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INDUCTION OF DROUGHT TOLERANCE IN *ZEA MAYS* L. BY FOLIAR APPLICATION OF TRIACONTANOL

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Abstract

In the present study, we assessed the effect of foliar application of triacontanol (TRIA) on various growth and physiochemical parameters of two maize (*Zea mays* L.) cultivars (cv. MMRI-Yellow and cv. Hybrid S-515) under different irrigation levels i.e., normal watering (control) and watering at 60% of the field capacity (drought). Seeds of the two maize cultivars were sown in plastic pots filled with sandy loam soil (2 kg in each). Foliar application of TRIA (0, 2 and 5 µM) was performed after two weeks of drought stress to 28-day-old plants. Data of 58-day-old maize plants was collected for analysis of various growth and physiochemical attributes. Drought stress significantly decreased growth and superoxide dismutase (SOD) activity while increased the activities of catalase (CAT) and peroxidase (POD) and the contents of total phenolics, total soluble proteins, glycinebetaine (GB) and free proline. Foliar treatment with TRIA further increased CAT and POD activities whereas decreased the contents of hydrogen peroxide (H_2O_2), malondialdehyde (MDA), total phenolics and GB in the maize plants when under drought stress. Of the two maize cultivars, cv. MMRI-Yellow excelled the growth under both normal and drought stress (60% of the field capacity). Overall, TRIA (5 µM) was much more effective in modulating various growth and physiochemical attributes, and thus improving drought tolerance in maize plants.

Key words: Triacontanol, Maize, Drought, Chlorophyll, Antioxidants.

Introduction

Drought reduces crop yield more than any other abiotic stress (Lambers *et al*., 2008). Drought reduces turgor pressure and causes wilting of cells leading to reduction in the cell expansion and growth (Aslam *et al*., 2014; Srivastava & Srivastava, 2014). Drought-induced oxidative stress oxidises various macromolecules such as proteins, lipids and nucleic acid and damages cell membranes (Peifang *et al*., 2015). However, plants have adapted antioxidative defence system comprising of enzymatic antioxidants and some low-molecular weight antioxidants to cope with drought-mediated oxidative stress (El-Beltagi & Mohamed, 2013). The organic compounds such as proline and glycinebetaine are known as osmoprotectants that play significant role in the osmotic regulation, protection of membranes and stability of enzymes (El Tayeb, 2006).

Abiotic stresses alter hormonal balance to modulate growth in plants. However, exogenous application of plant growth regulators could regulate hormonal balance to induce stress tolerance such as in spring wheat when under salt stress (Iqbal & Ashraf, 2013a, 2013b). Foliar application of TRIA has been known to regulate various physiochemical process under both normal and stressful environments in different crop species such as in *Erythrina variegata* L. seedling (Muthuchelian *et al.,* 1997), soybean (Krishnan & Kumari, 2008), sweet basil (Borowski & Blamowski, 2009), common duckweed (Kilic *et al.,* 2010), maize (Ertani *et al.,* 2013), sunflower (Aziz *et al.,* 2013), canola (Zulfiqar & Shahbaz, 2013) and wheat (Perveen *et al.,* 2014). The exogenously applied TRIA maintained water homeostasis through increased uptake of water, essential nutrients, and accumulation/synthesis of compatible organic compounds in wheat (Perveen *et al*., 2014). Furthermore, foliar application of TRIA at different growth stages stimulated growth in ginger (Singh *et al*., 2011), wheat (Ries, 1991) and chickpea (Singh *et al*., 1991).

Maize (*Zea mays* L.) is a multipurpose cereal that is globally used as food, feed, fuel and fodder (Liu *et al*., 2001). Depending upon the intensity and duration of drought stress, reduction in kernel yield is 10 to 76% in maize (Bolaoos, 1993). Being a C_4 plant, maize is an efficient water user that normally requires about 500 to 800 mm water to complete its life cycle (80 to 110 days) (Critchley & Klaus, 1991). Based on the beneficial effects of TRIA under salt stress (Perveen *et al*., 2010, 2012a, 2012b, 2013, 2014), it was hypothesized that foliar application of TRIA could improve growth of maize plants under drought stress. The principal objective of the present study was to assess whether foliar application of TRIA modulates physiochemical attributes to regulate growth of maize under normal and drought stress (60% of field capacity).

Materials and Methods

An experiment was conducted during the years 2013-2014 under natural climatic conditions at Government College University Faisalabad, Pakistan. Seeds of two maize cultivars, namely, cv. MMRI-Yellow and cv. Hybrid S-515 were sown in the plastic pots containing 2 kg sandy loam soil having electrical conductivity (EC) 1.31 dS m^{-1}, TSS (total suspended solids) 13.10 me L^{-1}, CO_3 0.84 me L^{-1}, HCO_3 4.84 me L^{-1}, Cl^- 5.84 me L^{-1}, Na^+ 6.24 me L^{-1}, Ca^{2+} and Mg^{2+} 5.88 me L^{-1} and sodium adsorption ratio (SAR) 3.84 $mmol^{1/2}$ $L^{-1/2}$. The experiment was laid-out in a completely randomized design with three replicates per treatment. After complete germination, four equal sized seedlings were kept per pot. Fourteen days old plants were subjected to drought stress (60% field capacity) in addition to control (100% field capacity). Foliar application of three concentrations (0, 2 and 5 µM) of TRIA (molecular weight 438.33; 99% pure of SIGMA- Aldrich USA) at the rate of 25 ml/plant was done after two weeks of drought stress. Fresh leaf samples of 58-d old plants were collected in plastic zipper bags and kept in freezer (-20°C) for various

biochemical determinations. In addition, plants were cut to separate shoots and roots to determine root and shoot fresh weights and the same plants were kept in an oven at 65°C for one week to determine dry weights. The total leaf area (cm^2) was calculated according to the method of Carleton & Foote (1965).

Chlorophyll contents: Fresh leaf tissue (0.5 g) was crushed with 80% acetone (10 ml). The supernatant was taken and the absorbance was noted at 480, 445 and 663 nm with the help of spectrophotometer. The chlorophyll contents were calculated as described earlier (Arnon, 1949).

Membrane permeability (%): Fresh leaf tissue (0.5 g) was cut into 1 cm pieces and floated in 10 ml of distilled water in the test tubes and vortexed for 5 s to obtain electrical conductivity (EC_o). The test tubes were kept overnight at 4°C and EC_I determined. The and tissue was autoclaved for 1 h, cooled at normal temperature and EC_2 determined. The membrane permeability was calculated as described by Yang *et al*. (1996).

