

INFLUENCE OF FOLIAR APPLICATION OF TRIACONTANOL ON GROWTH ATTRIBUTES, GAS EXCHANGE AND CHLOROPHYLL FLUORESCENCE IN SUNFLOWER (*HELIANTHUS ANNUUS* L.) UNDER SALINE STRESS

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Abstract

To appraise the effect the foliar application of triacontanol (TRIA) on sunflower (*Helianthus annuus* L.) under saline stress, a pot experiment was conducted under natural conditions. Two sunflower cultivars (SMH-907 and SMH-917) were grown in sand culture medium supplemented with full strength Hoagland's nutrient solution for 25 days, after which time, two saline regimes [control (0 mM) and saline (150 mM)] were applied. Three TRIA levels [0 (water spray), 50 and 100 μ M] were applied as foliar spray on 44-day old plants. Plants were harvested after 4 weeks of TRIA application. The root growing medium saline stress significantly reduced shoot and root fresh weights and their lengths, net CO₂ assimilation rate (*A*), transpiration rate (*E*), water use efficiency (WUE), co-efficient of non-photochemical quenching (*q_N*) and non-photochemical quenching (*NPQ*) in both sunflower cultivars. Foliar-applied TRIA markedly increased shoot and root fresh weights and lengths, *A*, *E*, stomatal conductance and WUE in both cultivars under both saline and non-saline regimes. TRIA did not show prominent effect on chlorophyll fluorescence attributes such as electron transport rate and photochemical quenching of sunflower plants. The TRIA level 100 μ M was more effective as compared to the others and sunflower cultivar SMH-917 showed better performance than those of cv. SMH-907 in terms of different attributes measured in this study.

Introduction

Triacontanol is a potential plant growth regulator which has been reported to significantly affect plant growth and development (Verma *et al.*, 2009; Shahbaz *et al.*, 2013). For example, exogenously applied triacontanol (TRIA) has been shown to enhance growth of many crops including wheat, maize, rice (Naeem *et al.*, 2009; Perveen *et al.*, 2010, 2011). TRIA application not only can enhance yield but also quality characteristics of crops as observed in wheat, tomato, cotton, etc. (Ries, 1985; Naeem *et al.*, 2009). Foliar application of TRIA has been reported to enhance chlorophyll contents, total soluble sugars, protein, nucleic acids, photosynthetic rate and chlorophyll fluorescence in wheat parameters (Perveen *et al.*, 2010) and soybean (Krishnan & Kumari, 2008). TRIA generally can influence the enzymes which regulate growth (Chen *et al.*, 2002) and metabolic processes in plants (Morre *et al.*, 1991). TRIA not only improves crop growth and yield under non-stress conditions, but it also can up-regulate crop growth under stressful environments. For example, exogenously applied TRIA has been reported to play a positive role in enhancing the plant growth in *Erythrina variegata* (Muthuchelian *et al.*, 1996), *Ocimum bacillicum* (Borowski & Blamowski, 2009), and common duck weed (Kilic *et al.*, 2010).

Like in most mesophytes, growth and yield of sunflower (*Helianthus annuus* L.) is adversely affected by salt stress. For example, gas exchange characteristics, proline contents and carbohydrates have been found to be reduced in sunflower under saline conditions (Hassnein *et al.*, 2009). However, from the literature no report was found regarding the role of TRIA in sunflower under saline stress. Thus, the main objective of present study was to assess whether or not foliar application of TRIA could enhance growth, chlorophyll contents and gas exchange characteristics in sunflower under saline stress.

Materials and Methods

An experiment was performed in a net-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 effect of foliar application of TRIA on sunflower (*Helianthus annuus* L.) subjected to saline stress. The climatic conditions were: mean day and night temperature 29°C and 15°C, 9 h and 15 h was light and dark period, respectively. Two sunflower cultivar achenes namely SMH-907 and SMH-917 were obtained from NARC (National Agricultural Research Centre) Islamabad, Pakistan. The experiment was laid out in a completely randomized design with four replications. Twelve achenes were sown in each pot filled with thoroughly washed river sand and allowed them to germinate. In each pot plants were thinned to maintain 6 plants per pot after 21 d of germination. Full strength Hoagland's nutrient solution was applied every week @ two liter per pot to fulfill the nutritional requirement. After 25 d of sowing, two salinity levels [(0 mM (Control) and 150 mM NaCl] prepared in full strength Hoagland's nutrient solution were applied. Salinity level was gradually enhanced as an aliquot of 50 mM per day up to the desired level. Then 150 mM was applied in Hoagland's nutrient solution (full strength) every week @ two liter per pot till the end of the experiment. Three levels of TRIA (0 μ M (control), 50 μ M, and 100 μ M) were applied to 44-d old plants. The TRIA levels were prepared following the method described in Perveen *et al.* (2010). Plants were harvested 72 d after sowing. Two plants per pot were harvested to record shoot and root fresh biomass. The plants were washed with distilled water before recording the biomass. In addition, following attributes were also determined.

