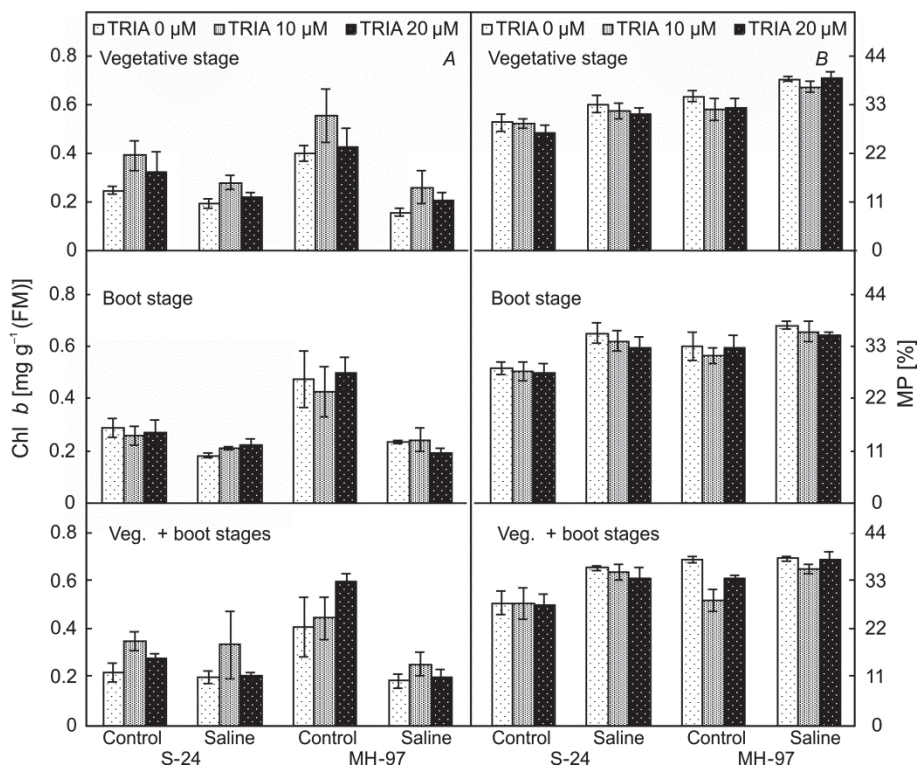

Fig. 6. Chlorophyll (Chl) *b* (*A*) and membrane permeability (MP) (*B*) of wheat plants foliarly sprayed with TRIA at different growth stages with or without salt stress (mean ± SE; *n* = 4).

under saline conditions (Fig. 7*A,B*). Generally, the effect of foliar application of TRIA was similar in both cultivars and salt-stressed plants concerning Chl fluorescence parameters, such as ETR, q_P, and q_N, at different stages (Figs. 7*A,B*; 8*A,B*). Salt stress itself did not alter q_P and q_N in both cultivars (Fig. 8*A,B*). However, the response of

both wheat cultivars to foliar-applied TRIA was different, *e.g.*, q_P increased consistently with increasing TRIA concentration in cv. MH-97 under saline conditions. In contrast, under control conditions, q_P increased in MH-97, while it decreased in S-24 with 10 μM TRIA (Fig. 8*A*). The cv. MH-97 was high in q_N value as com-

pared with cv. S-24. Although the effect of foliar-applied TRIA on q_N was insignificant at the V stage, the cultivar response to TRIA treatment was variable, *e.g.*, q_N increased in cv. S-24, while it decreased in MH-97 (Fig. 8*B*).

Discussion

The foliar application of TRIA is effective in enhancing crop production under abiotic stresses including salt stress. However, TRIA-promoting effect is variable among crops and even in different species of the same crop. In the current study, the cultivars showed variable response to varying foliar-applied TRIA concentrations; cv. MH-97 showed more positive response at 10 µM, while cv. S-24 at 20 µM TRIA, particularly under control conditions. Foliar-applied TRIA at different growth stages has been reported to enhance growth and yield in most of economically important crop species, such as coriander (Sarada *et al.* 2008), ginger (Singh *et al.* 2012), *Opium poppy* (Srivastava and Sharma 1990), pearl millet (Sivakumar *et al.* 2002), tomato (Khan *et al.* 2009), and hyacinth beans (Naeem *et al.* 2009). TRIA-mediated increase in growth could be due to its role in modulating the activities of different enzymes (Perveen *et al.* 2011, Singh *et al.* 2012) and enhancing photosynthetic rate (Singh *et al.* 2012). TRIA elicits a second messenger 9-β-L (+) adenosine, which is structurally similar to cytokinins, plant hormones, reported to be actively involved in the promotion of growth and other attributes in plants (He and Loh 2000).