Hydrogen peroxide (H_2O_2) contents: Fresh leaf tissue (0.1 g) was extracted with 5 ml of 0.1% (w/v) trichloroacetic acid (TCA) by using pestle and mortar in an ice bath. The extracted material was centrifuged at 12000 × *g* for 15 min. To 0.5 ml of supernatant, 0.5 ml of potassium phosphate buffer and 1 ml of 1 M potassium iodide (KI) was added. The mixture was vortexed and the absorbance was noted at 390 nm using a spectrophotometer (Velikova *et al*., 2000).

Malondialdehyde (MDA) contents: The NDA contents were determined by following the method of Carmak & Horst (1991). In finely ground 0.5 g fresh leaf tissue, 10 ml of 0.1% (w/v) TCA was added and the mixture centrifuged for 10 min at 12000 × *g*. To 1 ml of the supernatant, 4 ml of 0.5% thiobarbituric acid (TBA) was added. The reaction mixture was kept in a water bath at 95 °C for 30 min. The samples were cooled and the absorbance of the supernatant was noted at 532 and 600 nm using a spectrophotometer. The absorption coefficient of 156 $mmol^{-1}$ cm^{-1} was used to calculate the MDA contents. Following formula was used for MDA calculation.

$$MDA\ (nmol) = \Delta\ (A\ 532\ nm - A\ 600\ nm)/1.56 \times 105$$

Total soluble proteins: Fresh leaf tissue (0.5 g) was extracted with 10 ml of potassium phosphate buffer (pH 7.8) in an ice bath and centrifuged at 10000 × *g* for 10 min. The total protein contents were determined as describe earlier (Bradford, 1976) using bovine serum albumine (BSA) as standard.

Nitrate reductase (NR) activity: Fresh leaf tissue (0.5 g) was homogenised in 10 ml of potassium phosphate buffer (50 mM) with the help of prechilled pestle and mortar in an ice bath. The homogenate was centrifuged at 6000 × *g* for 5 min at 4°C. The NR activity was determined by following Jaworski (1971) and the absorbance was read at 545 nm by using a spectrophotometer.

Antioxidant enzymes: Enzymes extract was obtained by finely grinding fresh leaf (0.5 g) in 10 ml of 50 mM phosphate buffer (pH 7.8) in an ice bath. Then homogenate was centrifuged at 12000 × *g* at 4°C for 20 min. The extract was used for the assay of following antioxidant enzymes activities.

Superoxide dismutase (SOD): The SOD activity was determined by the method of Giannopolitis & Ries (1977). One unit of SOD was considered equivalent to the amount of enzyme that cause 50% inhibition in nitroblue tetrazolium (NBT) photoreduction when compared to the blank (without enzyme extract). The reaction solution for SOD contained 50 mM phosphate buffer (pH 7.8), distilled water, methionine 13 mM, NBT 50 µM, enzyme extract 50 µl and riboflavin1.3 µM. The reaction mixture was kept under 15 fluorescent lamps for 15 min and read the absorbance at 560 nm with a spectrophotometer.

Catalase (CAT) and peroxidase (POD) activities: Activity of CAT and POD was determined on protein basis by following the Chance & Maehly (1955) method. For CAT activity, the reaction solution contained 1.9 ml of 50 mM buffer (pH 7.00), 1 ml H_2O_2 (5.9 mM) and 100 µl enzyme extract. The change in the absorbance of reaction mixture was read after every 20 s at 240 nm with a spectrophotometer. For determination of POD activity, the reaction solution contained distilled H_2O, 250 µl of 50 mM phosphate buffer (pH 7.8), 100 µl of 40 mM H_2O_2, 100 µl of 20 mM guaicol and 50 µl enzyme extract. The change in enzyme activity was determined after every 20 s at 470 nm using a spectrophotometer.

Total phenolics: Fresh leaf material (0.5 g) was ground in 10 ml of 80% acetone and centrifuged at 15000 × *g* at 4 °C for 15 min. To 0.1 ml of supernatant, added 2 ml of distilled water and 0.5 ml Folin-Ciocalteau's reagent and vortexed vigorously. To the above mixture, added 2.5 ml of 20% Na_2CO_3 and vortexed for 10 s and made final volume up to 5 ml with distilled water. Then incubated the test tubes for 20 min and read the absorbance at 750 nm using a spectrophotometer (Julkenen-Titto, 1985).

Free amino acids: Fresh leaf (0.5 g) material was ground in 10 ml of phosphate buffer (pH 5.0) and centrifuged at 12000 × *g* for 15 min at 4°C. To 1 ml of extract, added 1 ml of pyridine (10 %) and 1 ml of the ninhydrin (2%) and kept in hot water bath for 30 min and read the absorbance at 570 nm using a spectrophotometer (Moore & Stein, 1957).

Free proline contents: Fresh leaf (0.5 g) was homogenized in 10 ml of 3 % sulphosalicylic acid (w/v) and filtered with Whatman No. 2 filter paper. The free proline contents were determined as described earlier (Bates *et al*., 1973). The free proline contents were aspirated from chromophore layer and read the absorbance at 520 nm with a spectrophotometer.

Glycinebetaine (GB): Dry leaf (0.5 g) was ground in 10 ml of distilled water and filtered and used for the determination of GB following the method of Grieve & Grattan (1983). Briefly, the reaction mixture contained 1 ml of filtrate, 1 ml of 2 N H_2SO_4 and 0.2 ml of potassium iodide (KI) and kept it on an ice bath. After 90 min, 2.8

ml chilled distilled water and 6 ml of 1, 2-dichloromethane was added and read the absorbance of the supernatant at 365 nm using a spectrophotometer.

Statistical analysis: Data of all the parameters was subjected to statistical analysis using MSTAT computer program (MSTAT Development Team, 1989) according to Snedecore & Cochran (1980).

Results

Shoot fresh and dry weights considerably ($p \leq 0.001$) decreased in both maize cultivars under drought stress (Fig. 1; Table 1). Cultivar MMRI-yellow had higher shoot fresh and dry weights compared with hybrid S-515. Exogenous TRIA (5 µM) increased fresh weight of shoot ($p \leq 0.01$) and root ($p \leq 0.05$) in both maize cultivars under water deficit and control conditions (Fig. 1; Table 1).

Root fresh and dry weights noticeably decreased in both maize cultivars under drought stress (Fig. 1; Table 1). The cultivar MMRI-Yellow showed higher root fresh and dry matter ($p \leq 0.001$) than that of hybrid S-515. Foliar application of TRIA significantly increased root fresh weight in both maize cultivars under drought stress (Fig 1; Table 1).

Table 1. Growth, chlorophyll contents, relative membrane permeability, hydrogen peroxide, malondialdehyde, soluble proteins, activities of antioxidant enzymes, and contents of free amino acids, free proline and glycinebetaine of maize (*Zea mays* L.) plants exogenously sprayed with triacontanol (TRIA) under two levels of water stress conditions.