Shoot and root lengths (cm): Shoot and root lengths were recorded using a measuring scale.

Gas exchange characteristics: A portable infra-red gas analyzer (ACD LCA-4 Analytical Development, Hoddesdon, UK) was used to measure the gas exchange characteristics. The second intact leaf from top of each plant was used. The gas exchange parameters were measured from 10:30 to 1:00 h using the following specific settings: leaf surface area 6.25 cm^2 , leaf chamber gas flow rate into the leaf chamber (U) $251 \mu\text{mol s}^{-1}$; water vapor pressure ranged from 6.0 to 8.9 mbar, leaf chamber temperature range was 28.4 to 32.4°C ; ambient pressure 98.8 kPa; concentration of ambient CO_2 was $350 \mu\text{mol mol}^{-1}$; molar flow of air per unit leaf area (Us) $22.06 \text{ mol m}^{-2} \text{ s}^{-1}$; PAR (Qleaf) at the leaf surface was up to $942 \mu\text{mol m}^{-2} \text{ s}^{-1}$.

Photosystem II activity: Chlorophyll fluorescence attributes were determined using multi-mode chlorophyll fluorometer (model, OS5P Opti-Sciences, Inc. Winn Avenue Huelson, USA) and determined the coefficient of nonphotochemical quenching (q_N), nonphotochemical quenching (NPQ), photochemical quenching (q_P), efficiency of photosystem-II (F_v/F_m), and electron transport rate (ETR). The measurements were determined by polyphasic rise in fluorescence transients according to Strasser *et al.*, (1995).

Statistical analysis of data: A COSTAT computer software (Cohort Software, Berkeley, CA) was used to analyze the data. Least significance difference test was used according to Snedecor & Cochran (1980) to compare the mean values.

Results

Imposition of saline stress (150 mM NaCl) to the root growing medium caused a significant reduction in shoot fresh weight of two sunflower cultivars, SMH-907 and SMH-917. Foliar-applied various levels of triacontanol (TRIA) (0, 50 and $100 \mu\text{M}$) markedly enhanced shoot fresh weight of both sunflower cultivars under both saline and non-saline conditions (Table 1; Fig. 1). Both sunflower cultivars did not differ significantly in this attribute.

Root fresh weight of both sunflower cultivars decreased significantly under saline regimes (Table 1; Fig. 1). The foliar application of different TRIA levels has a varying effect on the root fresh weight of the two cultivars. For example, $50 \mu\text{M}$ TRIA level was effective for cv. SMH-917 in enhancing root fresh weight under non-saline conditions, while the other TRIA levels remained non-effective to both cultivars under both saline and non-saline conditions.

Saline regimes adversely affected the shoot and root lengths of both sunflower cultivars (Table 1; Fig. 1). Foliar-applied TRIA markedly improved shoot length but it did not have any prominent effect on root length. Although $50 \mu\text{M}$ TRIA significantly increased root length in cv. SMH-917 under non-saline regimes, the other TRIA levels did not show any prominent effect on this growth trait. Of both sunflower cultivars, cv. SMH-907 showed better performance in shoot length as compared to cv. SMH-917 (Table 1; Fig. 1).

Saline stress significantly decreased net CO_2 assimilation rate (A) in both sunflower cultivars. However, the cultivar difference was not prominent with respect to this gas exchange attribute (Table 1; Fig. 1). Foliar application of TRIA caused a substantial increase in photosynthetic rate of both sunflower cultivars under both saline and non-saline conditions, but effect was not prominent on cv. SMH-917 under saline conditions. The increase in photosynthetic rate was significantly high when TRIA was applied @ $100 \mu\text{M}$ under both saline regimes.

