INTRODUCTION 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 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 (H₂O₂), 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 (Kilkic 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 C₄ 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) (Crittchley & 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, CO₂ 0.84 me L⁻¹, HCO₃⁻ 4.84 me L⁻¹, Cl⁻ 5.84 me L⁻¹, Na⁺ 6.24 me L⁻¹, Ca²⁺ and Mg²⁺ 5.88 me L⁻¹ and sodium adsorption ratio (SAR) 3.84 mmol L⁻¹ 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 (cm²) 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 (ECᵢ). The test tubes were kept overnight at 4°C and ECᵢ determined. The and tissue was autoclaved for 1 h, cooled at normal temperature and ECₑ determined. The membrane permeability was calculated as described by Yang et al. (1996).

Hydrogen peroxide (H₂O₂) 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) trichloroacetic acid (TCA) by using pestle and mortar in an ice bath. The extracted material was centrifuged at 12000 × g for 10 min. 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.

\[
\text{MDA (nmol)} = \Delta (A 532 \text{ nm} – A 600 \text{ nm})/1.56 \times 105
\]

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 described 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 H₂O₂ (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 H₂O, 250 μl of 50 mM phosphate buffer (pH 7.8), 100 μl of 40 mM H₂O₂, 100 μl of 20 mM guaiacol 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% Na₂CO₃ 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 (Julkkenen-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 H₂SO₄ 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.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>df</th>
<th>Shoot f. wt.</th>
<th>Shoot dry wt.</th>
<th>Root f. wt.</th>
<th>Root dry wt.</th>
<th>Shoot length</th>
</tr>
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<tbody>
<tr>
<td>Cultivars (Cvs)</td>
<td>1</td>
<td>23.5***</td>
<td>16.70***</td>
<td>0.530***</td>
<td>0.029***</td>
<td>121.7***</td>
</tr>
<tr>
<td>Drought (D)</td>
<td>1</td>
<td>229.3***</td>
<td>7.78***</td>
<td>0.135**</td>
<td>0.042***</td>
<td>97.35***</td>
</tr>
<tr>
<td>TRIA (TRIA)</td>
<td>2</td>
<td>10.14***</td>
<td>0.396*</td>
<td>0.056*</td>
<td>0.001ns</td>
<td>23.9*</td>
</tr>
<tr>
<td>Cvs × D</td>
<td>1</td>
<td>21.6ns</td>
<td>2.24***</td>
<td>0.040ns</td>
<td>0.000ns</td>
<td>0.000ns</td>
</tr>
<tr>
<td>Cvs × TRIA</td>
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<td>1.703ns</td>
<td>0.042ns</td>
<td>0.034ns</td>
<td>0.000ns</td>
<td>0.000ns</td>
</tr>
<tr>
<td>D × TRIA</td>
<td>2</td>
<td>0.068ns</td>
<td>0.145ns</td>
<td>0.048*</td>
<td>0.000ns</td>
<td>0.000ns</td>
</tr>
<tr>
<td>Cvs × D × TRIA</td>
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<td>0.003ns</td>
<td>0.0103ns</td>
<td>0.0002ns</td>
<td>0.000ns</td>
<td>0.000ns</td>
</tr>
<tr>
<td>Error</td>
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<td>1.108</td>
<td>0.110</td>
<td>0.012</td>
<td>0.000</td>
<td>3.373</td>
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<th>Source of variation</th>
<th>df</th>
<th>Root length</th>
<th>Total leaf area plant$^{-1}$</th>
<th>Chl a</th>
<th>Chl b</th>
<th>Total Chl</th>
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<td>3.867ns</td>
<td>804.9***</td>
<td>0.003ns</td>
<td>0.001ns</td>
<td>0.000ns</td>
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<tr>
<td>Drought (D)</td>
<td>1</td>
<td>6.417*</td>
<td>4847.2***</td>
<td>0.000ns</td>
<td>0.000ns</td>
<td>0.000ns</td>
</tr>
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<td>TRIA (TRIA)</td>
<td>2</td>
<td>1.40ns</td>
<td>53.90ns</td>
<td>0.04***</td>
<td>0.000ns</td>
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</tr>
<tr>
<td>Cvs × D</td>
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<td>1.521ns</td>
<td>116.2ns</td>
<td>0.001ns</td>
<td>0.000ns</td>
<td>0.000ns</td>
</tr>
<tr>
<td>Cvs × TRIA</td>
<td>2</td>
<td>0.254ns</td>
<td>22.3ns</td>
<td>0.000ns</td>
<td>0.000ns</td>
<td>0.000ns</td>
</tr>
<tr>
<td>D × TRIA</td>
<td>2</td>
<td>0.647ns</td>
<td>145.79*</td>
<td>0.003ns</td>
<td>0.000ns</td>
<td>0.000ns</td>
</tr>
<tr>
<td>Cvs × D × TRIA</td>
<td>2</td>
<td>0.134ns</td>
<td>4.60ns</td>
<td>0.013*</td>
<td>0.000ns</td>
<td>0.011ns</td>
</tr>
<tr>
<td>Error</td>
<td>24</td>
<td>0.942</td>
<td>41.38</td>
<td>0.0034</td>
<td>0.000</td>
<td>0.004</td>
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<table>
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<th>Source of variation</th>
<th>df</th>
<th>MP%</th>
<th>H$_2$O$_2$</th>
<th>MDA</th>
<th>Soluble proteins</th>
<th>NR activity</th>
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<td>0.145ns</td>
<td>28.53***</td>
<td>2.271*</td>
<td>1.738*</td>
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<tr>
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<td>16.3ns</td>
<td>0.066ns</td>
<td>2.36ns</td>
<td>5.173**</td>
<td>0.343ns</td>
</tr>
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<td>TRIA (TRIA)</td>
<td>2</td>
<td>73.6ns</td>
<td>0.185*</td>
<td>2.617*</td>
<td>1.724*</td>
<td>0.372ns</td>
</tr>
<tr>
<td>Cvs × D</td>
<td>1</td>
<td>58.9ns</td>
<td>0.002ns</td>
<td>9.46**</td>
<td>1.371ns</td>
<td>0.000ns</td>
</tr>
<tr>
<td>Cvs × TRIA</td>
<td>2</td>
<td>42.8ns</td>
<td>0.009ns</td>
<td>0.770ns</td>
<td>0.195ns</td>
<td>0.677ns</td>
</tr>
<tr>
<td>D × TRIA</td>
<td>2</td>
<td>6.508ns</td>
<td>0.004ns</td>
<td>0.497ns</td>
<td>2.463*</td>
<td>0.012ns</td>
</tr>
<tr>
<td>Cvs × D × TRIA</td>
<td>2</td>
<td>3.94ns</td>
<td>0.021ns</td>
<td>0.998ns</td>
<td>1.643*</td>
<td>0.262ns</td>
</tr>
<tr>
<td>Error</td>
<td>24</td>
<td>0.942</td>
<td>41.38</td>
<td>0.0034</td>
<td>0.000</td>
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<table>
<thead>
<tr>
<th>Source of variation</th>
<th>df</th>
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<th>CAT</th>
<th>POD</th>
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<td>0.001ns</td>
<td>98.92**</td>
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<td>Drought (D)</td>
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<td>1.633**</td>
<td>18.77**</td>
<td>0.243***</td>
<td>215.4***</td>
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<tr>
<td>TRIA (TRIA)</td>
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<td>0.032ns</td>
<td>0.376ns</td>
<td>0.045**</td>
<td>20.40*</td>
</tr>
<tr>
<td>Cvs × D</td>
<td>1</td>
<td>0.233ns</td>
<td>20.38**</td>
<td>0.003ns</td>
<td>177.4***</td>
</tr>
<tr>
<td>Cvs × TRIA</td>
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<td>0.205ns</td>
<td>1.86ns</td>
<td>0.019ns</td>
<td>12.61ns</td>
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<tr>
<td>D × TRIA</td>
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<td>0.312ns</td>
<td>8.75*</td>
<td>0.023ns</td>
<td>51.48***</td>
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<tr>
<td>Cvs × D × TRIA</td>
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<td>0.032ns</td>
<td>3.30ns</td>
<td>0.004ns</td>
<td>54.34***</td>
</tr>
<tr>
<td>Error</td>
<td>24</td>
<td>0.160</td>
<td>2.03</td>
<td>0.007</td>
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<tr>
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<th>df</th>
<th>Free amino acids</th>
<th>Free proline</th>
<th>GB</th>
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<tr>
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<td>68.22**</td>
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<td>54.20***</td>
<td>87.73**</td>
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<tr>
<td>TRIA (TRIA)</td>
<td>2</td>
<td>0.252ns</td>
<td>11.06**</td>
<td>2.94ns</td>
</tr>
<tr>
<td>Cvs × D</td>
<td>1</td>
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<td>0.539ns</td>
<td>41.79*</td>
</tr>
<tr>
<td>Cvs × TRIA</td>
<td>2</td>
<td>0.099ns</td>
<td>5.665*</td>
<td>2.60ns</td>
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<td>D × TRIA</td>
<td>2</td>
<td>0.216ns</td>
<td>3.408ns</td>
<td>31.88*</td>
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<tr>
<td>Cvs × D × TRIA</td>
<td>2</td>
<td>0.119ns</td>
<td>1.977ns</td>
<td>4.382ns</td>
</tr>
<tr>
<td>Error</td>
<td>24</td>
<td>0.961</td>
<td>1.653</td>
<td>7.87</td>
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</table>