Source of variation	df	Shoot f. wt.	Shoot dry wt.	Root f. wt.	Root dry wt.	Shoot length
Cultivars (Cvs)	1	23.53***	16.700***	0.530***	0.029***	121.7***
Drought (D)	1	229.3***	7.78***	0.135**	0.042***	97.35***
Triacontanol (TRIA)	2	10.14**	0.396*	0.056*	0.001ns	23.9**
Cvs × D	1	0.216ns	2.24***	0.040ns	0.000ns	4.551ns
Cvs × TRIA	2	1.703ns	0.042ns	0.034ns	0.000ns	0.48ns
D × TRIA	2	0.068ns	0.145ns	0.048*	0.000ns	0.105ns
Cvs × D × TRIA	2	0.003ns	0.0103ns	0.002ns	0.000ns	4.155ns
Error	24	1.108	0.110	0.012	0.000	3.373
Source of variation	**df**	**Root length**	**Total leaf area plant^{-1}**	**Chl *a***	**Chl *b***	**Total Chl**
Cultivars (Cvs)	1	3.867ns	804.99***	0.003ns	0.001ns	0.000ns
Drought (D)	1	6.417*	4847.2***	0.000ns	0.000ns	0.000ns
Triacontanol (TRIA)	2	1.40ns	53.90ns	0.04***	0.000ns	0.047***
Cvs × D	1	1.521ns	116.2ns	0.001ns	0.000ns	0.0034ns
Cvs × TRIA	2	0.254ns	22.3ns	0.000ns	0.000ns	0.000ns
D × TRIA	2	0.567ns	145.9*	0.003ns	0.000ns	0.001ns
Cvs × D × TRIA	2	0.134ns	4.60ns	0.013*	0.000ns	0.011ns
Error	24	0.942	41.38	0.0034	0.000	0.004
Source of variation	**df**	**MP%**	**H_2O_2**	**MDA**	**Soluble proteins**	**NR activity**
Cultivars (Cvs)	1	0.256ns	0.145ns	28.53***	2.271*	1.738*
Drought (D)	1	16.3ns	0.066ns	2.36ns	5.173**	0.343ns
Triacontanol (TRIA)	2	73.6ns	0.185*	2.617*	1.724*	0.372ns
Cvs × D	1	58.9ns	0.002ns	9.46**	1.371ns	0.000ns
Cvs × TRIA	2	42.8ns	0.009ns	0.770ns	0.195ns	0.677ns
D × TRIA	2	6.508ns	0.004ns	0.497ns	2.463*	0.012ns
Cvs × D × TRIA	2	3.94ns	0.021ns	0.998ns	1.643*	0.262ns
Error	24	27.12	0.051	0.751	0.467	0.399

Source of variation	df	SOD	CAT	POD	Total phenolics
Cultivars (Cvs)	1	0.0107ns	1.68ns	0.001ns	98.92***
Drought (D)	1	1.633**	18.77**	0.243***	215.4***
Triacontanol (TRIA)	2	0.032ns	0.376ns	0.045**	20.40*
Cvs × D	1	0.233ns	20.38**	0.003ns	177.4***
Cvs × TRIA	2	0.205ns	1.86ns	0.019ns	12.61ns
D × TRIA	2	0.312ns	8.75*	0.023ns	51.48***
Cvs × D × TRIA	2	0.032ns	3.30ns	0.004ns	54.34***
Error	24	0.160	2.03	0.007	4.790

Source of variation	df	Free amino acids	Free proline	GB
Cultivars (Cvs)	1	1.082ns	47.94***	68.22**
Drought (D)	1	1.289ns	54.20***	87.73**
Triacontanol (TRIA)	2	0.252ns	11.06**	2.94ns
Cvs × D	1	0.255ns	0.539ns	41.79*
Cvs × TRIA	2	0.099ns	5.665*	2.60ns
D × TRIA	2	0.216ns	3.408ns	31.88*
Cvs × D × TRIA	2	0.119ns	1.977ns	4.382ns
Error	24	0.961	1.653	7.87

df = degrees of freedom; ***, **, and * significant at 0.001, 0.01 and 0.05 levels respectively

ns = non-significant; Chl *a*, *b* and *a*/*b* ratio = chlorophyll *a*, *b* and chlorophyll *a*/*b* ratio respectively; MP (%) = relative membrane permeability (%); H_2O_2 = hydrogen peroxide; MDA = malondialdehyde; NR = nitrate reductase activity; SOD = superoxide dismutase; CAT = catalase; POD = peroxidase; GB = glycinebetaine

