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RESEARCH ARTICLE

Triacontanol modulates photosynthesis and osmoprotectants in canola (*Brassica napus* L.) under saline stress

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An experiment was conducted to assess the effect of pre-sowing seed treatment with triacontanol (TRIA) in canola (*Brassica napus* L.) cultivar (RBN-3060) under saline stress. Canola seeds were soaked in three levels of TRIA (0, 0.5, and 1 mg L^{-1}) for 12 hours. Three levels of salt stress (0, 100, and 150 mM NaCl) in full strength Hoagland's nutrient solution were applied to 56-days-old plants. Salt stress caused a significant reduction in growth, gas exchange, photochemical quenching (*qP*), and shoot and root K^+ contents, while increased leaf glycine betaine, free proline, and shoot Na^+ contents. Pre-sowing seed treatment with TRIA increased shoot fresh weight, number of seeds per plant, photosynthetic rate, transpiration rate, ratio of chlorophyll *a*/*b*, *qP*, electron transport rate, shoot and root K^+ contents, and free proline and glycine betaine contents of canola plants at various TRIA levels under nonsaline or saline conditions.

Keywords: canola; mineral nutrients; proline; salinity; triacontanol (TRIA)

Introduction

Salinity stress reported to reduce plant growth mainly due to osmotic stress (Shabala et al. 2012), specific ion (Na^+ and Cl^- ion) toxicity (interference in essential nutrients uptake; Shahbaz et al. 2011; Babu et al. 2012), and hormonal imbalance (Babu et al. 2012). However, plants escape salt stress by various adaptive mechanisms (multigenic in nature) including selective accumulation or exclusion of ions; synthesis of compatible solutes (e.g. proline and glycine betaine); induction of antioxidant defense system that protects plants from oxidative stresses by scavenging oxygen-free radicals; and adaptive regulation of stress-related hormones such as abscisic acid, ethylene, and jasmonic acid (Parida & Das 2005; Ashraf et al. 2012; Shahbaz & Zia 2011).

Canola (*Brassica napus* L.) is the most significant crop due to importance for its high-quality oil, but its production is noticeably decreased under environmental stresses including salt stress. It is a moderately salt-tolerant crop (Francois 1994). In view of increasing awareness of the health advantages of canola oil and existing salt tolerance potential, its demand has undoubtedly increased during the last two decades. This has resulted in increased cultivation of canola on soils where salinity problems already exist. Thus, there is a need for further improvement in the salt tolerance of canola (Francois 1994; Ashraf & McNeilly 2004). Farhoudi and Sharifzadeh (2006) reported that pre-sowing seed treatment with NaCl stimulates seed germination, seedling emergence, and growth in canola.

Phytohormones have been reported playing regulatory role in growth, development, reproduction, programmed cell death, and survival under changing environmental conditions (Sharma et al. 2005). Abiotic stresses such as salinity stress alter level of plant growth hormones, resulting in reduced growth and final yield of crop plants (Gupta et al. 1993; Javid et al. 2011). Hormone priming is one of the seed treatment techniques that enhance abiotic stress tolerance through major physiological and biochemical changes inside the seed (Hela et al. 2012). It is simple, low-cost, and environmentally friendly technique (Shahbaz et al. 2012). Among major factors that affect response of pre-sowing seed treatment to salt stress include seed maturation time period, suitable priming media, precise effective concentration, and priming period (Kazemi & Eskandari 2012).

Triacontanol (TRIA) is a plant hormone (Ries et al. 1977) that stimulates plant growth at very low concentration when exogenously applied to various plant species like groundnut (Verma et al. 2009), chickpea (Singh et al. 1991), pigeonpea (Pujari et al. 1998), rice, maize, and wheat (Perveen et al. 2011). TRIA has been reported to enhance photosynthesis (Eriksen et al. 1981) and water and mineral nutrients uptake (Ries 1985; Ivanov & Angelov 1997; Chen et al. 2003), regulate activities of various enzymes (Naeem et al. 2011), and increase the level of various organic compounds in leaf tissues (Kumaravelu et al. 2000; Chen et al. 2002, 2003). Under saline conditions, TRIA has been reported to enhance growth, biomass, photosynthetic pigments, and uptake of K^+ and Ca^{2+} essential

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mineral contents instead of sodium (Banuelous & Bangerth 1986; Muthuchelian et al. 1996).

Seed treatment with TRIA on various crops has variable effects like Cavusoglu et al. (2007, 2008) have reported that seed treatments with TRIA induce salt stress tolerance in radish and barley seedlings while nonsignificant effect was observed by Perveen et al. (2010, 2011, 2012a, 2012b) on wheat. Reports about the pre-sowing seed treatments of TRIA on canola are scarce in literature. It was hypothesized that pre-sowing seed treatment with TRIA could mitigate the malicious effects of salinity stress on canola. The major objective of the present study was to evaluate the TRIA-induced modulation in various growth, physiological, and biochemical attributes of canola plants under saline stress.

