Original Article

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Triazole induced changes on biochemical and antioxidant metabolism of *Zea mays* L. (Maize) under drought stress

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ABSTRACT

The effects of triazole compounds namely triadimefon (TDM), tebuconazole (TBZ), and propiconazole (PCZ) on drought stress, biochemical, and antioxidant metabolism were studied in *Zea mays* L. (Maize) under pot culture. Plants were subjected to 4 days interval drought stress and drought with TDM at 15 mg/L, TBZ at 10 mg/L, and PCZ at 15 mg/L separately by soil drenching on 30, 40, and 50 days after sowing (DAS). Irrigation at 1 day interval was kept as control. The plant samples were collected on 40, 50, and 60 DAS and separated into root, stem, and leaf for estimating the amino acid (AA) and proline (PRO) contents and the activities of antioxidant enzymes. The drought and drought with triazole treatments increased the AA and PRO contents, superoxide dismutase, catalase, and ascorbate peroxidise activities when compared to control. Our results of this investigation have good significance, as this increase the innate biochemical and antioxidant metabolism of this maize plant.

KEY WORDS: Antioxidant enzymes, drought, biochemical, propiconazole, tebuconazole, triadimefon

INTRODUCTION

Water is a major limiting factor affecting plant growth, development, and yield mainly in arid and semi-arid regions where plants are often exposed to periods of water deficit drought (Wang *et al.*, 2004). Drought, cold, and salinity are major forms of stress from abiotic sources that adversely affect plant growth and productivity (Nakashima *et al.*, 2012) of which drought is considered as the most devastating. Drought or soil water deficit can be chronic in climatic regions with low water availability or random and unpredictable due to changes in weather conditions during the period of plant growth. The effects of drought are expected to increase with climate change and growing water scarcity. Water is an increasingly scarce resource given current and future human population and societal needs, putting an emphasis on sustainable water use (Rosegrant and Cline, 2003). Thus, an understanding of drought stress and water use in relation to plant growth is of importance for sustainable agriculture. The need for water conservation and evaluation of the existing and/or newly developed germplasm of crop plants for their tolerance to drought has become urgent (Morison *et al.*, 2008; Sivritepe *et al.*, 2008). Under drought stress, plants tend to close their stomata to avoid unnecessary water loss (Rasmussen *et al.*, 2013). Plants, being sessile, have evolved specific acclimation and adaptation mechanisms to respond to and survive short- and long-term drought stresses. Analysis of these protective mechanisms will contribute to our knowledge of tolerance and resistance to stress. The complex responses to environmental stress, from perception to transcriptional and physiological changes, need to be considered at a global systems biology level to study the multiple interactive components in this biological process (Krishnan and Pereira, 2008).

Maize (*Zea mays* L.) is one of the most important crops for livestock and humans worldwide. Its growth and development are seriously affected by adverse environmental conditions (Ron and Walter, 2007). It is primarily a cross-pollinating species, a feature that has contributed to its broad morphological variability and geographic adaptability. Kernels may be colorless (white) or yellow, red, blue, or variegated with these colors in mottled or striated patterns (Salvador, 1997). Traditionally maize is used for human consumption (white maize)

(Rehman *et al.*, 2002) and animal feed (yellow maize) (Dredge, 2004). This crop is also versatile and is used in many inedible products, including rubber, plastics, biofuel (Bant, 2007), alcohol fermentation, clothing, food additives, and adjuncts and literally thousands of other forms (Abbas *et al.*, 2006).

Triazoles are the largest and most important group of systemic compounds, developed in the 1960s for the control of fungal diseases in plants and animals. Commercial triazole derivatives (such as paclobutrazol [(2RS, 3RS)-1-(4-chlorophenyl)-4,4- dimethyl-2-(1,2,4-triazolyl)-pentan-3-ol]) have been recommended for use as either fungicides or plant growth regulators (Fletcher *et al.*, 1986). Several of these compounds, including paclobutrazol, uniconazole, tetraconazole, and triadimefon (TDM), cause remarkable growth responses in plants. Changes caused by triazoles are mediated through cytochrome P-450 group of enzyme inhibition (Zhu *et al.*, 2004). Characteristic of morphological and anatomical effects of the triazole include reduced shoot elongation and trichome length, increased epicuticular wax, larger chloroplasts, and increased root growth (Grossmann, 1990). Triazoles affect the isoprenoid pathway and alter the levels of certain plant hormones by inhibiting gibberellin synthesis, reducing ethylene evolution and increasing cytokinin levels (Kamountsis and Chronopoulou-Sereli, 1999). Triazole treated plants have a more efficient free-radical scavenging system that enables them to detoxify active oxygen (Kopyra and Gwozdz, 2003). Morphological and physiological changes associated with triazole treatment in various plants, include the inhibition of plant growth, decreased internodal elongation, increased chlorophyll levels, enlarged chloroplasts, thicker leaf tissue, increased root to shoot ratio, increased antioxidant potentials, and an enhancement in alkaloid production (Muthukumarasamy and Panneerselvam, 1997; Muthukumarasamy *et al.*, 2000; Jaleel *et al.*, 2006). The drought stress amelioration by triazole compounds is of major research interest because these compounds have the innate potentiality for increasing antioxidant enzymes and molecules in oxidative stressed plants (Fletcher *et al.*, 2000).