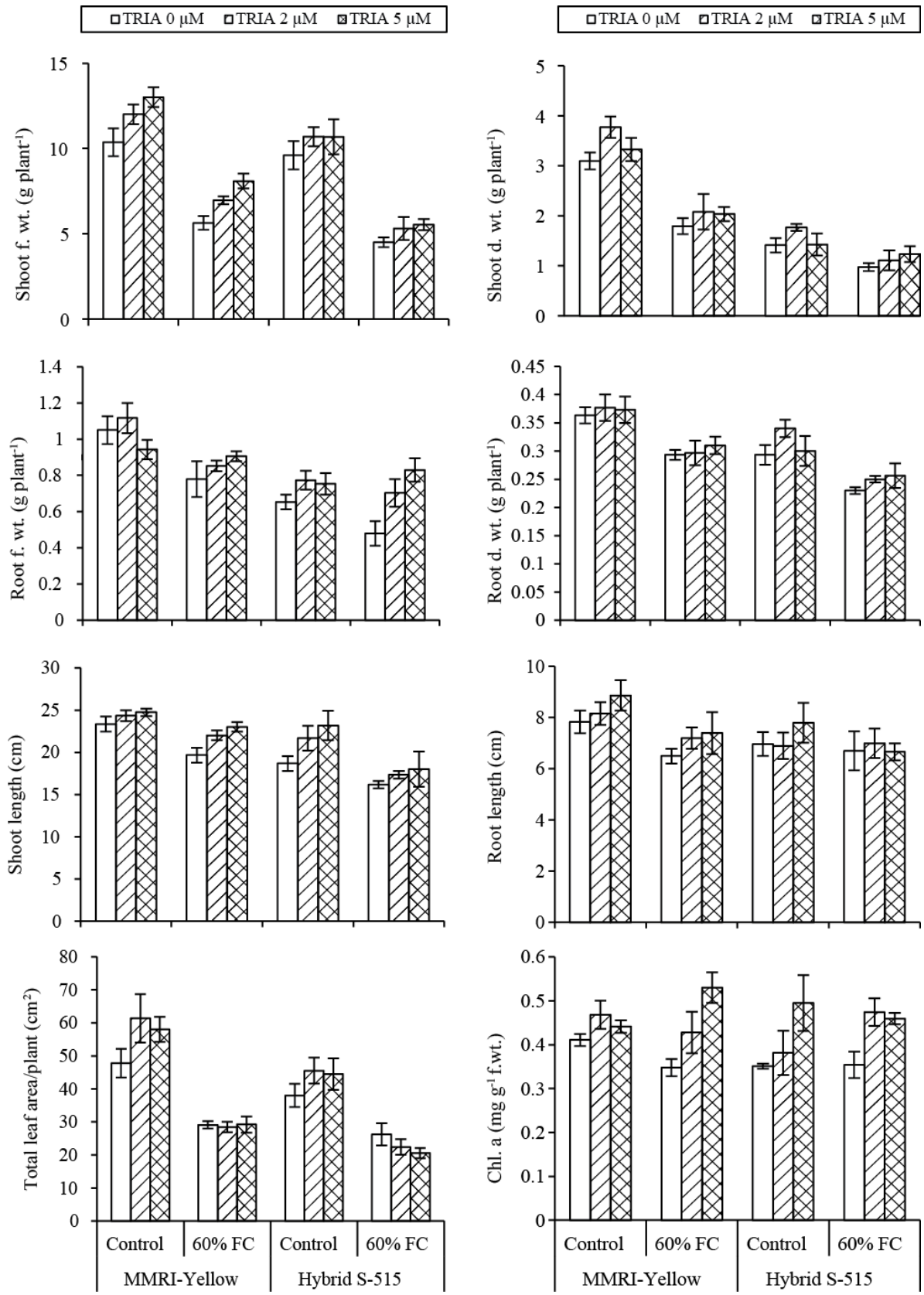

Fig. 1. Shoot and root fresh and dry weights, shoot and root lengths, total leaf area per plant and chlorophyll *a* contents of 58-day-old maize (*Zea mays* L.) plants foliary-sprayed with triacontanol under drought-stressed and non-stressed conditions.

Drought significantly altered shoot and root lengths in both the cultivars under drought stress. The cultivars also differed significantly ($p \leq 0.001$) with respect to this attribute. The cv. MMRI-yellow had greater shoot and root lengths under both control and water deficit conditions. Foliar application of TRIA significantly ($p \leq 0.01$) increased shoot length in both the cultivars (Fig. 1; Table 1).

Total leaf area plant^{-1} significantly decreased in both maize cultivars under drought stress (Fig. 1; Table 1). Foliar application of TRIA significantly ($p \leq 0.05$) increased leaf area per plant under non-stressed conditions (Fig. 1; Table 1).

Chlorophyll (Chl) □*a* , □*b*□ and total chlorophyll contents did not change under drought stress in both maize cultivars. However, foliar application of TRIA significantly ($p \leq 0.001$) increased Chl □*a* (Fig. 1) and total chlorophylls (Fig. 2; Table 1) in both maize cultivars under both drought stressed and non-stressed conditions.

Oxidative stress attributes such as relative membrane permeability (RMP), hydrogen peroxide (H_2O_2) and malondialdehyde contents (MDA) did not change under drought stress in both maize cultivars (Fig. 2). However, foliar application of TRIA significantly ($p \leq 0.05$) decreased H_2O_2 and MDA contents. Cultivars differed significantly regarding the accumulation of MDA contents. The cv. hybrid S-515 had higher MDA contents when compared with cv. MMRI-Yellow under both drought regimes.

Soluble proteins significantly ($p \leq 0.01$) increased under drought stress in both maize cultivars. Foliar application of TRIA significantly ($p \leq 0.05$) increased soluble protein contents particularly in cv. MMRI-Yellow. The foliar application of TRIA did not affect NR activity under water stress. However, cv. MMRI-yellow had higher NR activity than that of cv. Hybrid S-515 (Fig. 3; Table 1).

Drought stress significantly decreased SOD while it significantly (p□0.01) increased CAT activity in the cv. MMRI-Yellow while POD activity in both cultivars under both drought stressed and non-stressed conditions. The cultivar MMRI-Yellow had higher CAT activity under drought stress (Fig. 3; Table 1).

Total phenolic contents noticeably ($p \leq 0.001$) increased under drought stress in MMRI-yellow cultivar (Fig. 3; Table 1). Foliar application of TRIA significantly ($p \leq 0.001$) increased total phenolics in the hybrid S-515 under non-stressed conditions (Fig. 3; Table 1). The foliar application of TRIA did not alter free amino acids under drought stressed and non-stressed conditions (Fig. 3; Table 1).

Drought stress significantly altered free proline and GB contents in both maize cultivars (Fig. 3; Table 1). Foliar application of TRIA significantly (p□0.01) increased proline contents in the hybrid S-515 under both drought stressed and non-stressed conditions. However cv. Hybrid S-515 accumulated more GB contents when under drought. Foliar application of TRIA significantly (p□0.05) decreased GB contents under drought stress while increased under non-stressed conditions.

Discussion

Drought is a sever menace of agricultural crops and reduces crop yields up to 70% throughout the world (Praba *et al*., 2009; Akram *et al*., 2013; Osakabe *et al*., 2014). The foliar application of TRIA increased shoot fresh and dry weights in both maize cultivars. Drought-mediated decrease in tissue water contents leads to reduced turgor pressure thereby causing growth reductions (Delfine *et al*., 2002). In mugnbeen, foliar application of TRIA improved seedling growth under PEG-6000-induced drought stress (Dheera *et al*., 2012). In the present study, drought stress did not alter Chl contents, however, foliar application of TRIA increased total Chl and Chl □*a* contents. A number of factors such as degradation of photosynthetic machinery, destruction of Chl structure, Chl photo-oxidation, inhibition of Chl synthesis or increase in the activity of chlorophyllase could reduce photosynthetic pigments under drought stress. Furthermore, reduction in chlorophyll contents under drought stress is dependent upon the stress intensity, duration of stress, growth stage and genetic resistance of plants to drought stress (Kabiri *et al*., 2014). In contrast, drought decreased photosynthetic pigments in pea (*Pisum sativum* L.) (Karatas *et al*., 2014).

