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TRIACONTANOL POSITIVELY INFLUENCES GROWTH, YIELD, BIOCHEMICAL ATTRIBUTES AND ANTIOXIDANT ENZYMES OF TWO LINSEED (*LINUM USITATISSIMUM* L.) ACCESSIONS DIFFERING IN DROUGHT TOLERANCE

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Abstract

Foliar application of three triacontanol (TRIA) levels i.e., 0, 1.0 and 0.1 µM was performed on two linseed (*Linum usitatissimum* L.) accessions (20888 and 22186) at week 6 and 9 from date of sowing under water deficit stress (50% field capacity). Data of growth and physiochemical parameters was determined after two weeks of foliar treatment of TRIA, while yield was obtained at maturity. Results of current study revealed that growth and yield significantly decreased in two linseed accessions under water deficit stress. However, foliar application of TRIA significantly increased shoot and root fresh and dry weight, shoot and root length, seed weight per plant, total number of seeds per plant, 1000-seed weight and number of capsules per plant, chlorophyll *a*, and *b* contents, activities of antioxidant enzymes (superoxide dismutase, peroxidase and catalase), anthocyanin contents, total soluble proteins, free amino acids and total phenolics while decreased relative water content (%), carotenoids, hydrogen peroxide (H_2O_2) and malondialdehyde (MDA) contents in both linseed accessions. Overall, foliar application of 1.0 µM TRIA proved more effective in increasing growth and yield of two linseed accessions under drought stress. Of the two linseed accessions 20888 exhibited high growth and yield than linseed 22186 under drought stress or non-stress conditions.

Key words: Hydrogen peroxide, Malondialdehyde, Superoxide dismutase, Peroxidase, Catalase.

Introduction

Drought stress is one of the major environmental constraints that negatively affects growth, yield, quality, quantity and nutritional profile of various oilseed crops such as peppermint (Khorasaninejad *et al.*, 2011), maize (Ali *et al.,* 2013), seasame (Ozkan & Kulak, 2013), chamomile (Farhoudi *et al.,* 2014), groundnut (Karimian *et al.,* 2015), sunflower (Eslami *et al.*, 2015), *Glycine max* (Shukla *et al.*, 2017), Carthamus (Nazari *et al.*, 2017), oilseed rape (Raza *et al.*, 2017) and canola (Raza *et al.*, 2015; Akram *et al.*, 2017). Drought stress can interfere with various physiological processes, including membrane integrity (Zang *et al*., 2019), and enzyme activity (Kong *et al*., 2021) which could lead to plant growth inhibition. Although drought at vegetative stage reduces plant biomass, however, water deficiency at flowering stage drastically reduced yield (Sadiq *et al.*, 2017). A common response of plant to abiotic stresses is accumulation of compatible osmolytes (Elewa *et al.*, 2017). In addition to this, a strong antioxidant defense system is considered to be indicator of abiotic stress tolerance (Khan *et al.*, 2017). Drought can cause physiological, physicochemical, and morphological variations in plants, which negatively affects plant production in arid and semiarid regions. To combat this under the increasing global threat of water shortage and rapid population expansion, it is important to develop strategies to meet global food demand (Ijaz *et al.,* 2019). It triggers the activation of downstream pathways, mainly through phytohormones and their signaling networks, which further initiate the biosynthesis of secondary metabolites (SMs) (Chen *et al*., 2021). Advancement of production and maintaining production stability of crops under normal as well as drought stress conditions, is vital for the food security of the growing global population (Gaff *et al.*, 1987).

Local oil production of Pakistan stood at 0.833 million tones, which accounted for 27% of the total availability while the remaining 73% was made available through imports. According to an estimate Pakistan has imported about 70% edible oil during 2012 (Anon., 2012). Alsi is the common name for linseed (*Linum usitatissimum* L.) and has its place in genus *Linum* of the family Linaceae. Linseed ranked at 6th position in oilseed crops. Linseed possesses good amount of proteins, fibers, lignin, omega-3 fatty acids, vitamins, linolenic acid, antioxidants and other metabolites that scavenge free radicals and have role in antioxidant defense system (Morris, 2007; Kaithwas & Majumdar, 2013; Ganorkar *et al.,* 2013). Total oil contents in seeds of linseed are in the range of 35-50% known as linseed oil (Muir & Westcott, 2003). Major oil components include 50% α-linolenic acid and omega-3 fatty acids that make up 61% of total fatty acid profile (Ayad *et al.,* 2013). Under abiotic stresses, the level of linolenic acid decreased (Hasham *et al.,* 2011), however, foliar treatment with various growth regulating agents can enhance seed yield in linseed plants (El-Lethy *et al*., 2010). According to Anon., (2012), typical countrywide harvest of linseed was 692 kg per hectare.