Materials and methods

The present study was conducted to assess the response of canola (*Brassica napus* L.) to salinity by seed pre-treatment with TRIA. Seeds of canola cultivar (RBN-3060) were obtained from Ayub Agricultural Research Institute, Faisalabad. The study was carried out in the wire house of Botanical Garden, Department of Botany, University of Agriculture, Faisalabad during the year 2010–2011 under natural climatic conditions. Seeds were soaked in different levels of TRIA (i.e. 0, 0.5, 1.0 mg L^{-1}) for 12 hours. Fifteen seeds of canola were directly sown in each plastic pot (23 cm in diameter and 29 cm deep) containing 10 kg of well-washed river sand. Full-strength Hoagland's nutrient solution was applied to fulfill nutrients requirement. After germination, the plants were thinned so as to maintain four plants of almost uniform size per pot. Three salt stress levels 0, 100, and 150 mM were applied in full-strength Hoagland's nutrient solution through rooting medium on 56-days-old plants. The data for various growth, physiological, and biochemical attributes like gas exchange characteristics and fluorescence and chlorophyll contents were recorded after three weeks of salt treatment. Two plants per pot were uprooted carefully from each pot, thoroughly washed with distilled water and their fresh weights were recorded. The samples were oven-dried at 65°C for 72 hours to record their dry weights, and means of both plants were taken as data for each replicate. At maturity yield parameters like yield per plant (g), 100-seed weight (g) and number of seeds per plant were recorded.

Gas exchange characteristics

Various gas exchange attributes such as g_s = stomatal conductance (mmol m^{-2} s^{-1}), C_i = sub-stomatal conductance (µmol mol^{-1}), A = net CO_2 assimilation rate (µmol CO_2 m^{-2} s^{-1}), E = transpiration rate (mmol H_2O m^{-2} s^{-1}), and A/E = net CO_2 assimilation rate/transpiration rate (µmol CO_2/mmol H_2O) were made on third leaf from top of each plant. The readings were made from 11:00 am to 1:30 pm with the following specification/adjustments: leaf surface area 6.25 cm^2, ambient CO_2 level (Cref) 354.4 µmol mol^{-1}, leaf chamber temperature varying from 31.5°C to 37.8°C, leaf chamber gas flow rate (v) 392.8 mL min^{-1}, molar flow of air per unit leaf area (Us) 404.84 mol m^{-2} s^{-1}, ambient pressure (P) 99.2 kPa, water vapor pressure (e ref) into chamber ranging from 20.5 to 23.1 mbar, and Q leaf 1048 µmol m^{-2} s^{-1}.

Photosynthetic pigments

The chlorophyll 'a' and 'b' were determined with the method as described by Arnon (1949) method. The fresh leaves were cut into 0.5 cm segments and extracted overnight in 5 mL 80% acetone at −10°C. The extract was centrifuged at 14000 x *g* for 5 minutes, and the absorbance of the supernatant was read at 645 and 663 nm using a spectrophotometer (IRMECO U2020, Germany).

The chlorophyll *a* and *b* were calculated by the following formulae:

$$\text{Chl. } a \text{ (mg g}^{-1}\text{ f.wt)} = [12.7\ (\text{OD } 663) - 2.69\ (\text{OD } 645)] \times \text{V}/1000 \times \text{W}$$

$$\text{Chl. } b \text{ (mg g}^{-1}\text{ f.wt)} = [22.9\ (\text{OD } 645) - 4.68\ (\text{OD } 663)] \times \text{V}/1000 \times \text{W}$$

$$\text{Carotenoid (mg mL}^{-1}\text{)} = \text{A}$$

W = weight of the fresh leaf tissue (g)

V = volume of the extract (mL)

Photosystem II activity

Fluorescence parameters were calculated by the method of Schreiber et al. (1986) and Genty et al. (1989). Before measurement, the leaf samples were kept in darkness for 15 minutes. Actinic light of 400 µmol $m^{-2}s^{-1}$ and saturating pulse light of 8000 µmol $m^{-2}s^{-1}$ were used. Fluorescence was measured with the multi-mode chlorophyll fluorometer (model, OS5P Opti-Sciences, Inc. Winn Avenue Huolson, USA) and determined the coefficient of nonphotochemical quenching (*qN*), nonphotochemical quenching (*NPQ*), photochemical quenching (*qP*), efficiency of photosystem-II (*Fv*/*Fm*), and electron transport rate (*ETR*).