Foliar application of TRIA decreased H_2O_2 and MDA contents in both cultivars under drought stress. The TRIA can act as an antioxidizing agent (Ramanarayan *et al*., 2000). The TRIA has been shown to reduce oxidative damages in a number of crop species such as in *Triticum aestivum* L. (Perveen *et al*., 2014), *Spinaceae oleracea* L. (Ramanarayan *et al*., 2000) and *Arachis hypogaea* L. (Verma *et al*., 2011) under stressful environments. Foliar application of TRIA increased soluble proteins under drought stress. Long term drought decreased soluble proteins (Surendar *et al*., 2013; Karatas *et al*., 2014). The drought-mediated decrease in soluble proteins could be due to protein degradation and/or decreased synthesis (Pandey & Chikara, 2014). In this context, TRIA might have inhibited the activity of protease to enhance total soluble proteins under drought stress. The beneficial effects of TRIA on soluble proteins are widely documented in a number of plants such as green gram (Kumaravelu *et al*., 2000), groundnut (Verma *et al*., 2011) and soybean (Krishnan & Kumari, 2008).

Nitrate reductase (NR) is a metallo protein that gives best estimate of level of organic nitrogen compounds in plants as it converts nitrate to nitrite in the cytosol (Beevers & Hageman, 1969). The NR activity correlated with growth and yield in different plants (Garg, 2013). Both age of plants and water deficit conditions have been reported to decrease NR activity in plants (Singh & Kataria, 2012). In the present study, the foliar application of TRIA did not alter NR activity under drought stress. However, cv. MMRI-Yellow had higher NR activity when compared with Hybrid S-515 cultivar. In maize, the application of plant hormones did not induce NR activity. For instance, kinetin application did not alter NR activity in maize (Sharma & Sopory, 1987). Nonetheless, the higher NR activity in the cultivar MMRI-Yellow might have contributed towards its better performance under drought stress when compared with Hybrid S-515 cultivar.

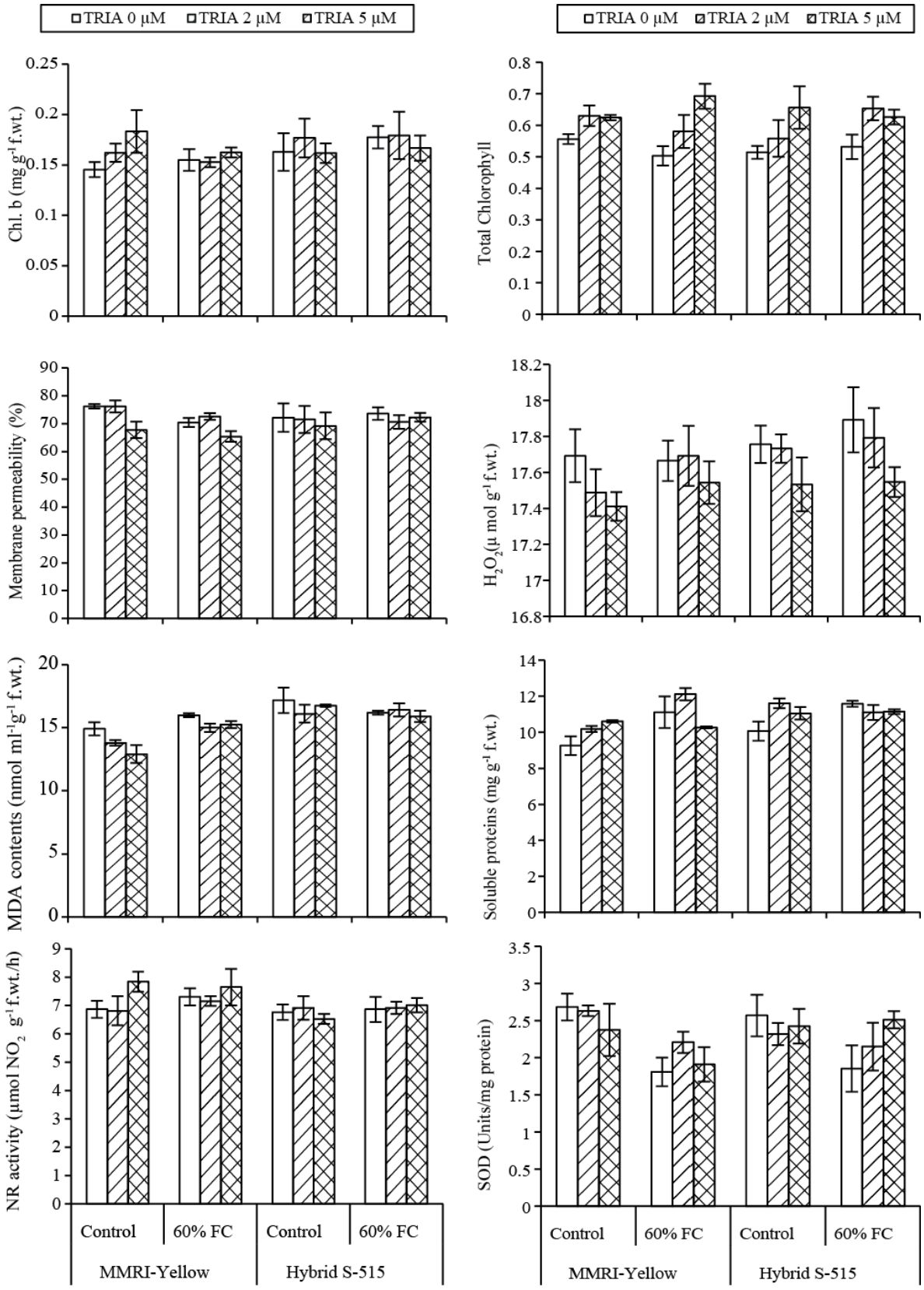

Fig. 2. Contents of chlorophyll *b*, total chlorophyll, membrane permeability, hydrogen peroxide, malondialdehyde, proteins, nitrate reductase and superoxide dismutase activity of 58-day-old maize (*Zea mays* L.) plants foliary-sprayed with triacontanol under drought-stressed and non-stressed conditions.