Phytohormones and/or plant growth regulator's (PGRs) increase abiotic stresses tolerance and regulate various physiological and biochemical processes. Foliar application with plant growth regulators (PGRs) increase abiotic stresses tolerance in plants (Soliman & Shanan, 2017). Triacontanol (TRIA), one of relatively new PGRs is reported to increase resistance against environmental stresses in different plant species (Keramat *et al.,* 2017; Karam & Keramat, 2017; Perveen *et al.,* 2016, 2017). TRIA regulate various metabolic processes such as photosynthesis, uptake of water and mineral nutrients, synthesis of various organic compounds and antioxidant defense system (Maresca *et al.,* 2017). The TRIA exogenously applied caused an increase in plant biomass, photosynthetic pigments, gas exchange parameters, mineral nutrient acquisition, leaf carbonic anhydrase (CA), nitrate reductase (NR) activity, osmolytes accumulation, modulates antioxidant enzyme activities, yield and quality attributes (Naeem *et al*., 2019; Waqas *et al*., 2016; Zaid *et al*., 2019).

Keeping in view the importance of oilseed crops in general and linseed in particular the current study was conducted to evaluate the effect of foliar application of triacontanol on growth, yield and physio-biochemical parameters of two linseed accessions (20888 and 22186) differing in drought tolerance under natural climatic conditions.

Materials and Methods

To evaluate the impactof foliar treatment of triacontanol (TRIA) on linseed (*Linum usitatissimum* L.) accessions, pot experiment was conducted under natural conditions. There were 2 linseed accessions (i.e., 20888 & 22186), two drought levels i.e., normal watering and 50% FC (field capacity) and three levels of triacontanol i.e., 0, 1.0, and 0.1 µM. Drought stress was applied to two week old seedling, while 3 levels of triacontanol i.e., 0, 1.0, and 0.1 µM were applied at 42 (6 week old plants) and 63 (9 week old plants) days from sowing of both linseed accessions under drought stress or non-stress conditions.

Growth and yield attributes: Data for different growth parameters such as shoot and root fresh weight and dry weight and shoot and root length were determined. At maturity yield traits such as seed weight per plant, 1000 seed weight, total number of seeds per plant and number of capsule per plant were calculated.

Chlorophyll pigments: Chlorophyll '*a'* and '*b'* contents were measured by Arnon (1949) procedure. Fresh leaves (0.5 g) were chopped into smaller pieces. Added 10 ml of 80% acetone and kept the samples for one night at room temperature. Then filtered the solution and took reading at 480nm, 645nm and 663nm with the help of spectrophotometer (Hitachi, U-2800).

Anthocyanin contents: Fresh leaves (0.1 g) were homogenized in 5 ml of phosphate buffer, centrifuged and measured the absorbance at 600 nm using a spectrophotometer.

Relative water contents (RWC%): Jones & Turner (1978) method was used to measure the leaf relative water contents. First of all leaf fresh weight was calculated by electronic balance, then leaf was placed in distilled water for overnight and turgid weight was measured. Turgid leaves were placed in dry air for one hour and then placed in oven at 72°C for 2 days and dry weight was determined. The relative water content of leaf were measured using the formula:

$$\text{Leaf relative water content (\%)} = \frac{\text{Leaf fresh weight - Leaf dry weight}}{\text{Leaf turgid weight - Leaf dry weight}} \text{ x } 100$$

Free proline contents: Bates *et al*., (1973) protocol was used for the assessment of proline contents. Fresh leaf 0.5 g was ground with 5 ml sulfosalicylic acid. The 2 ml of ninhydrin and 2 ml of glacial acetic acid was added in 2 ml of leaf extract in test tubes and heated in water bath for 1 hour at 100°C. After cooling the solution added 4 ml toulene and vortexed for 2 minutes. Took reading at 520 nm with spectrophotometer.

Hydrogen peroxide (H_2O_2) contents: H_2O_2 contents were calculated by Velikova e*t al*., (2000) method. Fresh leaves (0.5 g) were ground in 0.1% TCA (trichloroacetic acid). At 12000 rpm samples were centrifuged for 10 minutes in centrifuge machine. To 0.5 ml of each plant sample added 1 ml of potassium iodide and 0.5 ml of phosphate buffer in test tubes, vortex the solution and took the reading at 390 nm wavelength with the use of spectrophotometer.

Malondialdehyde (MDA) contents: Cakmak & Horst (1991) determined the oxidative damage in plants by calculating the MDA contents. Fresh leaf (0.5 g) was homogenized with 3 ml of TCA (trichloroacetic acid). After centrifugation at 12,000 × *g* for 10 min added 1 ml of 0.5% tribarbituric acid (prepared in 20% TCA) added 0.5 ml of supernatant and heated the solution at 95°C in water bath for half an hour. Then allowed the samples to cool at room temperature again centrifuged and recorded the reading at 532 nm and 600 nm wavelength with a spectrophotometer.

Total soluble protein contents: By using Bradford (1976) method total protein contents were calculated. Fresh leaves 0.5 g were homogenized in 10 ml of 50 mM potassium phosphate buffer and centrifuged at 6,000 × *g* for five min at 4ºC. Took 100 µl of leaf extract and added 2 ml of Bradford's reagent and measured the absorbance at 595nm with a spectrophotometer.

Total free amino acids contents: Moore & Stein (1957) method was used to determine the total free amino acid contents. Fresh leaves (0.5 g) were ground in citrate buffer (10 ml) and centrifuged at 15,000 × *g* for ten min processed with ninhydrin solution and took the reading at 570 nm using a spectrophotometer.