Transpiration rate (E) of the two sunflower cultivars decreased significantly under various saline regimes. Although $50 \mu\text{M}$ TRIA caused a significant increase in transpiration rate in cv. SMH-917 under non-saline regimes, the other TRIA levels did not show any prominent effect on this attribute. Both sunflower cultivars showed a uniform performance in transpiration rate (Table 1; Fig. 1).

Root medium applied saline stress did not affect stomatal conductance (g_s) of the two sunflower cultivars i.e. SMH-907 and SMH-917 (Table 1; Fig. 1). Foliar applied TRIA slightly enhanced g_s in cv. SMH-907 under both saline and non-saline conditions, while $100 \mu\text{M}$ TRIA caused a substantial reduction in cv. SMH-917. Overall, the difference between both cultivars was non-significant with respect to g_s .

Sub-stomatal CO_2 concentration (C_i) and relative intercellular CO_2 (C_i/C_a) in both sunflower cultivars showed almost uniform values under saline regimes (Table 1; Fig. 1). Exogenous application of TRIA as foliar spray did not alter both C_i and C_i/C_a in the sunflower plants. Of both cultivars, cv. SMH-917 was higher than cv. SMH-907 in both gas exchange attributes.

Root growing medium salinity adversely affected water use efficiency (WUE) in sunflower cultivars SMH-917, while the effect was not prominent in cv. SMH-907 (Table 1; Fig. 1). Foliar application of TRIA significantly enhanced WUE of both sunflower cultivars under non-stress and salt stress conditions. Of various TRIA levels, $100 \mu\text{M}$ TRIA caused a marked increase in WUE of the sunflower plants under both saline regimes.

Maximum quantum yield of PSII (F_v/F_m) in both sunflower cultivars increased significantly under saline stress (Table 1; Fig. 2). Although TRIA had a significant effect, the effect was not uniform in both sunflower cultivars under saline regimes. Of both cultivars, SMH-907 was slightly higher as compared to cv. SMH-917 in F_v/F_m .

Root growing medium saline stress slightly increased electron transport rate (ETR) in cv. SMH-917 while the effect was not prominent in cv. SMH-907. Of both cultivars, cv. SMH-907 was superior to cv. SMH-917 under non-saline conditions, however, both cultivars responded uniformly under saline stress. The foliar application of TRIA did not affect this attribute under either saline or non-saline environment (Table 1; Fig. 2). Foliar-applied TRIA as well as root growing medium saline stress had non-significant effect on photochemical quenching (q_P) of the sunflower cultivars (Table 1; Fig. 2). The cultivars showed a significant difference as cv. SMH-917 showed higher value of q_P than that in cv. SMH-907.