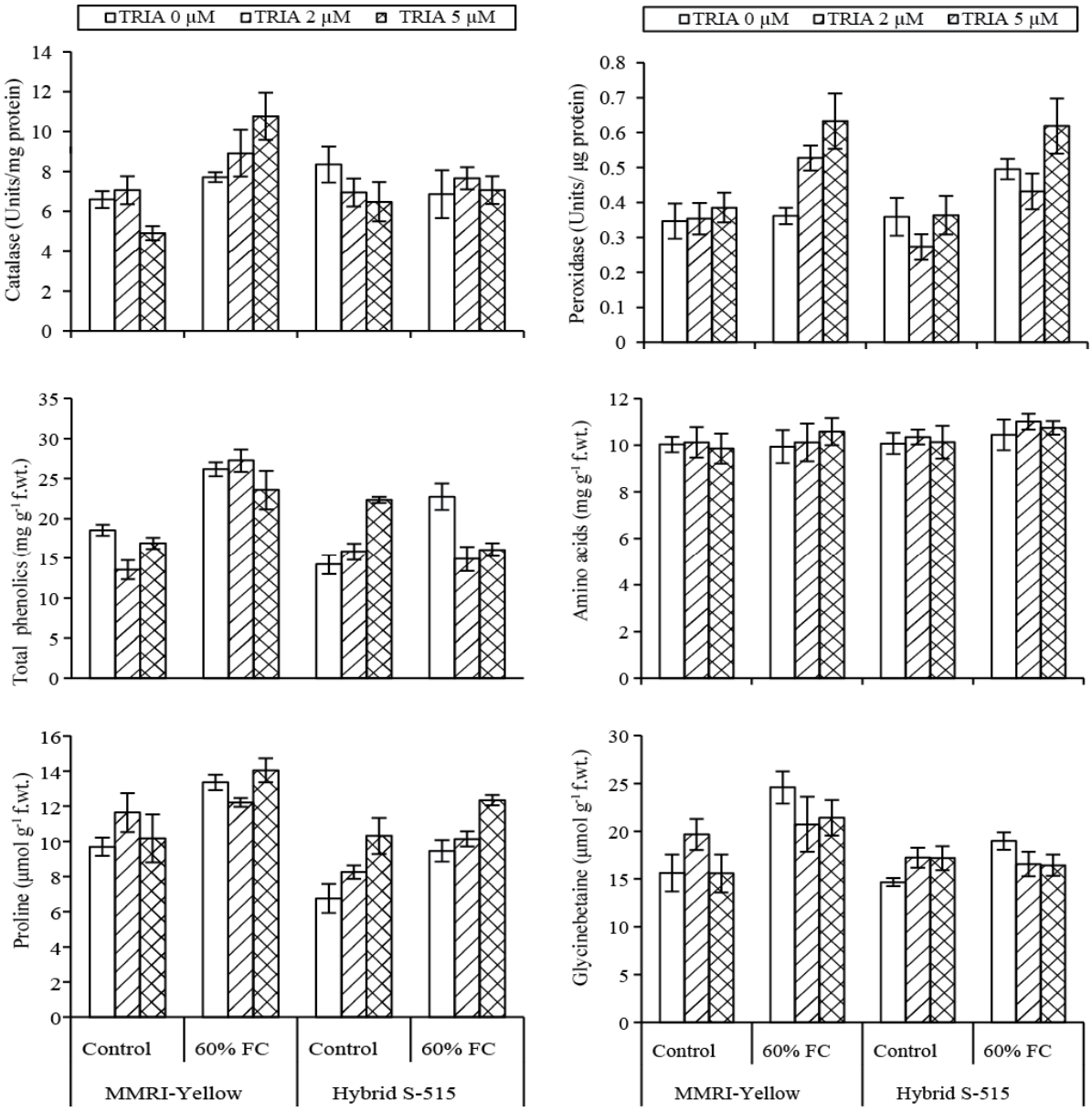

Fig. 3. Activities of catalase and peroxidase enzymes, and contents of total phenolics, free amino acids, free proline and glycinebetaine of 58-day-old maize (*Zea mays* L.) plants foliary-sprayed with triacontanol under drought-stressed and non-stressed conditions. FC = field capacity.

In the present study, drought stress decreased SOD while increased CAT and POD activities and total phenolics. Foliar application of TRIA enhanced activities of CAT while decreased total phenolic contents when under drought. Total phenolics tend to decrease by treatment with TRIA or TRIA-based bio-stimulant in wheat (Perveen *et al*., 2014) and maize (Ertani *et al*., 2013). An increased activity of antioxidant enzymes has been found to be related to the biomass production and improvement to drought stress in rice (Lum *et al*., 2014).

Free amino acids play role in the mitigation of reactive oxygen species (Sandhya *et al*., 2010). Higher amino acid pool is due to the breakdown of structural proteins. Accumulation of free amino acids under water deficit conditions help plants to osmotically adjust cellular environment and related to tolerance capability of crop plants such as wheat, pepper and sorghum (Yadav *et al*., 2005). Interestingly, in the present study, drought stress and foliar application of TRIA did not alter free amino acids. However, drought stress significantly increased free proline and glycinebetaine (GB) contents while TRIA increased free proline and decreased GB contents under drought stress. The application of TRIA decreased free proline in the soybean (Krishnan & Kumari, 2008). Proline acts as an osmoticum and help plants to osmotically adjust under water limited environments. Accumulation may be due to increased synthesis or decreased degradation of proline under stressful environments (Szabados & Savoure, 2009). Proline stabilizes proteins structure, enhances antioxidant defence system and maintains cellular pH under stressful

conditions. Proline content is a good indicator for screening tolerant cultivars under water stress (Rahdari *et al*., 2012). The results suggested that foliar application of TRIA helped plants to osmotically adjust cellular environment through accumulation of osmotic including proline rather than amino acids. In addition, TRIA has a potential to increase its effect by integrating with cytokinins and gibberellic acid (Veram *et al*., 2009; Aftab *et al*., 2010) as well as by increasing a signalling molecules like Ca^{2+} (Perveen *et al*., 2014).

In conclusion, drought stress negatively affected growth and altered soluble proteins, NR activity, total phenolics, free proline and GB contents in both maize cultivars. Of different levels, 5 µM TRIA was much more effective in modulating growth and physiochemical attributes and thus induced drought tolerance in maize plants. Of the two maize cultivars, cv. MMRI-Yellow performed better under both control and drought stressed conditions.

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References

Aftab, T., M.M.A. Khan, M. Idrees, M. Naeem, M. Singh and M. Ram. 2010. Stimulation of crop productivity, photosynthesis and artemisinin production in *Artemisia annua* L. by triacontanol and gibberellic acid application. *J. Plant Interac*., 4: 273-281.

Akram, H.M., A. Ali, A. Sattar, H.S.U. Rehman and A. Bibi. 2013. Impact of water deficit stress on various physiological and agronomic traits of three Basmati rice (*Oryza sativa* L) cultivars. *J. Anim. Plant Sci*., 23(5): 1415-1423.

Arnon, D.T. 1949. Copper enzyme in isolated chloroplasts polyphenoloxidase in *Beta vulgaris*. *Plant Physiol*., 24: 1-15.

Aslam, M., M. Zeeshan, M.A. Maqbool and B. Farid. 2014. Assessment of drought tolerance in maize (*Zea may* L.) genotypes at early growth stages by using principle component and biplot analysis. *The Experiment*, 29(1): 1943-1951.