Biochemical effects of the triazole include detoxification of active oxygen (Kraus and Fletcher, 1994), increased levels of proline (PRO) (Mackay *et al.*, 1990), and antioxidants (Senaratna *et al.*, 1988). The application of TDM caused a partial recovery of the damaging effect of drought stress by its influence on the antioxidant system (Mohamadi and Rajaei, 2013). Triazoles protect plants against various stresses including drought, low and high temperatures, ultraviolet light, and air pollution. They have been referred to as plant "multi-protectants" because of their ability to induce tolerance in plants to environmental and chemical stresses (Gupta *et al.*, 2004). Therefore, spraying PBZ with 50 mg/L or 6-BA with 30 mg/L at the heading stage could increase grain yields and improve grain qualities in the two super-hybrid rice (Pan *et al.*, 2013). This study investigated the use of TDM, tebuconazole (TBZ), and propiconazole (PCZ) as one of the triazole less has been tested on tolerance to the drought stress in *Z. mays* L.

MATERIALS AND METHODS

Biological Material and Drought Stress Applications

The hybrid maize seeds variety NK 6240 were obtained from Syngenta India Private Limited and used for this investigation. The experimental seeds were surface sterilized with 0.2% mercuric chloride solution for 5 min with frequent shaking and thoroughly washed with tap water. In the preliminary study, under lab condition, 2, 5, 10, 15, and 20 mg/L of triazole compounds were tested and among the concentration tested, 15 mg/L of TDM, 10 mg/L of TBZ and 15 mg/L of PCZ treatments were prepared as optimum doses and used for further study. Plastic pots of 40 cm diameter and 45 cm height size were used for pot culture study. The pots were filled with 10 kg of soil mixture containing red soil; sand and farm yard manure in 1:1:1 ratio and the pots were arranged in a completely randomized block design. Totally 250 pots were used, and one set containing 50 pots was kept as control, and another set of 50 pots was used for drought stress inducement and the remaining three sets of 150 pots were used for drought stress with triazoles treatment. The treatments were given as soil drenching, 30 days after sowing (DAS). The plants were left for 30 DAS with alternative day irrigation. From 30th to 60th day, control plants were irrigated on every alternative day, drought treated and drought with triazole treated plants were irrigated at every four days interval. After drought treatment, all the pots were irrigated on the alternative day, and it last up to harvest. Plants were uprooted randomly on 40th, 50,th and 60th DAS, washed with water and separated into root, stem, and leaf for estimating biochemical and antioxidant metabolism.

Biochemical Analysis

Estimation of total free amino acid (AA) content

Extraction and estimation of AA content were followed by the method suggested by Moore and Stein (1948). 0.5 g of plant material was taken in a pestle and mortar and homogenized with 10 ml of 80% boiling ethanol.

The extract was centrifuged at 800 g for 15 min, and the supernatant was made up to 10 ml with 80% ethanol and used for the estimation of free AAs. 1 ml of ethanol extract was taken in a 25 ml test tube and neutralized with 0.1 N sodium hydroxide using methyl red indicator, to which 1 ml ninhydrin reagent was added. The contents were boiled in a boiling water bath for 20 min and then 5 ml of diluted reagent was added, cooled, and diluted to 25 ml with distilled water. The absorbance was read at 570 nm in a spectrophotometer. The standard graph was prepared by using glycine. The AA content was calculated using the standard graph. The results were expressed in milligrams per gram of dry weight.

Determination of PRO content

The PRO content was estimated by the method of Bates *et al*. (1973). The plant material was homogenized in 3% aqueous sulfosalicylic acid and the homogenate was centrifuged at 10,000 rpm. The supernatant was used for the estimation of the PRO content. The reaction mixture consisted of 2 ml of acid ninhydrin and 2 ml of glacial acetic acid, which was boiled at 100°C for one h. After termination of the reaction in an ice bath, the reaction mixture was extracted with 4 ml of toluene and the absorbance was read at 520 nm.

Antioxidant Enzyme Activities

Superoxide dismutase activity (SOD, EC: 1.15.1.1)

The crude enzyme extract was prepared for assay of SOD by the method suggested by Hwang *et al*. (1999). The enzyme protein was determined according to Bradford (1976) for all the three enzymes for expressing the specific activity of enzymes. SOD (EC 1.15.1.1) activity was assayed according to Beauchamp and Fridovich (1971). The reaction mixture contained 1.17×10^{-6} M of riboflavin, 0.1 M of methionine, 2×10^{-5} M of potassium cyanide and 5.6×10^{-5} M of nitroblue tetrazolium salt dissolved in 3 ml of 0.05 M sodium phosphate buffer (pH 7.8). 3 ml of the reaction medium were added to 1 ml of 5-enzyme extract. The mixtures were illuminated in glass test tubes by two sets of Phillips 40-W fluorescent tubes in a single row. Illumination was started to initiate the reaction at 30°C for one h. Identical solutions that were kept under dark served as blanks. The absorbance was read at 560 nm in the spectrophotometer against the blank. SOD activity was expressed in units (U/mg protein).