Free proline determination

The free proline was estimated according to the method of the Bates et al. (1973). Fresh leaf tissues (0.5 g) were homogenized in 10 mL 3% sulfosalicylic acid and filter through Whatman filter paper. The extract (2 mL) was reacted with 2 mL acid ninhydrin solution (1.25 g ninhydrin in 30 mL glacial acetic acid and 20 mL 6 M orthophosphoric acid) and 2 mL of glacial acetic acid in a test tube for 1 hour at 100°C. The reaction was terminated in an ice bath. The reaction mixture was extracted with 4 mL toluene, mixed vigorously by shaking the mixture for 1 minute. The chromophore containing toluene was

aspirated from the aqueous phase, warmed at room temperature, and absorbance was read at 520 nm using toluene as a blank. The proline concentration was determined from a standard curve and calculated on fresh weight basis as follows:

$$\text{Proline } (\mu\text{mol g}^{-1}\text{ f.wt.}) = (\text{g proline mL}^{-1} \times \text{mL of toluene}/115.5)/(\text{g of sample}).$$

Glycine betaine determination

Glycine betaine was determined following Grieve and Grattan (1983) method. Fresh leaf material (0.5 g) was grinded in 10 mL distilled water and filtered. After filtration, 1 mL of the extract was mixed with 1 mL of 2N H_2SO_4 and 0.2 mL potassium triiodide and cooled in an ice bath for 90 minutes. Then 2.8 mL of the ice cooled distilled water and 6 mL of 1-2 dichloroethane (cooled at -10°C) were added to the mixture. The samples were vortexed in test tubes for 1 minute. Two layers were formed, the upper aqueous layer was discarded and optical density of the lower (organic layer) layer was measured at 365 nm. The concentration of glycine betaine was calculated against standard curve.