Gas-exchange characteristics were adversely affected in wheat due to the salt stress (Zheng *et al.* 2009, Perveen *et al.* 2010, Kanwal *et al.* 2011). It has been reported that long-term exposure to salt decreased photosynthetic rate, which might be due to reduced g_s (Ouerghi *et al.* 2000). Leaf stomata closure due to toxic Na^+ and Cl^- ions decreases g_s that further decreases photosynthesis thereby causing reduction in growth (Shahbaz and Zia, 2011, Shahbaz *et al.* 2011, Ashraf *et al.* 2012). TRIA plays some role in stomata regulation by upregulating photosynthetic genes (Chen *et al.* 2002). Exogenous application of TRIA increased CO_2 exchange rate and total Chl under normal and saline conditions (Srivastava and Sharma 1990, Perveen *et al.* 2010). In the present study, P_N decreased under salt stress, however, TRIA foliar-spray increased it at all growth stages, especially at VB stages in both cultivars under both saline and nonsaline conditions. Similarly, Srivastava and Sharma (1990) reported increased CO_2 fixation rate by foliar-applied TRIA at all growth stages in *Opium poppy*. It could be inferred from the results presented here that increased growth under salt stress might be due to the increase in photosynthesis due to foliar-applied TRIA. Tolerant genotypes of wheat are known to possess higher photosynthetic capacity than sensitive ones under NaCl stress (Zheng *et al.* 2009). In the current study, TRIA increased g_s more in salt-sensitive cultivar, MH-97, compared with the salt-tolerant cultivar, S-24, under salt-stress conditions although the performance of cultivar S-24 was better than that of MH-97 in P_N and g_s.

Chl *a* and *b* contents decreased under salinity stress in both cultivars similarly to results reported by Zheng *et al.* (2009). Chl reduction could be due to salt stress-induced activity of chlorophyllase (Reddy and Vora 1986), enhanced H_2O_2 production, and Chl photodamage

Table 2. Mean squares from analysis of variance of data for chlorophyll fluorescence attributes of wheat (*Triticum aestivum* L.) plants foliarly sprayed with triacontanol at various growth stages with or without salt stress. Cvs – cultivars; S – salinity; GS – growth stages; TRIA – triacontanol. F_v/F_m – efficiency of photosystem II; ETR – electron transport rate; q_P – photochemical quenching efficiency; q_N – nonphotochemical quenching coefficient. *, **, and *** – significant at 0.05, 0.01, and 0.001, respectively; ns – not significant.