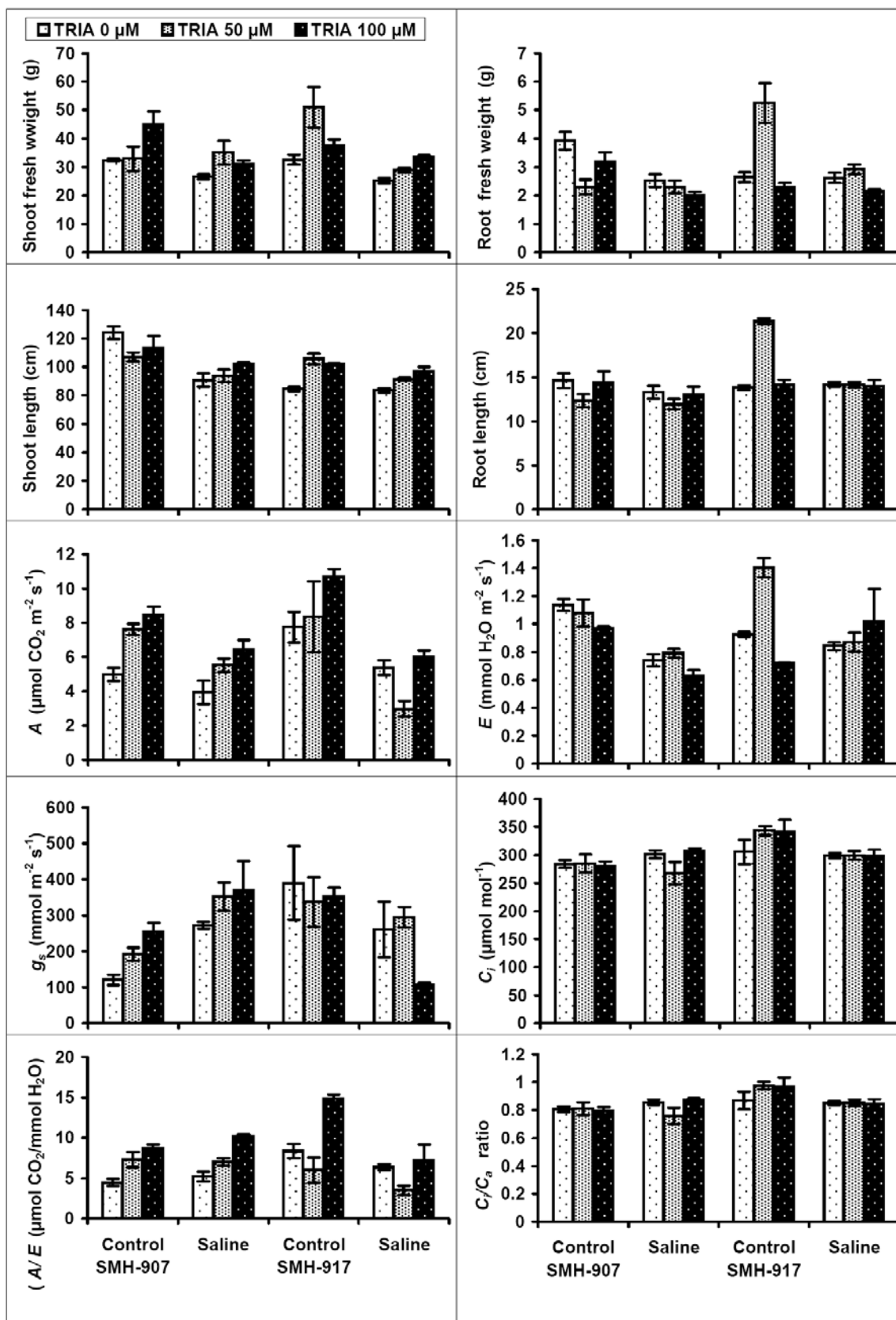


Fig. 1. Growth and gas exchange attributes of sunflower (*Helianthus annuus* L.) when plants were foliary applied with triacontanol under saline or non-saline conditions.

Table 1. Mean squares from analyses of variance of data for growth attributes, gas exchange and chlorophyll fluorescence of sunflower (*Helianthus annuus* L.) when plants were foliary applied with triacontanol under saline or non-saline conditions.

Source of variations	df	Shoot fresh weight	Root fresh weight	Shoot length	Root length	A
Cultivars (Cvs)	1	10.97ns	0.923ns	1476.7***	48.70***	5.689ns
Salinity (S)	1	868.1***	8.739***	2019.9***	34.49***	103.0***
Triacontanol (TRIA)	2	316.8**	2.587**	241.9*	5.261ns	24.57***
Cvs × S	1	85.94ns	0.001ns	491.7**	5.063ns	17.66*
Cvs × TRIA	2	79.36ns	6.873***	482.1**	39.16***	9.134*
S × TRIA	2	12.28ns	0.306ns	85.05ns	13.16**	4.584ns
Cvs × S × TRIA	2	302.6**	4.169***	300.0*	22.77***	1.005ns
Error	36	39.52	0.334	59.93	1.940	2.39
Source of variations	df	E	g _s	C _i	A/E	C _i /C _a
Cultivars (Cvs)	1	0.061ns	10740.0ns	8957.1***	3.834ns	0.072***
Salinity (S)	1	0.603***	24.08ns	1558.3ns	33.90**	0.012ns
Triacontanol (TRIA)	2	0.167**	4743.5ns	402.0ns	95.71***	0.003ns
Cvs × S	1	0.168*	238290.0***	5008.2*	66.04***	0.040*
Cvs × TRIA	2	0.066ns	45433.0*	1257.8ns	27.36***	0.010ns
S × TRIA	2	0.150**	15666.0ns	1331.0ns	6.183ns	0.010ns
Cvs × S × TRIA	2	0.196**	6320.5ns	740.7ns	14.53*	0.006ns
Error	36	0.028	10789.7	679.8	3.185	0.005
Source of variations	df	Fv/Fm	ETR	q _p	q _N	NPQ
Cultivars (Cvs)	1	0.120***	15.84**	0.328***	0.044***	0.034**
Salinity (S)	1	0.238***	52.71***	0.002ns	0.07***	0.146***
Triacontanol (TRIA)	2	0.051***	3.12ns	0.006ns	0.016**	0.015*
Cvs × S	1	0.029***	102.0***	0.014*	8.585ns	0.014*
Cvs × TRIA	2	0.050***	2.689ns	0.007*	0.009*	5.825ns
S × TRIA	2	0.051***	3.719ns	0.001ns	0.002ns	0.023**
Cvs × S × TRIA	2	0.112***	60.57***	0.001ns	0.003ns	0.018**
Error	36	0.002	1.658	0.002	0.002	0.003