Total phenolic contents: Julkenen-Titto (1985) method was used for the determination of total phenolic contents. Fresh leaves (0.25 g) were ground in 5 ml methanol solution and centrifuged at 10,000 × *g* for 15 min. the 0.5 ml Folin Ciocalteau's phenol reagent and 2.5 ml of sodium carbonate (20%) solution was added in each sample of 0.1 ml .Final volume maintained upto 5 ml by adding distilled water, vortex it for 10 minutes and noted the absorbance at 750 nm with a spectrophotometer.

Superoxide dismutase (SOD) activity: Superoxide dismutse activity was measured using Giannopolitis & Ries (1977) procedure. The reaction mixture i.e., phosphate buffer (50 mM), distilled water, methionine (13 mM), nitroblue tetrazolium (50µM), enzyme extract (50µl) and riboflavin (1.3 µM) was placed under light (15 fluorescent lamps at 78 µmol $m^{-2}s^{-1}$) and spectrophotometer was used to take absorbance at 560 nm.

Catalase (CAT) and peroxidase (POD) activities: Chance & Maehly (1955) procedure was applied for the measurement of catalase and peroxidase activities. Reaction mixture for catalase contains 50 mM phosphate buffer, 5.9 mM H_2O_2 and 0.1 ml enzyme extract. Absorbance of reaction mixture was measured after every 20 second interval at 240 nm. The reaction solution for peroxidise contains 20 mM guaiacol, 50 mM phosphate buffer, 40 mM H_2O_2 and 50 µl enzymes extract. Guaiacol addition starts the oxidation. Absorbance was taken after every 20 sec at 470 nm with a spectrophotometer.

Statistical analysis

Data was evaluated statistically by COSTAT computer program (V. 6.303, Company, CoHart Software), and means were compared by least significant difference (Snedecor & Cochran, 1980).

Results

Growth characteristics: Statistical analysis of data of present study revealed that there was reduction in shoot and root fresh weight, shoot and root length of both linseed accessions significantly at ($p \leq 0.001$) under drought stress of 50% field capacity i.e., 20888 and 22186 (Table 1, Fig. 1). However, TRIA foliarly applied significantly ($p \leq 0.001$) increased shoot and root fresh and dry weights and shoot and root lengths. The linseed accession 20888 indicated higher shoot and root fresh and dry weights and shoot and root lengths values. Maximum increase in all measured growth parameters were observed at 1.0 µM TRIA treatment under both in limited water stress and non-stress conditions (Table 1, Fig. 1).

Yield and yield components: Drought stress of 50% field capacity significantly decreased yield and yield component i.e., seed weight per plant, number of seeds per plant (Fig. 1), number of capsules per plant and 1000 seed weight (Fig. 2) of both linseed accessions (20888 & 22186) (Table 1, Fig. 1). The treatment with triacontanol in both linseed accessions at 1.0 µM caused significantly increase in seed weight per plant, number of seeds per plant, number of capsules per plant and 1000 seed weight (Table 1, Fig. 2).

Chlorophyll *a* and *b* contents (mg/g f. wt.): Drought stress significantly ($p \leq 0.01$) decreased chlorophyll *a* contents in both linseed accessions (20888 & 22186), whereas, chlorophyll *b* contents did not change in both linseed accessions (20888 & 22186). The linseed 20888 showed more chlorophyll *a* contents at 1.0 µM followed by 0.1 µM under water deficit stress conditions. Foliar application of triacontanol significantly ($p \leq 0.01$) increased chl *a* and *b* contents in both linseed accessions at 1.0 µM under both control and drought stress condition (Table 1, Fig. 2).

Carotenoid and anthocyanin contents (mg/g f. wt.): Carotenoid contents significantly ($p \leq 0.01$) decreased under both drought stress and by foliar application of triacontanol in both linseed accessions. However, maximum reduction was observed in linseed 22186 than that of 20888 under drought stress. There was significant increase in Anthocyanin contents under both drought stress and with foliar application of TRIA (1.0 µM) in both accessions. The linseed 20888 showed high anthocyanin contents than that of linseed 22186 under drought stress or non-stress condition (Table 1, Fi g. 2).

Percentage relative water contents (RWC%): Drought stress did not affect relative water contents (%) significantly, however, RWC decreased significantly ($p \leq 0.05$) by foliar application of TRIA in both linseed accessions (20888 & 22186) under control conditions (Table 1, Fig. 2).

Free proline contents: Drought stress increased free proline contents significantly ($p \leq 0.01$) in both linseed accessions under drought stress, however, remained unaffected with the foliar application of triacontanol under both drought stress and control conditions (Table 1, Fig. 2).

Hydrogen peroxide (H_2O_2) and malondialdehyde (MDA) contents: Hydrogen peroxide (H_2O_2) contents significantly decreased under both drought stress ($p \leq 0.01$) and with the foliar application of TRIA ($p \leq 0.001$) in both linseed accessions (Table 1, Fig. 3). However, linseed 22186 showed low hydrogen peroxide contents than that of 20888. Malondialdehyde (MDA) contents were not affected under drought stress in both linseed accessions, however, significantly ($p \leq 0.05$) decreased with the exogenous application of triacontanol (Table 1, Fig. 3).