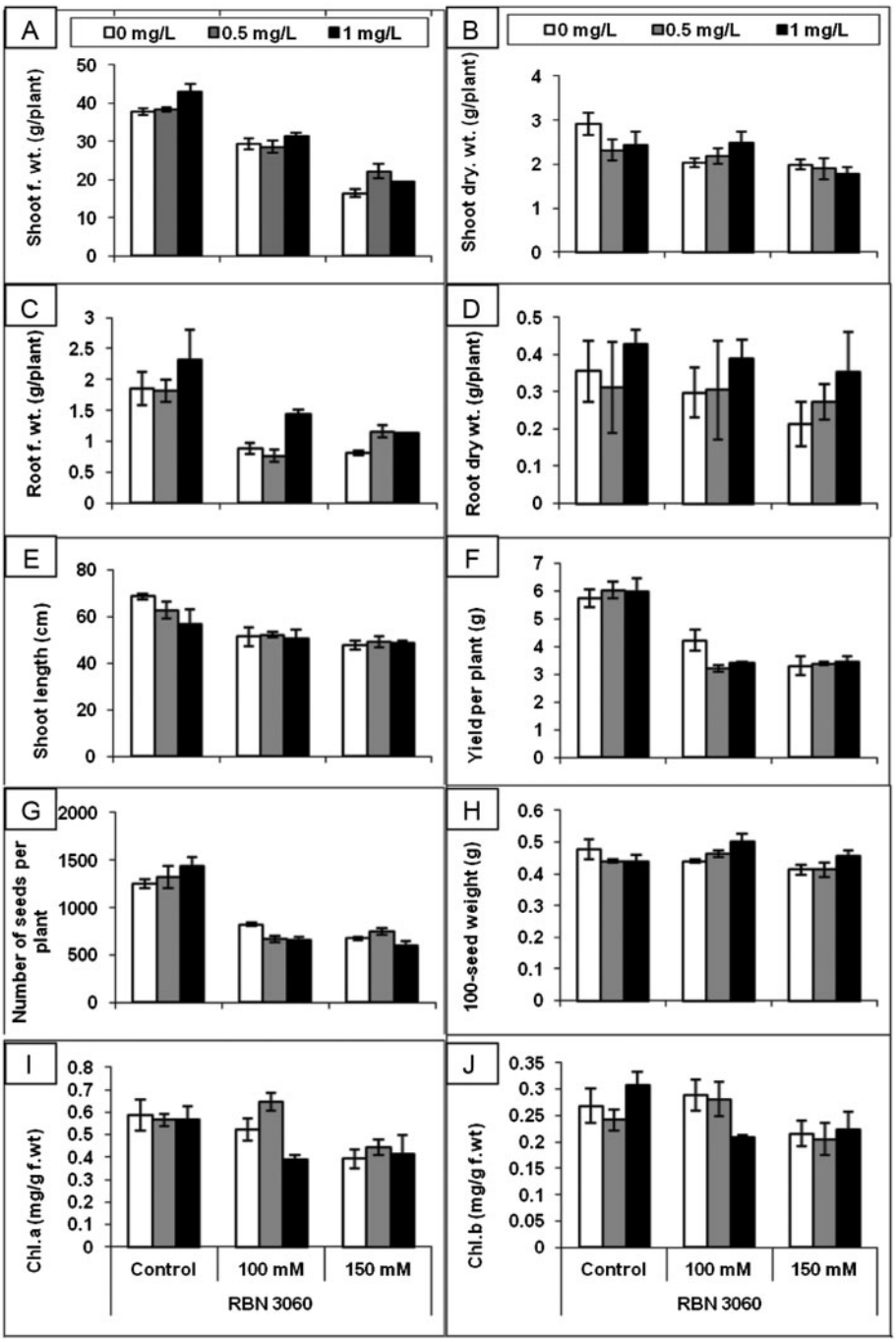

Figure 1. Growth and chlorophyll pigments of canola (*Brassica napus* L.) when 12 hours pre-treated seeds with triacontanol were subjected to grow for 3 weeks under saline conditions.

Mineral contents

The oven-dried ground material of both shoots and roots was digested with digestion mixture following Allen et al. (1986). The dry ground material (0.1 g) of shoots or roots was digested with 1.5 mL of digestion mixture at 200°C on hot plate using $HCIO_4$ (35%). The volume of the digested material was maintained up to 50 mL with deionized water. The extract was filtered and used for the estimation of sodium, potassium, and calcium ions. All three minerals (Ca^{2+}, Na^{+}, and K^{+}) were determined by using flame photometer (Jeneway, PFP-7, UK).

Statistical Analysis

Costat software was applied for carrying out statistical analysis of variance of data collected during experiment. The mean data were compared with completely randomized design with four replicates (Steel & Torrie 1980).

Results

Application of different salt levels (0, 100, and 150 mM) significantly reduced growth and yield attributes like shoot and root fresh and dry weights (Figure 1A–D), shoot length (Figure 1E), yield and number of

Table 1. Growth, chlorophyll contents, gas exchange characteristics, chlorophyll fluorescence, free proline, glycine betaine, and shoot and root mineral contents of canola (*Brassica napus* L.) when 12 hours pre-treated seeds with triacontanol were subjected to grow for 3 weeks under saline conditions.

Source of variation	df	Shoot fresh weight	Shoot dry weight	Root fresh weight	Root dry weight	Shoot length
Salinity (S)	2	1158.6***	2.106***	1.264***	0.050ns	702.8***
Triacontanol (TRIA)	2	22.02*	0.042ns	0.102ns	0.008ns	18.16ns
S × TRIA	4	15.27ns	0.164ns	0.031ns	0.002ns	13.79ns
Error	27	5.755	0.134	0.052	0.015	20.23
Source of variation	df	Yield per plant	100-Seed weight	Number of seeds/plant	Chl. *a*	Chl. *B*
Salinity (S)	2	23.44***	0.005*	1657***	0.076**	0.011*
Triacontanol (TRIA)	2	0.138ns	0.003ns	1141ns	0.027ns	0.0007ns
S × TRIA	4	0.549ns	0.003ns	4519*	0.020ns	0.006ns
Error	27	0.303	0.002	1402	0.010	0.003
Source of variation	df	Chl *a*/*b* ratio	*A*	*E*	g_s	*Ci*
Salinity (S)	2	0.333ns	16.05***	0.159***	1579**	1385ns
Triacontanol (TRIA)	2	0.466*	2.691*	0.080**	2614ns	753.6ns
S × TRIA	4	0.246ns	3.136**	0.291***	3047ns	560.1ns
Error	27	0.133	0.600	0.0112	1804	488.2
Source of variation	df	*Ci*/*Ca*	WUE	*Fv*/*Fm*	*ETR*	*qP*
Salinity (S)	2	0.011ns	18.01***	0.050ns	21.17ns	0.020**
Triacontanol (TRIA)	2	0.006ns	8.555**	0.012ns	18.00ns	0.006ns
S × TRIA	4	0.004ns	9.851***	0.022ns	89.34*	0.019***
Error	27	0.004	1.042	0.031	26.91	0.003
Source of variation	df	*qN*	*NPQ*	Proline	Glycinebetaine	Shoot Na^{+}
Salinity (S)	2	0.0000ns	0.012ns	1422***	1449***	4118***
Triacontanol (TRIA)	2	0.0014ns	0.006ns	1194ns	1919***	196.5ns
S × TRIA	4	0.001ns	0.002ns	6979**	2840ns	360.0ns
Error	27	0.002	0.004	1673	1907	382.8
Source of variation	df	Root Na^{+}	Shoot K^{+}	Root K^{+}	Shoot Ca^{2+}	Root Ca^{2+}
Salinity (S)	2	8125**	4450***	1210.5***	13.75ns	10.05ns
Triacontanol (TRIA)	2	969.4ns	2847***	498.0*	11.13ns	9.26ns
S × TRIA	4	1040ns	1079*	874.8***	8.67ns	8.35ns
Error	27	1284	300	126.7	5.62	8.43