*, **, *** = Significant at 0.05, 0.01, and 0.001 levels, respectively
ns = Non-significant

Salt stress markedly reduced co-efficient of non-photochemical quenching (q_N) and non-photochemical quenching (NPQ) in both sunflower cultivars (Table 1; Fig. 2). Foliar applied TRIA caused a slight decrease in both attributes in both sunflower cultivars under both saline and non-saline conditions except in cv. SMH-917 under saline conditions where TRIA treatment slightly increased both the above-mentioned attributes.

Discussion

Triacontanol (TRIA) is an effective plant growth regulator which can significantly enhance plant growth (Khan *et al.*, 2009; Naeem *et al.*, 2009; Perveen *et al.*, 2010, 2011, 2012b; Shahbaz *et al.*, 2013). In the present study, the foliar application of TRIA (50 µM and 100 µM) enhanced growth, measured as shoot fresh and dry weights, in both sunflower cultivars (SMH-907 and SMH-917) under both saline and non-saline conditions. Foliar-applied TRIA-induced enhancement in growth has already been observed in several crops like ginger (Singh *et al.*, 2012), hyacinth beans (Naeem *et al.*, 2009), tomato (Khan *et al.*, 2009), mungbean (Reddy *et al.*, 2002), rice (Pandey *et al.*, 2001), etc. TRIA-induced improvement in growth of sunflower as observed in the present study might be due to one of the many reasons such as up-regulation of many genes related to photosynthesis (Singh *et al.*, 2012), modulation in activities of antioxidants (Perveen *et al.*, 2011) by triggering a secondary messenger or a metabolite

which is responsible for activity of those enzymes involved in carbohydrate metabolism and many physiological responses (Naeem *et al.*, 2009). In our current study, saline stress markedly reduced the growth of sunflower plants which might be due to hormonal imbalance (Babu *et al.*, 2012), osmotic stress (Shabala *et al.*, 2012), and toxicity of specific ions e.g. Na⁺ and Cl⁻ (Shahbaz & Zia, 2011; Shahbaz *et al.*, 2011; Babu *et al.*, 2012).

Saline stress adversely affected gas exchange attributes in both sunflower cultivars. Salinity-induced reduction in gas exchange attributes has already been observed in many crops such as canola (Shahbaz *et al.*, 2013), sunflower (Akram *et al.*, 2009, 2012), radish (Noreen *et al.*, 2012), wheat (Kanwal *et al.*, 2011; Perveen *et al.*, 2012a; Kausar & Shahbaz, 2013; Shahbaz & Ashraf, 2013), etc. The decreased photosynthetic rate may be due to closure of leaf stomata which is caused by toxic Na⁺ and Cl⁻ ions (Tavakkoli *et al.*, 2011) and the reduced leaf expansion may cause build-up of undesired photosynthates in young tissues which act as signals in the process of down-regulating photosynthesis (Munns & Tester, 2008). In the present study, the foliar application of TRIA enhanced net CO₂ assimilation rate (A), water use efficiency (A/E) and transpiration rate (E) in both sunflower cultivars under both saline and non-saline conditions. TRIA application is effective in enhancing net CO₂ assimilation rate. However, the underlying mechanism by which TRIA accelerates rate of photosynthesis is not clear yet. One of the possible

reasons is the role of TRIA in up-regulating Rubisco activity (Houtz *et al.*, 1985). For example, Chen *et al.* (2002) observed the up-regulation of *rbc* gene by TRIA application in rice seedlings which was ascribed to enhanced rate of photosynthesis. Increase in photosynthetic rate by exogenous application of TRIA has also been observed in opium poppy (Srivastava & Sharma, 1990), wheat (Perveen *et al.*, 2010), and canola (Shahbaz *et al.*, 2013).