Total soluble proteins and free amino acid contents: Total soluble protein contents significantly ($p \leq 0.001$) increased under both drought stress and with foliar application of varying triacontanol levels in both linseed accessions (Table 1, Fig. 3). Linseed 20888 showed more total soluble protein contents than that of 22186 under both drought stress and non-stress conditions. Statistical data showed that free amino acid contents were not affected under drought stress, however, foliar treatment of triacontanol in two linseed 20888 & 22186 accessions caused increase free amino acid contents under both water deficit stress and non-stress conditions (Table 1, Fig. 3).

Total phenolic contents: The data obtained from statistical analysis showed that under both drought stress and with the foliar application of triacontanol, total phenolic contents was significantly ($p \leq 0.001$) increased in both linseed accessions. Linseed accession 20888 showed more total phenolic contents than that of 22186 under water deficit stress conditions (Table 1, Fig. 3).

Table 1. Mean squares from analysis of variance (ANOVA) of data on growth, yield, RWC (%), chlorophyll *a*, *b*, carotenoids, anthocyanin, H_2O_2, MDA, free proline, total soluble protein, total free amino acids, total phenolics, activities of SOD, POD and CAT of two linseed (*Linum usitatissimum* L.) accessions foliarly-sprayed with triacontanol under drought stress and non-stress conditions.

Source of variation	Df	Shoot f. wt.	Shoot dry wt.	Root f. wt.	Root dry wt.	Shoot length
Accessions (Acc)	1	2.89***	0.93***	0.08***	0.013ns	2037.31***
Drought (D)	1	7.29***	3.36***	0.23***	0.04***	9383.15***
Triacontanol (Tria)	2	2.40***	1.57***	0.06***	0.02**	1584.81***
Acc × D	1	2.25***	0.53**	0.006ns	0.006ns	102.81*
Acc × Tria	2	0.0108ns	0.001ns	0.020*	0.003ns	8.47ns
D × Tria	2	0.22*	0.26**	0.018*	0.021**	231.79***
Acc × D × Tria	2	0.0025ns	0.008ns	0.006ns	0.003ns	24.02ns
Error	24	0.057	0.043	0.003	0.003	16.40

Source of variation	Df	Root length	Seed weight/ plant (g)	Total number of seeds/plant	Number of capsules/ plant	1000 seed weight
Accessions (Acc)	1	1537.94***	14.31***	105.9***	27944.6***	14.31***
Drought (D)	1	1044.36***	109.9***	601.9***	158802.2***	109.9***
Triacontanol (Tria)	2	227.38***	32.38***	135.8***	35830.1***	32.38***
Acc × D	1	416.84***	0.302ns	13.87**	3660.2**	0.302ns
Acc × Tria	2	16.87**	3.393**	16.54***	4364.1***	3.393**
D × Tria	2	54.10***	9.771***	81.58***	21524.3***	9.771***
Acc × D × Tria	2	6.38ns	0.110ns	19.53***	5154.3***	0.110ns
Error	24	2.22	0.381	263.8	263.8	0.381

Source of variation	Df	Chl *a*	Chl *b*	Carotenoids	Anthocyanin contents	RWC %
Accessions (Acc)	1	0.138***	0.129ns	0.070***	17.12***	0.371ns
Drought (D)	1	0.069***	0.147ns	0.084***	208.6***	0.371ns
Triacontanol (Tria)	2	0.348***	3.920***	0.096***	28.95***	11.90*
Acc × D	1	9.969ns	0.034ns	0.001ns	1.086ns	0.371ns
Acc × Tria	2	0.037**	0.087ns	0.005*	0.098ns	0.130ns
D × Tria	2	0.013ns	0.111ns	0.006*	0.405ns	16.94**
Acc × D × Tria	2	0.012ns	0.002ns	0.003ns	0.132ns	0.130ns
Error	24	0.004	0.054	0.001	0.552	2.359

Source of variation	Df	H_2O_2	MDA	Proline	Soluble protein	Free amino acids
Accessions (Acc)	1	0.005*	0.203ns	4.236ns	0.697***	0.609ns
Drought (D)	1	0.007**	0.029ns	269.08***	63.52***	0.002ns
Triacontanol (Tria)	2	0.177***	0.379*	18.24ns	11.56***	12.46***
Acc × D	1	6.136ns	0.007ns	4.765ns	0.051ns	0.458ns
Acc × Tria	2	8.950ns	0.013ns	2.991ns	0.026ns	0.052ns
D × Tria	2	0.004*	0.007ns	28.46ns	0.446***	0.012ns
Acc × D × Tria	2	9.726ns	8.093ns	8.583ns	0.062*	0.024ns
Error	24	0.001	0.108	13.91	0.015	0.223

Source of variation	Df	Phenolics	SOD	POD	CAT
Accessions (Acc)	1	8.739*	0.108***	0.034***	8.730*
Drought (D)	1	242.6***	0.001***	0.037***	2.865ns
Triacontanol (Tria)	2	45.12***	0.021***	0.014**	1.054ns
Acc × D	1	0.326ns	0.056***	0.154***	8.014*
Acc × Tria	2	5.821*	0.052***	0.039***	0.027ns
D × Tria	2	8.832**	0.014***	0.006ns	2.367ns
Acc × D × Tria	2	5.540*	0.029***	0.032***	0.042ns
Error	24	1.346	0.001	0.002	1.253