ns = nonsignificant; df = degrees of freedom; Chl. *a* = chlorophyll *a*; Chl. *b* = chlorophyll *b;* Chl. a/*b* = chlorophyll *a*/*b* ratio; *Fv*/*Fm* = efficiency of photosystem-II; *qP* = photochemical quenching; *qN* = coefficient of nonphotochemical quenching; *NPQ* = nonphotochemical quenching; *ETR* = electron transport rate.
*Significant at 0.05 level.
**Significant at 0.01 level.
***Significant at 0.001 level.

seeds per plant (Figure 1F and G) and 100-seed weight (Figure 1H) of canola cultivar RBN-3060 (Table 1). Pre-sowing seed treatment with TRIA at 1 mg L^{-1} level significantly increased only shoot fresh weight (Figure 1A) and number of seeds per canola plant (Figure 1G) under nonsaline conditions (Table 1).

Imposition of saline stress through rooting medium caused a significant reduction in chlorophyll *a* (Figure 1I) and *b* contents (Figure 1J) particularly at 150 mM, while chlorophyll *a*/*b* ratio remained uniform in canola cultivar RBN-3060 (Figure 2A). Pre-sowing seed treatment with TRIA did not show a significant effect on chlorophyll contents (Table 1); however, chlorophyll *a*/*b* ratio significantly increased at 0.5 mg L^{-1} TRIA level under 100 mM salt stress level (Figure 2A).

Gas exchange characteristics like net CO_2 assimilation rate (*A*), transpiration rate (*E*), stomatal conductance, and water use efficiency (*A*/*E*) significantly decreased, while sub-stomatal CO_2 concentration (C_i) and relative intercellular CO_2 (C_i/Ca) remained unchanged under varying levels of salt stress (Table 1). Pre-sowing seed treatment with TRIA particularly at 1 mg L^{-1} significantly increased photosynthetic rate (*A*; Figure 2B) and transpiration rate (*E*; Figure 2C) under nonsaline conditions, while