Chlorophyll fluorescence is also considered as one of the potential indicators of stress tolerance including salt tolerance (Mehta *et al.*, 2010). In our experiment, saline stress significantly reduced the co-efficient of non-photochemical quenching (q_N) and non-photochemical quenching (NPQ) and increased maximum quantum yield of PSII and electron transport ratio (ETR). Although TRIA induced improvement in different chlorophyll fluorescence attributes has been reported in different plant species like *Erythrina variegata* (Muthuchelian *et al.*, 2003), mangrove seedlings (Moorthy & Kathiresan, 1993), and *Ocimum basilicum* (Borowski & Blamowski 2009). In our study, exogenous application of TRIA as foliar spray either did not affect chlorophyll fluorescence attributes or if affected, the effect being inconsistent in the two sunflower cultivars. Similar findings were also observed by Shahbaz *et al.*, (2013) in canola when TRIA was applied as pre-seed treatment under saline conditions. They observe slight increase in q_P and ETR under saline conditions, and non-significant effect of TRIA on F_v/F_m and q_N in canola.

In conclusion, saline stress reduced growth attributes, gas exchange characteristics and efficiency of photosystem-II. However, exogenous application of TRIA as foliar spray significantly enhanced growth, net CO_2 assimilation rate, stomatal conductance and water use efficiency in both sunflower cultivars. TRIA slightly enhanced efficiency of photosystem-II. Of all TRIA levels used, 100 μM was the most effective in regulating all the earlier mentioned attributes. Overall, cultivar SMH-917 showed better performance than cv. SMH-907 in response to TRIA application and salt stress.

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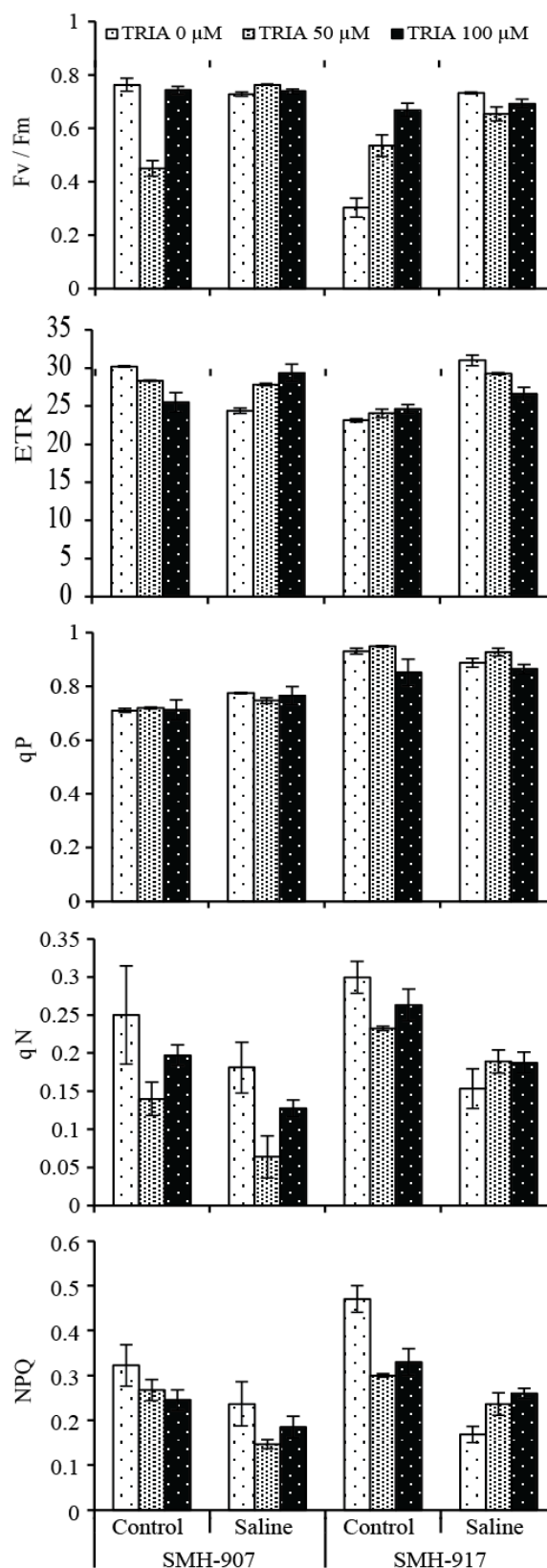


Fig. 2. Leaf chlorophyll fluorescence of sunflower (*Helianthus annuus* L.) when plants were foliarly applied with triacontanol under saline or non-saline conditions.

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