***, **, and *= Significant at 0.001, 0.01, and 0.05 levels respectively; df = Degrees of freedom; ns = Non-significant; Chl *a* = Chlorophyll *a*; Chl *b* = Chlorophyll *b*; H_2O_2 = Hydrogen peroxide; MDA = Malondialdehyde; RWC = Relative water content; SOD = Superoxide dismutase; POD = Peroxidase; CAT = Catalase

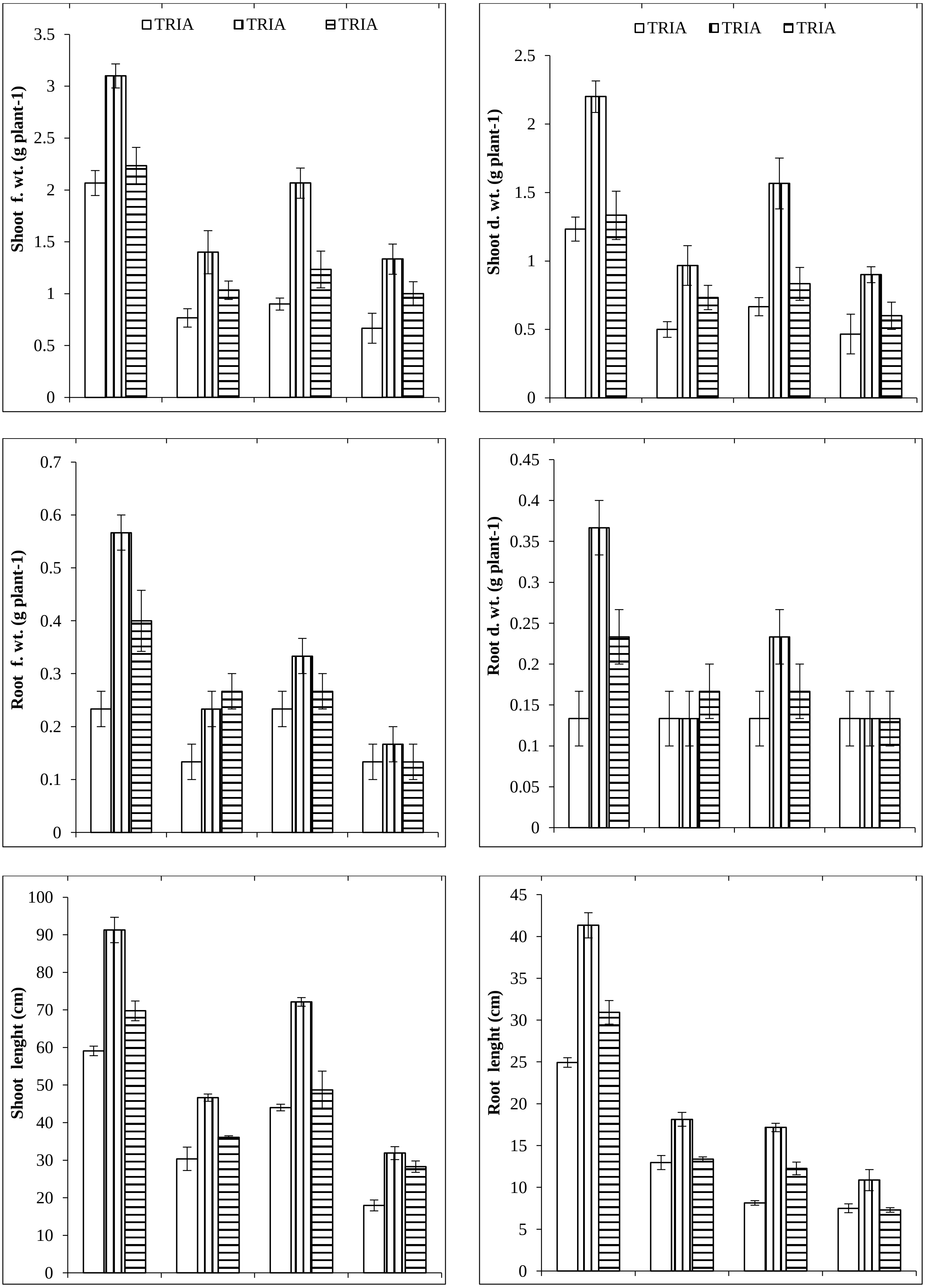

Fig. 1. Shoot fresh and dry weights, root fresh and dry weights, shoot and root lengths, seed weight and number of seeds per plant of linseed (*Linum usitatissimum* L.) plants foliarly sprayed with triacontanol under drought stress or non-stress conditions. FC = field capacity.