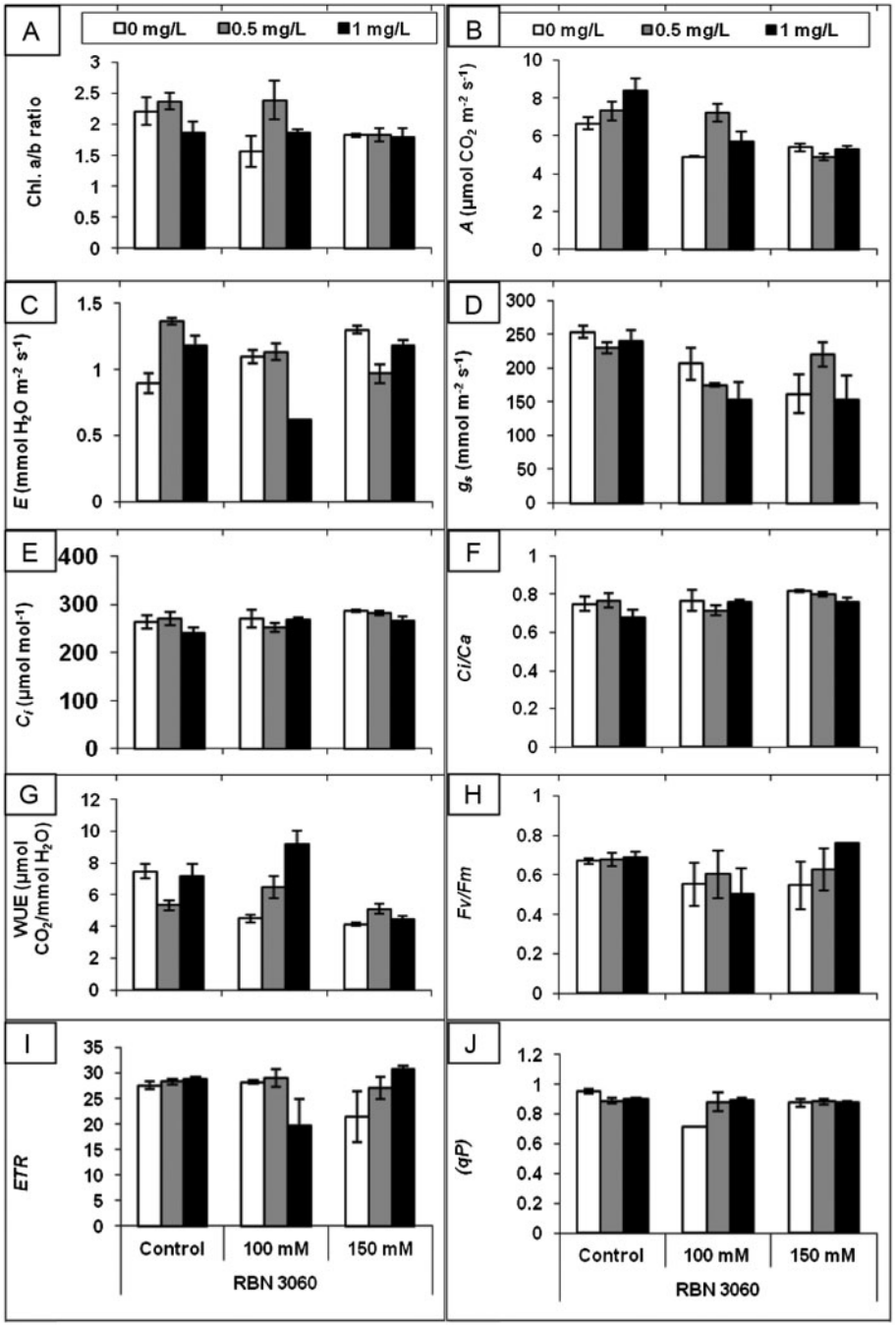

Figure 2. Chlorophyll *a*/*b* ratio, gas exchange characteristics, and chlorophyll fluorescence attributes of canola (*Brassica napus* L.) when 12 hours pre-treated seeds with triacontanol were subjected to grow for 3 weeks under saline conditions.