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 \((p<0.001)\) 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 \((p\leq0.01)\) 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 \((p\leq0.05)\) increased leaf area per plant under non-stressed conditions (Fig. 1; Table 1).

Chlorophyll \((\text{Chl})\) \(a\), \(b\) and total chlorophyll contents did not change under drought stress in both maize cultivars. However, foliar application of TRIA significantly \((p\leq0.001)\) increased \text{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 \((\text{RMP})\), hydrogen peroxide \((\text{H}_2\text{O}_2)\) and malondialdehyde contents \((\text{MDA})\) did not change under drought stress in both maize cultivars. However, foliar application of TRIA significantly \((p\leq0.01)\) increased \text{H}_2\text{O}_2 and \text{MDA} contents. Cultivars differed significantly regarding the accumulation of \text{MDA} contents. The cv. hybrid S-515 had higher \text{MDA} contents when compared with cv. MMRI-Yellow under both drought regimes.

Soluble proteins significantly \((p\leq0.01)\) increased under drought stress in both maize cultivars. Foliar application of TRIA significantly \((p\leq0.05)\) 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).

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 \((p \leq 0.05)\) 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 mugnbean, 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 \text{H}_2\text{O}_2 and \text{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 \text{Triticum aestivum L.} (Perveen et al., 2014), \text{Spinacea oleracea L.} (Ramanarayan et al., 2000) and \text{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 \((\text{NR})\) 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 Ca²⁺ (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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