Aziz, R., M. Shahbaz and M. Ashraf. 2013. Influence of foliar application of triacontanol on growth attributes, gas exchange and chlorophyll fluorescence in sunflower (*Helianthus annuus* L.) under saline stress. *Pak. J. Bot.,* 45: 1913-1918.

Bates, I.S., R.P. Waldren and I.D. Teare. 1973. Rapid determination of free proline for water stress studies. *Plant Soil*, 39: 205-207.

Beevers, L. and R.H. Hageman. 1969. Nitrate reduction and its role in nitrate assimilation in plants. *Physiol. Plant*., 74: 214-219.

Bolaòos, J., G.O. Edmeades and L. Martinetz. 1993. Eight cycles of selection for drought tolerance in lowland tropical, maize. III. Responses in drought adaptive physiological and morphological traits. *Field Crops Research*, 31: 269-286.

Borowski, E. and Z.K. Blamowski. 2009. The effect of triacontanol 'TRIA'and Asahi-SL on the development and metabolic activity of sweet basil (*Ocimum basilicum* L.) plants treated with chilling. *Folia Hortic*., 21: 39-48.

Bradford, M.M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Annal. Biochem*., 72: 248-254.

Carleton and Foote. 1965. A comparison of methods for estimating total leaf area of barley plants. *Crop Sci*., 5(6): 602-603.

Carmak, I. and J.H. Horst. 1991. Effects of aluminum on lipid peroxidation, superoxide dismutase, catalase, and peroxidase activities in root tips of soybean (*Glycine max*). *Physiol. Plant*, 83: 463-468.

Chance, B. and A. Maehly. 1955. Assay of catalase and peroxidase. *Meth. Enzymol*., 2: 764-817.

Critchley, W. and S. Klaus. 1991. A manual for the design and construction of water harvesting schemes for plant production.

Delfine, S., R. Tognettir, F. Loreto and A. Alvino. 2002. Physiological and growth responses to water stress in field grown bell pepper (*Capsicum annuum* L.). *J. Hort. Sci. Biotechnol*., 77(6): 697-704.

Dheera, S., K. Ekta and C.P. Malik. 2012. Effect of drought stress and its interaction with two phytohormones on *Vigna radiata* seed germination and seedling growth. *Int. J. Life Sci*., 1(3): 201-207.

EL-Beltagi, H.S. and H.I. Mohamed. 2013. Alleviation of cadmium toxicity in *Pisum sativum* L. seedlings by calcium chloride. *Not. Bot. Horti. Agrobo.,* 41: 157-168.

El-Tayeb, M.A. 2006. Differential response of two *Vicia faba* cultivars to drought: growth, pigments, lipid peroxidation, organic solutes, catalase and peroxidase activity. *Acta Agronomica Hungarica*, 54(1): 25-37.

Ertani, A., M. Schiavon, A. Muscolo and S. Nardi. 2013. Alfalfa plant derived biostimulant stimulate short-term growth of salt stressed *Zea mays* L. plants. *Plant and Soil*, 364: 145-158.

Garg, S.K. 2013. Role and hormonal regulation of nitrate reductase activity in higher plants: A review. *Plant Sci. Feed*, 3(1): 13-20.

Giannopolitis, C.N. and S.K. Ries. 1977. Superoxide dismutase. I. Occurrence in higher plants. *Plant Physiol*., 59: 309-314.

Grieve, C.M. and S.R. Grattan. 1983. Rapid assay for the determination of water soluble quaternary ammonium compounds. *Plant Soil*, 70: 303-307.

He, Y.W. and C.S. Loh. 2000. Cerium and lanthanum promote floral initiation and reproductive growth of *Arabidopsis thaliana*. *Plant Sci*., 159: 117-124.

Iqbal, M. and M. Ashraf. 2013a. Gibberellic acid mediated induction of salt tolerance in wheat plants: growth, ionic partitioning, photosynthesis, yield and hormonal homeostasis. *Environ. Exp. Bot*., 86: 76-85.

Iqbal, M. and M. Ashraf. 2013b. Alleviation of salinity-induced perturbations in ionic and hormonal concentrations in spring wheat through seed preconditioning in synthetic auxins. *Acta Physiol. Plant*., 35: 1093-1112.

Jaworski, E.G. 1971. Nitrate reductase assay in intact plant tissue. *Biochem. Biophysic. Res. Commun*., 43: 1274-1279.

Julkenen-Titto, R. 1985. Phenolic constituents in the leaves of northern willows: methods for the analysis of certain phenolics. *J. Agric. Food Chem*., 33: 213-217.

Kabiri, R., F. Nasibi and H. Farahbakhsh. 2014. Effect of exogenous salicylic acid on some physiological parameters and alleviation of drought stress in *Nigella sativa* plant under hydroponic culture. *Plant Protect. Sci.,* 50(1): 43-51.

Karataş, I., L. Öztürk, Y. Demir, A. Ünlükara, A. Kurunç and O. Düzdemir. 2014. Alterations in antioxidant enzyme activities and proline content in pea leaves under long-term drought stress. *Toxicol Ind Health*, 30: 693-700.

Kilic, N., K., E. Duygu and G. Donmez. 2010. Triacontanol hormone stimulates population, growth and brilliant blue r dye removal by common duckweed from culture media. *J. Hazard Mater*., 182: 525-530.

Krishnan, R.R. and B.D.R. Kumari. 2008. Effect of n-triacontanol on the growth of salt stressed soybean plants. *J. Biosci*., 19: 53-56.

Kumaravelu, G., V.D. Livingstone and M.P. Ramanujam. 2000. Triacontanol-induced changes in the growth, photosynthetic pigments, cell metabolites, flowering and yield of green gram. *Biology Plant*, 43: 287-290.

Lambers, H., F.S. Chapin and T.L. Pons. 2008. *Plant Physiol. Ecol., second edition*. New York: Springer.

Liu, D.H., W.S. Jiang and W.Q. Hou. 2001. Uptake and accumulation of copper by roots and shoots of maize. *J. Environ. Sci*., 13: 228-232.

Lum, M.S., M.M. Hanafi, Y.M. Rafii and A.S.N. Akmar. 2014. Effect of drought stress on growth, proline and antioxidant enzyme activities of upland rice. *J. Anim. Plant Sci*., 24(5): 1487-1493.

Moore, S. and W.H. Stein. 1957. A modified ninhydrin Reagent for the photometric determination of amino acids and related compounds. *J. Biol. Chem*., 211: 907-913.

MSTAT, Development Team. 1989. MSTAT user's guide: A microcomputer program for the design management and analysis of agronomic research experiments. Michigan State Univ. East Lansing, USA.

Muthuchelian, K., C. Murugan, N. Nedunchelian and G. Kulandaivellu. 1997. Photosynthesis and growth of *Erythrina variegata* as affected by water stress and triacontanol. *Photosynthetica,* 33: 241-248.