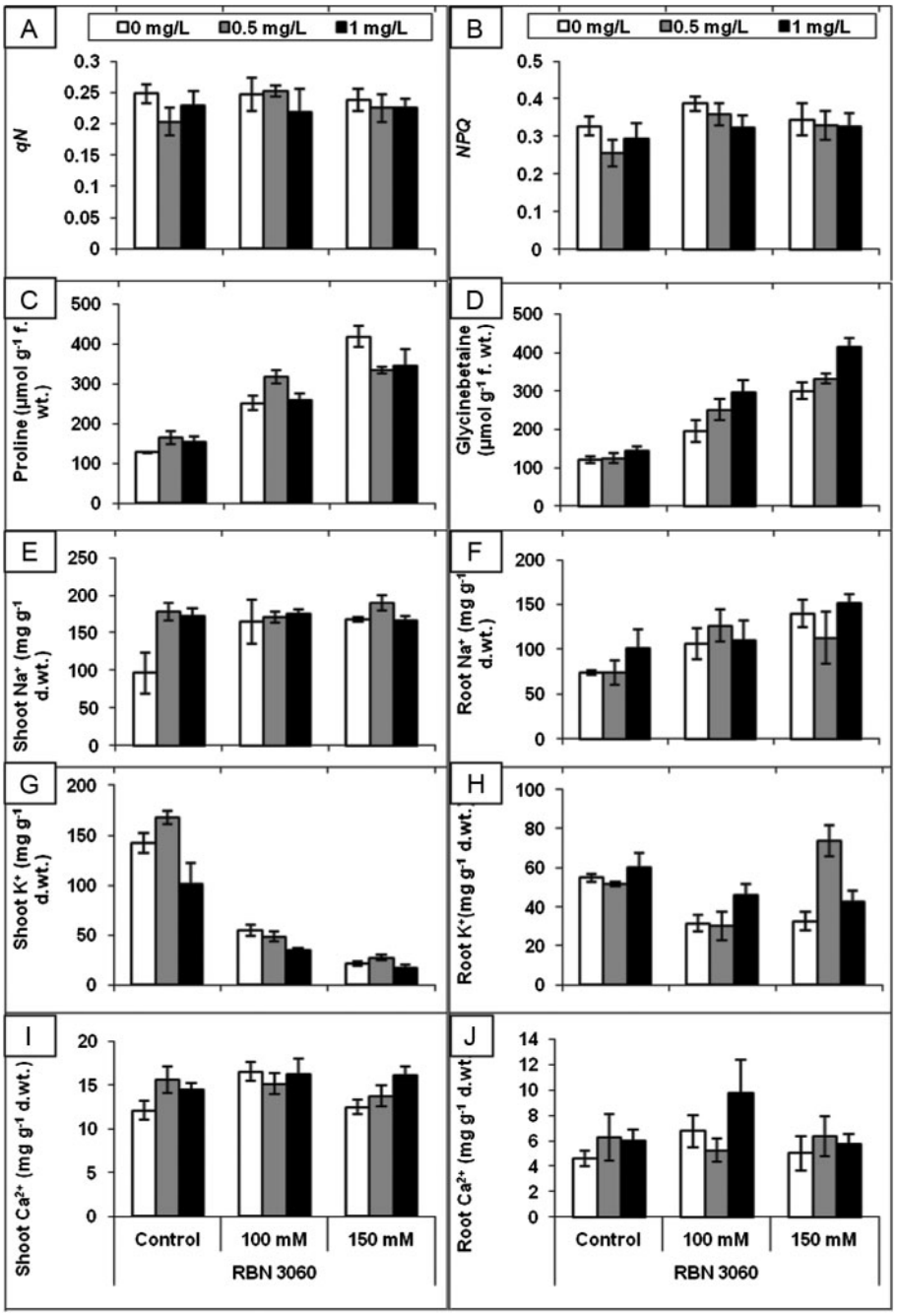

Figure 3. Chlorophyll fluorescence, free proline, glycine betaine, and shoot and root mineral contents of canola (*Brassica napus* L.) when 12 hours pre-treated seeds with triacontanol were subjected to grow for 3 weeks under saline conditions.

water use efficiency (*WUE*; Figure 2G) under saline (100 mM) conditions (Table 1).

Varying levels of salt stress showed nonsignificant effect on efficiency of PS-II (F_v/F_m; Figure 2H), *ETR* (Figure 2I), *qN* (Figure 3A) and *NPQ* (Figure 3B), while significantly decreased *qP* (Figure 2J). Pre-treatment of canola seeds with TRIA significantly increased only *qP* under lower (100 mM) salt level and *ETR* under higher (150 mM) salt level (Table 1). Of various TRIA levels, 1 mg L^{-1} TRIA was more effective as compared to others.

Osmoprotectants (proline and glycine betaine) accumulation in canola leaves significantly increased under varying levels of salinity stress (Table 1). Pre-sowing seed treatment with TRIA significantly increased leaf free proline (Figure 3C) under nonsaline conditions, while glycine betaine was increased (Figure 3D) under both saline and nonsaline conditions (Table 1).

Shoot and root Na^+ in canola cultivar RBN-3060 significantly increased under various saline regimes (Table 1). However, pre-sowing seed treatment with TRIA did not alter shoot and root Na^+ contents significantly under both saline and nonsaline conditions (Figure 3E and F). Shoot and root K^+ of canola cultivar were significantly reduced due to salt stress (Figure 3G and H). Pre-sowing seed treatment with TRIA and the interactive effect of salinity and TRIA

showed significant effect on shoot and root K^+. TRIA at 0.5 mg L^{-1} significantly improved the shoot and root K^+ at 150 mM NaCl (Figure 3G and H). Varying levels of salt stress as well as TRIA showed nonsignificant effect on shoot and root Ca^{2+} in canola cultivar (Table 1).

Discussion

TRIA is a plant hormone that has been known to regulate various growth processes under normal or stress conditions (Naeem et al. 2011; Verma et al. 2011). It is known to enhance uptake of water, cell division, cell elongation, and permeability of membranes (Hangarter et al. 1978). Pre-sowing seed treatment with TRIA has been reported to stimulate plant growth under saline conditions in seedlings of barley (Cavusoglu et al. 2007) and radish (Cavusoglu et al. 2008). However, in the present study pre-sowing seed treatment with different levels of TRIA did not ameliorate adverse effects of salt stress on canola plants except that at 1 mg L^{-1} shoot fresh weight and number of seeds per plant improved but only under nonsaline conditions. Charlton et al. (1980) reported that seed treatment with TRIA and its derivatives did not improve germination and growth in Leeds durum wheat. Similarly, Perveen et al. (2010, 2011, 2012a) reported that pre-sowing seed treatment with TRIA did not alter growth, contents of total soluble proteins, and activities of antioxidant enzymes (catalase and superoxide dismutase) in wheat under saline conditions. Although mechanism of TRIA action is not clearly known, however, Houtz et al. (1985) reported that TRIA stimulate plant growth through increased Rubisco activity. Furthermore, Chen et al. (2002) reported increased *RBC* genes expression in rice that encode Rubisco, a major enzyme of photosynthesis. In current study, pre-sowing seed treatment with TRIA did not stimulate growth under salt stress that could be due its complex mechanism of action. For example, Hoagland (1980) reported that TRIA could not induce growth in various weed and horticulture crops due to lack of its effective penetration through seed coat.