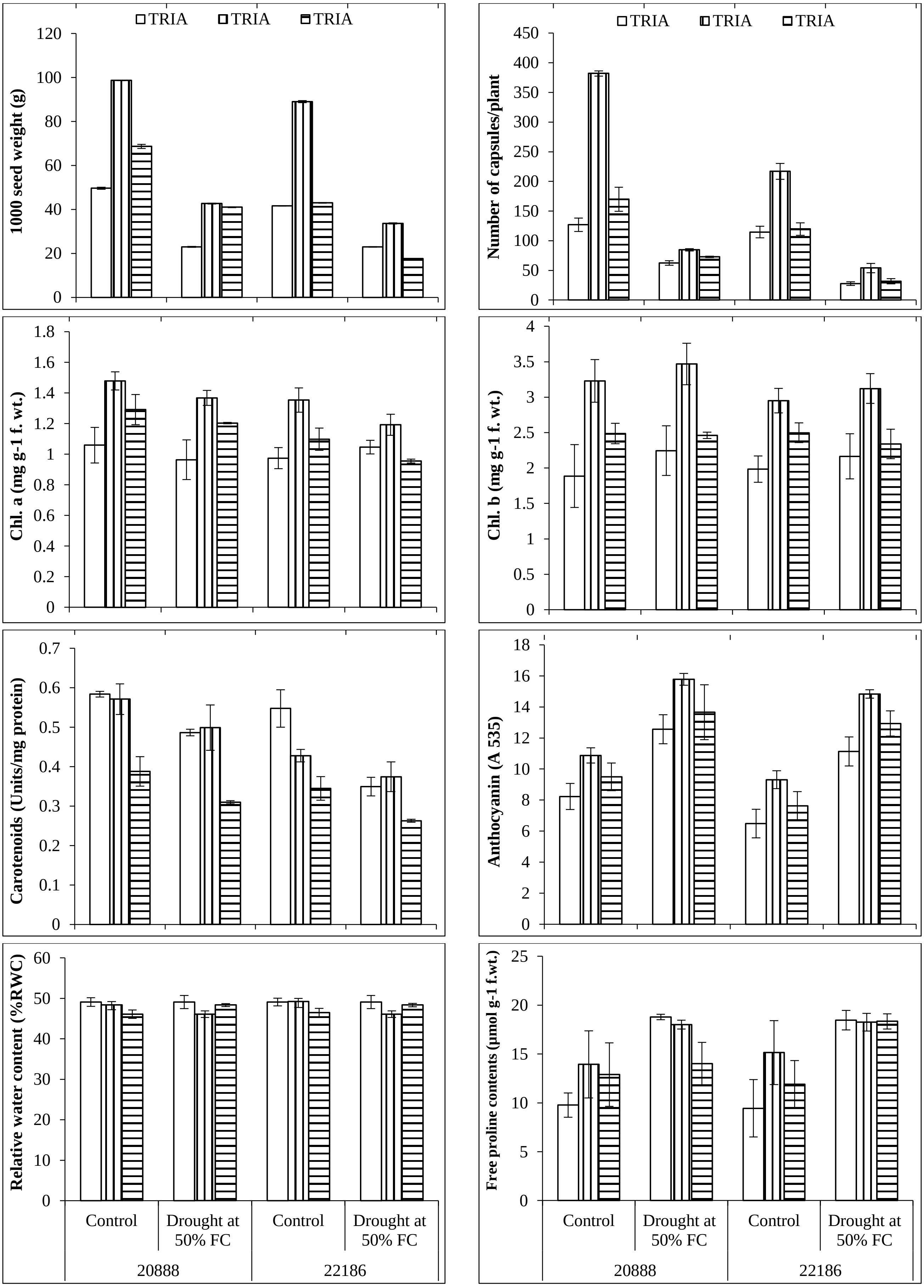

Fig. 2. Yield components, chlorophyll contents, carotenoids, anthocyanin, relative water contents and free proline contents of linseed (*Linum usitatissimum* L.) plants foliarly sprayed with triacontanol under drought stress or non-stress conditions.