Source of variation	df	F_v/F_m	ETR	q_P	q_N
Cvs	1	0.0006[ns]	26.87[***]	0.002[ns]	0.190[***]
S	1	0.004[ns]	18.63[**]	0.0004[ns]	0.011[*]
Cvs × S	1	0.006[*]	7.111[ns]	0.00004[ns]	0.014[*]
GS	2	0.00003[ns]	0.498[ns]	0.0003[ns]	0.003[ns]
Cvs × GS	2	0.001[ns]	3.445[ns]	0.003[ns]	0.0006[ns]
S × GS	2	0.001[ns]	2.189[ns]	0.0006[ns]	0.001[ns]
Cvs × S × GS	2	0.0008[ns]	0.991[ns]	0.001[ns]	0.002[ns]
TRIA	2	0.0065[*]	13.79[**]	0.004[ns]	0.004[ns]
Cvs × TRIA	2	0.005[*]	1.476[ns]	0.008[*]	0.013[**]
S × TRIA	2	0.0009[ns]	16.51[***]	0.001[ns]	0.0004[ns]
Cvs × S × TRIA	2	0.005[*]	20.93[***]	0.011[**]	0.009[*]
GS × TRIA	4	0.001[ns]	2.811[ns]	0.001[ns]	0.002[ns]
Cvs × GS × TRIA	4	0.004[*]	10.03[***]	0.002[ns]	0.004[ns]
S × GS × TRIA	4	0.005[**]	7.824[**]	0.002[ns]	0.004[ns]
Cvs × S × GS × TRIA	4	0.003[ns]	8.325[**]	0.005[*]	0.003[ns]
Error	108	0.001	1.882	0.002	0.002
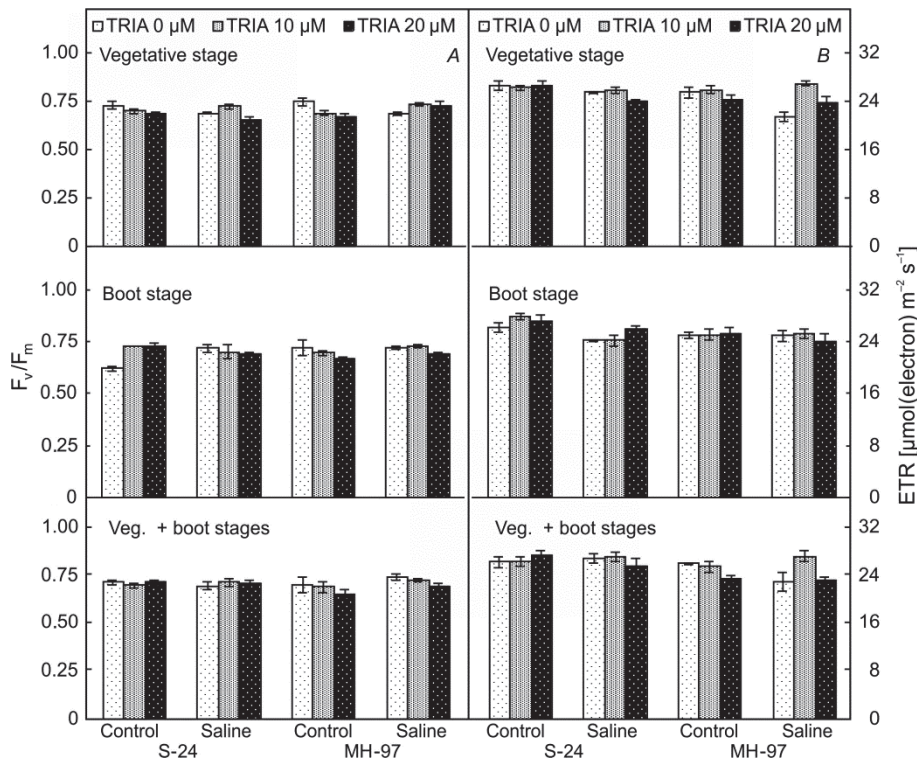
Fig. 7. Efficiency of photosystem II (*A*) and electron transport rate (*B*) of wheat plants foliarly sprayed with TRIA at different growth stages with or without salt stress (mean ± SE; $n = 4$).
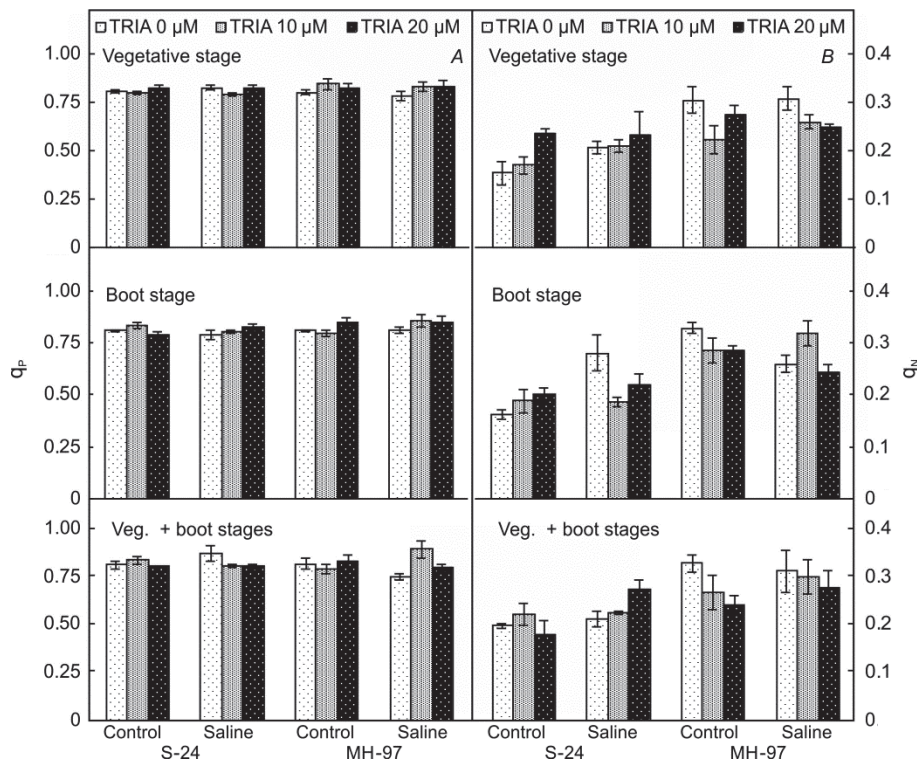
Fig. 8. Photochemical quenching (*A*) and coefficient of nonphotochemical quenching (*B*) of wheat plants foliarly sprayed with TRIA at different growth stages with or without salt stress (mean ± SE; $n = 4$).