It is generally known that photosynthetic capacity depends on photosynthetic pigments such as chlorophyll *a* and *b*, and salt-induced reduction in photosynthesis can be attributed to a decrease in chlorophyll contents (Delfine et al. 1999). This is in agreement with current study in which salt stress caused a substantial decline in chlorophyll contents of canola cultivar RBN-3060, but pre-treated seed with varying levels of TRIA did not alter chlorophyll contents under saline or nonsaline conditions except that chlorophyll *a*/*b* ratio under 100 mM salt stress level by seed treatment at 0.5 mg L^{-1} TRIA level. One of the reasons for nonsignificant effect on growth is that it did not affect chlorophyll contents causing nonsignificant effect on photosynthetic activity and ultimately to plant growth (Miniraj & Shanmugavelu 1987).

In our experiment, salt stress significantly reduced gas exchange attributes in canola cultivar RBN-3060. Similar pattern was observed by Akram et al. (2009) in sunflower. The decrease in photosynthetic rate is considered to be a result of oxidative damage to important photosynthetic cells (Iterbe-Ormaetxe et al. 1998; Kanwal et al. 2011). Besides, it was well-known fact that salt stress increased the oxygenase activity of Rubisco as compared to the carboxylase (Sivakumar et al. 2000), which causes less CO_2 fixation. In the present study, pre-treated seeds with TRIA increased gas exchange attributes of canola under nonsaline conditions, while in wheat, Perveen et al. (2010) reported that pre-sowing seed treatment with 10 μM TRIA increased photosynthetic rate and transpiration rate under both nonsaline and saline conditions, while stomatal conductance was increased under nonsaline conditions alone.

In this experiment, salt stress did not alter chlorophyll fluorescence attributes significantly except *qP*. Pre-sowing seed treatment with TRIA significantly increased only *qP* and *ETR*. Similarly, efficiency of PSII increased when TRIA was applied as foliar spray in tomato (Borowski et al. 2000) and rice (Muthuchelian et al. 1995; Kumaravelu et al. 2000; Chen et al. 2003).

Plants may accumulate compatible solutes such as glycine betaine and proline under salt stress to enhance their salt stress tolerance (Dos Reis et al. 2012; Shahbaz et al. 2012). In this experiment, salinity increased level of glycine betaine as well as proline in canola cultivar. Pre-sowing seed treatment with TRIA further enhanced proline accumulation under nonsaline conditions whereas glycine betaine under both saline and nonsaline conditions. In some previous studies, proline accumulation has been reported to be increased by exogenous application of TRIA, e.g. in cucumber (Borowski et al. 2000) and soybean (Krishnan and Kumari 2008); however, in contrast nonsignificant effect of TRIA was observed on glycine betaine accumulation in wheat under saline and nonsaline conditions (Perveen et al. 2012b).

High accumulation of Na^+ in plant tissues compete with K^+ uptake (Talei et al. 2012) and disturbs stomatal regulation and consequently photosynthesis, so increased uptake of potassium (an osmotic regulator) instead of sodium could be considered as a salt tolerance mechanism (Munns & Tester 2008; Ghogdi et al. 2012). Ries et al. (1993) reported that under normal conditions TRIA application stimulate K^+, Ca^{2+}, and Mg^{2+} accumulation in tomato, maize, and cucumber by eliciting a secondary messenger L(+)-adenosine. TRIA-induced increased accumulation of nutrients might result in enhanced crop yield (Miniraj & Shanmugavelu 1987; Aftab et al. 2010). However, optimal concentration of TRIA and plant age play important role in improved growth and yield of various plant species (Sagaral et al. 1978). For example, in wheat pre-sowing seed

treatment with TRIA exerted no significant effect on K^+ and Ca^{2+} contents, their uptake, and use efficiency under saline conditions (Perveen et al. 2012a). However, contrarily to these reports, foliar spray of TRIA increased Ca^{2+} and K^+ contents in soybean under salt stress (Krishnan & Kumari 2008). In the present investigation, pre-sowing seed treatment with TRIA improved shoot and root K^+ contents particularly at 0.5 mg L^{-1} TRIA level under 150 mM NaCl stress (Table 1; Figure 3). Since abscisic acid has been known to play role in stomatal closing under abiotic stresses, it could be suggested in the present study that increased leaf K^+ contents by pre-sowing TRIA treatment might have played some role in stomatal functioning. TRIA down-regulated genes of abscisic acid and stress-related proteins in rice (Chen et al. 2002).

Overall, salt stress exerted negative effect on growth, yield, photosynthesis, and essential nutrients accumulation in canola plants; however, pre-sowing seed treatment with TRIA was proved to be effective only in enhancing shoot fresh weight, number of seeds per plant, and *A* and *E* under nonsaline conditions, while ratio of chlorophyll *a*/*b*, *WUE*, *qP*, *ETR*, and shoot and root K^+ contents under saline conditions and osmoprotectants (proline and glycine betaine) under both nonsaline and saline condition of canola plants.

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