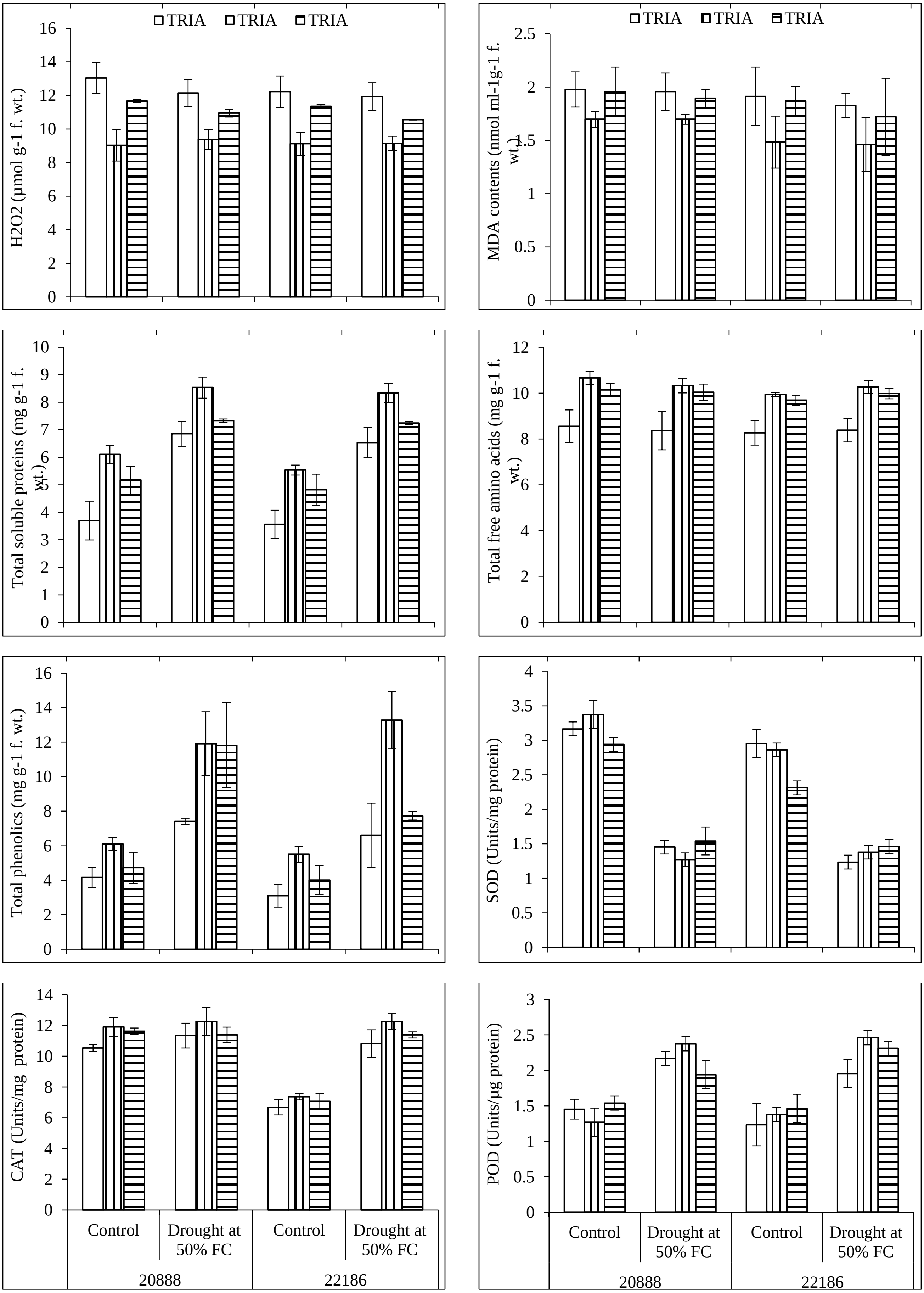

Fig. 3. Hydrogen peroxide, malondialdehyde, soluble proteins, amino acids, total phenolics, activities of SOD, CAT and POD antioxidant enzymes of linseed (*Linum usitatissimum* L.) plants foliarly sprayed with triacontanol under drought stress or non-stress conditions.

Activities of antioxidant enzymes: Statistical analysis of data showed that superoxide dismutase (SOD) activity significantly ($p≤0.001$) decreased, while activity of peroxidase (POD) enzyme significantly ($p≤0.001$) increased in both linseed accessions (20888 & 22186) under drought condition. However, catalase (CAT) activity significantly ($p≤0.05$) increased only in 22186 under drought stress condition (Table 1, Fig. 3). Foliar application of TRIA significantly increased SOD activity under control conditions, while decreased under drought stress particularly in linseed 22186. Similarly, TRIA as foliar application significantly enhanced POD activity in linseed 22186 under both drought stress and non-stress conditions. However, foliar treatment of TRIA did not affect CAT activity significantly. Linseed accessions exhibited significant difference in activities of antioxidant enzymes e.g., linseed 20888 showed higher values of SOD, POD and CAT activities than that of linseed 22186 particularly under control conditions.

Discussion

Abiotic stresses has been known as the major cause of crop yield reduction throughout the world (Zandalinas *et al.,* 2018). Among different environmental stresses drought stress can decrease yields of agricultural crops up to 70%, all over the world (Osakabe*et al.,* 2014; Perveen *et al.,* 2016; Mathobo *et al*., 2017).

Findings of the recent study undoubtedly showed that there was decrease in growth and yield of both the linseed accessions (20888 & 22186) under water deficit stress. Drought stress has been reported to decrease yield of soybean *(Glycine max)* (Shukla *et al*., 2017), plant height of linseed (*Linum usitatissimum* L.) cultivars (Kariuki *et al.,* 2016), 1000-seed weight in dry bean (*Phaseolus vulgaris* L.) (Gohari, 2014) and field beans (*Vicia faba* L.) (McEwen *et al*., 1990).The observance of reduction in 1000 seed weight revealed that under drought stress seeds mature early and there are smaller seeds. However, foliar application if TRIA significantly increased growth and yield components of both linseed accessions. Generally, the foliarly applied triacontanol as exogenous treatment was examine to stimulate growth and yield of different cereal crops under stress and non stress conditions (Ries, 1991; Kumaravelu *et al.,* 2000; Krishnan & Kumari, 2008). Furthermore, foliar treatment of TRIA enhanced growth and yield of coriander (Karam & Keramat, 2017), wheat (Perveen *et al.,* 2014) and canola (*Brassica napus* L.) (Shahbaz *et al*., 2013) under salt stress.

Under drought stress relative water contents (RWC) considered as one of the easiest agricultural trait that play a role in screening of the plants (Boutraa *et al*., 2010). However, in the current study, RWC (%) remained unaffected under drought stress by foliar application of TRIA. In our previous study, pre-sowing seed treatment with TRIA did not change relative water content in wheat (Perveen *et al*., 2012).