Osakabe, Y., K. Osakabe, K. Shinozaki and L.S. Phan Tran. 2014. Response of plants to water stress. *Front. Plant Sci.,* 5: 86.

Pandey, M., and S.K. Chikara. 2014. In vitro regeneration and effect of abiotic stress on physiology and biochemical content of *Stevia Rebaudiana*'Bertoni'. *J. Plant Sci. Res*., 1(3): 1-13.

Peifang, C., L. Hangyi and L. Yi. 2015. Physiological responses of seedling roots of the desert plant *Reaumuria soongorica* to drought stress[J]. *Acta Prataculturae Sinica*, 24(1): 72-80.

Perveen, S., M. Shahbaz and M. Ashraf. 2010. Regulation in gas exchange and quantum yield of photosystem II (PSII) in salt-stressed and non-stressed wheat plants raised from seed treated with triacontanol. *Pak. J. Bot*., 42(5): 3073-3081.

Perveen, S., M. Shahbaz and M. Ashraf. 2012a. Changes in mineral composition, uptake and use efficiency of salt stressed wheat (*Triticum aestivum* L.) plants raised from seed treated with triacontanol. *Pak. J. Bot*., 44: 27-35.

Perveen, S., M. Shahbaz and M. Ashraf. 2012b. Is pre-sowing seed treatment with triacontanol effective in improving some physiological and biochemical attributes of wheat (*Triticum aestivum* L.) under salt stress. *J. Appl. Bot. Food Qual.,* 85: 41-48.

Perveen, S., M. Shahbaz and M. Ashraf. 2013. Influence of foliar-applied triacontanol on growth, gas exchange characteristics, and chlorophyll fluorescence at different growth stages in wheat under saline conditions. *Photosynthetica*, 51: 541-551.

Perveen, S., M. Shahbaz and M. Ashraf. 2014. Triacontanol-induced changes in growth, yield, leaf water relations, antioxidative defense system and some key osmoprotectants in bread wheat (*Triticum aestivum* L.) under saline stress. *Turk. J. Bot*., 38: 896-913.

Praba, M.L., J.E. Cairns, R.C. Babu and H.R. Lafitte. 2009. Identification of physiological traits underlying cultivar differences in drought tolerance in rice and wheat. *J. Agron. Crop Sci*., 195: 30-46.

Rahdari, P., S.M. Hosseini and S. Tavakoli. 2012. The study effect of drought stress on germination, proline, sugar, lipid, protein and chlorophyll content in purslane (*Portulaca oleracea* L.) leaves. *J. Med. Plants Res*., 6(9): 1539-1547.

Ramanarayan, K., A. Bhut, V. Shripathi, G.S. Swamy and K.S. Rao. 2000. Triacontanol inhibits both enzymatic and nonenzymatic lipid peroxidation. *Phytochemistry*, 55: 59-66.

Ries, S.K. 1991. Triacontanol and its second messenger 9-β-L (+)-adenosine as plant growth substances. *Plant Physiol*., 95: 986-989.

Sandhya, V., Sk.Z. Ali, M. Grover, G. Reddy and B. Venkateswarlu. 2010. Effect of plant growth promoting Pseudomonas spp. on compatible solutes, antioxidant status and plant growth of maize under drought stress. *Plant Growth Regul*., 62: 21-30.

Sharma, A.K. and S.K. Sopory. 1987. Effect of phytochrome and kinetin on nitrite reductase activity in *Zea mays*. *Plant Cell Physiol*., *28(3)*: 447-454.

Singh, K., B.S. Afria and B.L. Kakralya. 1991. Seed and protein yield of macrosperma chickpea in response to treatment with growth substances under field conditions. *Indian J. Plant Physiol*., 34(2): 137-142.

Singh, M., M.M.A. Khan, Moinuddin and M. Naeem. 2011. Augmentation of nutraceuticals, productivity and quality of ginger (*Zingiber officinale* Rosc.) through triacontanol application. *Plant Biosystems,* 1-8. ISSN (Print) 1126-3504 - ISSN (Online) 1724-5575. DOI:10.1080/11263504. 2011. 575891.

Singh, N., and N. Kataria. 2012. Role of potassium fertilizer on nitrogen fixation in chickpea (*Cicer arietinum* L.) under quantified water stress. *J. Agric. Technol.,* 8(1): 377-392.

Snedecor, G.W. and G.W. Cochran. 1980. Statistical Methods. 7th edition. The Lowa State University Press. Ames, Lowa.

Srivastava, S. and M. Srivastava. 2014. Morphological changes and antioxidant activity of *Stevia rebaudiana* under water stress. *Amer. J. Plant Sci.,* 5: 3417-3422.

Surendar, K.K., D.D. Devi, I. Ravi, P. Jeyakumar and K. Velayudham. 2013. Water stress affects plant relative water content, soluble protein, total chlorophyll content and yield of *Ratoon Banana*. *Inter. J. Hort.*, 3: 96-103.

Szabados, L. and A. Savouré. 2009. Proline: a multifunctional amino acid, *Trends Plant Sci*., 15(2): 89-97.

Velikova, V., I. Yordanov and A. Edreva. 2000. Oxidative stress and some antioxidant systems in acid rain-treated bean plants: protective roles of exogenous polyamines. *Plant Sci*., 151: 59-66.

Verma, A., C.P. Malik, V.K. Gupta and Y.K. Sinsinwar. 2009. Response of groundnut varieties to plant growth regulator (BAP) to induce direct organogenesis. *World J. Agric. Sci*., 5(3): 313-317.

Verma, A., Malik, C.P., Gupta, V.K. and B.K. Bajaj. 2011. Effects of in vitro triacontanol on growth, antioxidant enzymes, and photosynthetic characteristics in *Arachis hypogaea* L. *Braz. J. Plant Physiol*., 23(4): 271-277.

Yadav, S.K., N.J. Lakshmi, M. Maheswari, M. Vanaja and B. Venkateswarlu. 2005. Influence of water deficit at vegetative, anthesis and grain filling stages on water relation and grain yield in sorghum. *Indian J. Plant Physiol*., 10: 2-20.

Yang, G., G. Rhodes and R.G. Joly. 1996. Effects of high temperature on membrane stability and chlorophyll fluorescence in glycinebetaine-deficient and glycinebetaine-containing maize lines. *Aust. J. Plant Physiol.,* 23(4): 437-443.

Zulfiqar, S. and M. Shahbaz. 2013. Modulation in gas exchange parameters and photosystem-II activity of canola (*Brassica napus* L.) by foliar-applied triacontanol under salt stress. *Agrochimica*, 57: 193-200.

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