(Hossain *et al*. 2011). TRIA increased Chl content in both wheat cultivars, however, salt-sensitive cultivar, MH-97, showed higher increase in pigment contents, particularly under nonsaline conditions. There are many reports, which show elevated Chl contents due to foliar application of TRIA (Krishnan and Kumari 2008, Borowski and Blamowski 2009, Naeem *et al*. 2009, Perveen *et al*. 2010). TRIA enhanced cell number through enhanced cell division that might be due to increase in Chl content and CO_2 fixation (Haugstad *et al*. 1983, Houtz *et al*. 1985, Perveen *et al*. 2010). In the present study, TRIA increased Chl content and photosynthetic capacity in both

549

wheat cultivars under both stress and nonstress condi-
tions. It could be inferred that the enhanced P_N might be
due to increased Chl content (Ivanov and Angelove 1997)
or TRIA-promoted Rubisco activity (though not app-
raised in the present study) that might have resulted in
improving the Calvin cycle functioning (Erikson *et al*.
1981).

Salt stress adversely affects structure and chemical
composition of plant cell membranes in wheat (Perveen
et al. 2012b). TRIA is believed to play a significant role
in inhibiting lipid peroxidation of biological membranes
by acting as an antioxidant compound (Khan *et al.* 2009).
Thus, in the present study, TRIA-induced decrease in
RMP could be due to its putative role in reduction of oxi-
dative stress under salt stress by a mechanism not known
yet. Similarly, foliar application of TRIA was very effec-
tive in improving membrane integrity in drought-stressed
jack pine seedlings (Rajasekaran and Blake 1999) and
decreasing electrolyte leakage in sweet basil (*Ocimum
basilicum* L.) under chilling stress (Borowski and
Blamowski 2009). Furthermore, in our previous study on
wheat (Perveen *et al.* 2012b), membrane permeability
decreased only under normal, nonsaline conditions, when
TRIA was applied as a seed pretreatment.

Chl fluorescence is an important criterion for
assessing tolerance of plants to salt stress (Mehta *et al.*
2010). Ashraf and Ashraf (2012) have reported that salt
stress reduces the activity of PSII in wheat at all growth
stages. Our data showed that most of the Chl fluorescence
attributes remained unaffected, but in contrast, ETR
decreased significantly at all growth stages under NaCl
(150 mM) stress. This was in agreement with results of

Saleem *et al*. (2011) for okra and of Zribi *et al*. (2009) for
tomato, where no significant effect of salt stress was
observed on F_v/F_m, q_P, and NPQ values. Similarly, Mehta
et al. (2010) reported insignificant effect of salt stress on
F_v/F_m in wheat. Exogenous application of TRIA (10 µM)
as a foliar spray at different growth stages reduced
salinity-induced damages by improving ETR in both
wheat cultivars, while it decreased q_N only in cv. MH-97
under saline conditions. It is likely that TRIA-induced
increase of P_N in the two wheat cultivars might be due to
increased efficiency of PSII (increased ETR and
decreased q_N value) under saline and nonsaline condition.
Foliar application of TRIA has been reported to improve
various Chl fluorescence parameters of different plant
species such as *Erythrina variegata* (Muthuchelian *et al.*
2003), mangroves (Moorthy and Kathiresan 1993), and
O. basilicum (Borowski and Blamowski 2009).

Conclusion: foliar spray of TRIA at different growth
stages can reduce the negative influence of salt stress on
wheat plants. This could be associated with TRIA-
induced regulatory role on gas exchange, pigment
contents, membrane permeability, and Chl fluorescence
parameters. The most effective stage for TRIA applica-
tion were V and VB stages. 10 µM TRIA used as a foliar
spray was proved as the most effective in both wheat
cultivars. Salt-tolerant cultivar S-24 was better in growth
and ETR, while the salt-sensitive cultivar MH-97 showed
higher value of membrane permeability and q_N under
saline conditions, but it showed more positive response to
TRIA in terms of E, g_s, and q_P under both stress and
nonstress conditions.

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