It is known fact that photosynthetic capacity depends on photosynthetic pigments such as chl*a* andchl *b* contents. Decline in chlorophyll content under drought stress has been considered to be due to reactive oxygen species led chloroplast deterioration (Mafakheri *et al*., 2010; Akram *et al*., 2017; Raza *et al*., 2017). In this study, foliar application of TRIA significantly decreased chlorophyll contents. It has been reported that foliar treatment of triacontanol increased chlorophyll contents in different crops such as mint (*Mentha arvensis* L.), basil (*Ocimum basilicum* L.) (Hashmi *et al.,* 2010) and wheat (*Triticum aestivum* L) (Perveen *et al*., 2013). TRIA-mediated increase in chlorophyll contents might be due to increase in size and number of chloroplast (Muthuchelian *et al*., 2003).

Proline is commonly considered to play important role in enhancing abiotic stress tolerance in plants (Szabados & Savoure, 2010). It has been reported that proline accumulated as compatible osmolyte under drought stress (Akram *et al.,* 2017; Elewa *et al.,* 2017). In this study, free proline contents increased by foliar application of triacontanol under drought stress in both linseed accessions. Similarly, foliar application of TRIA has been reported to increase free proline contents in maize (Perveen *et al.,* 2017) and canola (Shahbaz *et al.,* 2013) under salt stress.

It has been reported that TRIA inhibits peroxidation of membrane lipids (Ramanarayan *et al.,* 2000). However, in another report pre-sowing seed treatment with TRIA did not affect H_2O_2 and MDA contents in wheat under saline or non-saline conditions (Perveen *et al.,* 2011). In this study H_2O_2 contents decreased, while malondialdehyde contents remained unaffected under drought stress, however, foliarly-applied TRIA significantly decreased H_2O_2 and MDA contents in both linseed accessions under water deficit stress or non-stress conditions. Similar findings were observed in maize when treated with TRIA under water deficit conditions (Perveen *et al.,* 2016).

The decreased in soluble protein contents was observed after prolonged water stress (Surendar *et al.,* 2013; Karatas *et al.,* 2014). However, in current study, soluble protein contents increased both under drought stress and by foliar application of triacontanol. It has been reported that the foliar application of TRIA increase total soluble protein contents in maize (Perveen *et al.,* 2017). Free amino acids accumulation help crop plants to osmotically regulate their cellular environment and related to drought stress tolerance (Yadav *et al.,* 2005).

To scavenge reactive oxygen species plants used strong antioxidant defense system (Asadi *et al.,* 2017; Bansal & Srivastava, 2017). Phenolic compounds are induced under environmental stresses and produced by phenylpropanoids pathway in plants (Yuan *et al.,* 2010). Drought stress has been reported to increased total phenolic contents (Akram *et al.,* 2017). In this study, total phenolic contents increased by foliar application of TRIA in both linseed accessions under drought stress. Similarly, total phenolic contents increased by foliar application with TRIA in maize (*Zea mays* L.) under salt stress (Perveen *et al.,* 2017). In other studies, however, total phenolic contents decreased when treated with TRIA under abiotic stresses such as in wheat (Perveen *et al*., 2014) and maize (Ertani *et al.,* 2013; Perveen *et al.,* 2016).

In this study, it has been shown that drought stress decreased SOD activity, while increased CAT and POD activity in maize plants (Perveen *et al.,* 2016). In the present investigation, foliar application of TRIA increased SOD activity under drought stress, while decreased under non-stress conditions (in linseed accession 22186), however, increased POD activity under both drought stress and non-stress conditions. In our previous studies, there was increased POD activity with exogenous application of TRIA as pre-sowing seed treatment under non-saline conditions (Perveen *et al.,* 2011), while in maize plants foliarly-applied TRIA increased both CAT and POD activity under water deficit condition (Perveen *et al.,* 2016). Triacontanol has been considered to regulate antioxidant enzyme activities in various crop species such as in peanut (*Arachis hypogaea* L.) (Verma *et al.,* 2011), spinach (*Spinaceae oleracea* L.) (Ramanarayan *et al.,* 2000) and wheat (*Triticuma estivum* L.) (Perveen *et al.,* 2014; 2017) etc.

In the present study, drought stress decreased growth, yield, chlorophyll *a*, carotenoids and hydrogen peroxide (H_2O_2) contents, whereas increased total soluble proteins, total phenolics, anthocyanin and proline contents was observed in both linseed accessions. Activity of superoxide dismutase (SOD) decreased, while those of catalase (in linseed 22186) and peroxidase (POD) increased in both linseed accessions under drought stress. Foliar application of TRIA increased shoot and root fresh and dry weights, shoot and root lengths, seed weight per plant, number of seeds per plant, number of capsules per plant and 1000 seed weight in the two linseed accessions under drought stress. Of the varying TRIA levels, 1.0 µM TRIA showed significant effect in increasing growth and yield of two linseed accessions. TRIA-mediated increase in growth and yield might be due to increased synthesis of photosynthetic pigments, total phenolics, total soluble proteins, total free amino acids and activities of antioxidant enzymes (SOD and POD), and reduced oxidative damage (decrease in H_2O_2 and MDA) in the two linseed accessions (20888 and 22186). From the two linseed accessions, the linseed 20888 exhibited more tolerance to drought stress than that of 22186.

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