Catalase activity (CAT, EC: 1.11.1.6)

CAT was measured according to Chandlee and Scandalios (1984), with modification. The assay mixture contained 2.6 ml of 50 mM potassium phosphate buffer (pH 7.0), 0.4 ml of 15 mM H_2O_2 and 0.04 ml of enzyme extract. The decomposition of H_2O_2 was followed by the decline in absorbance at 240 nm. The enzyme activity was expressed in U/mg protein.

Ascorbate peroxidase activity (APX, EC: 1.11.1.11)

APX (EC 1.11.1.1) activity was determined according to Asada and Takahashi (1987). The reaction mixture (1 ml) contained 50 mM of potassium phosphate buffer (pH 7.0), 0.5 mM of ascorbic acid, 0.1 mM of H_2O_2, and 200 µl of enzyme extract. The absorbance was read as the decrease at 290 nm against the blank, correction was done for the low, non-enzymatic oxidation of ascorbic acid by H_2O_2 (extinction coefficient: 2.9/mM/cm). The enzyme activity was expressed in U/mg protein.

Statistical Analysis

Statistical analysis was performed using the one-way analysis of variance followed by the Duncan's multiple range test. The values are mean ± standard error for seven samples in each group. $P \leq 0.05$ were considered as significant.

RESULTS AND DISCUSSION

Biochemical Analysis

Effect of drought and drought with triazole combination on AA content

Drought stress increased the AA content when compared to control in *Z. mays* [Figure 1]. The AA content increased under drought condition in *Arachis hypogaea* (Asha and Rao, 2002). Accumulated AA may be occurring in response to the change in the osmotic adjustment of their cellular contents (Shao *et al*., 2007). AAs and other soluble nitrogenous compounds play an essential role in plant metabolism being the primary product of inorganic nitrogen assimilation and precursors of protein and nucleic acids. Because of the importance of soluble nitrogenous compounds, there has been much interest in the influence of environmental stress on their metabolism. A common response of plants to environmental stress is an accumulation of AAs (Aspinall and Paleg, 1981). The AAs accumulation plays a very important role in drought tolerance, probably through the osmotic adjustment in different plant species such as *Radix astragali* (Tan *et al*., 2006). The complexity of tolerance to water deficit and supports the statements of many authors that the flexibility of cell metabolism and its fast acclimation to changes in environmental conditions is a first essential step in stress avoidance (Zlatev and Lidon, 2012).

Increased AA content to a higher level immediately after treatment, later it declined as the day progresses. The AA content has been shown to increase under drought condition in coconut (Kasturi Bai and Rajagopal, 2000)

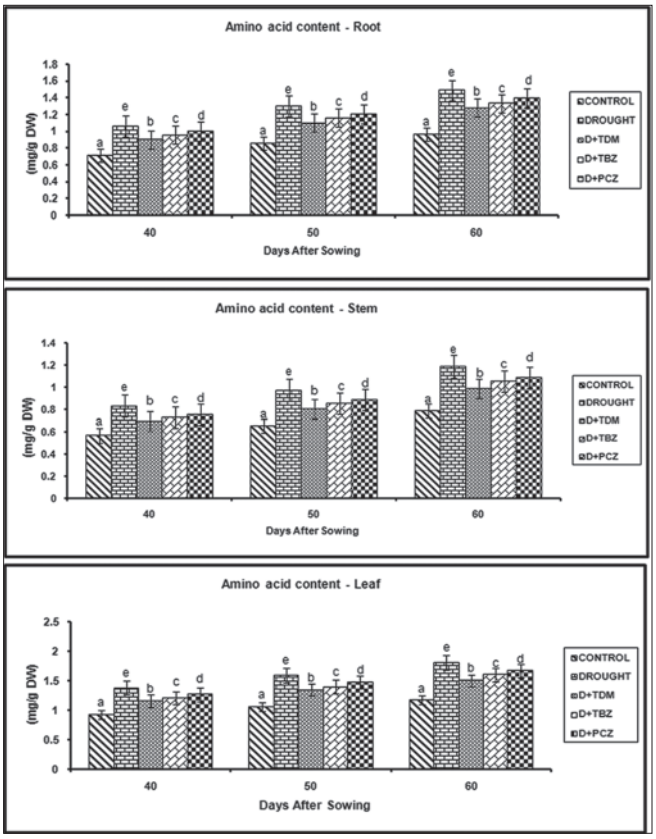

Figure 1: Effect of drought and drought with triazole combination on the amino acid content of *Zea mays.* Values are given as mean ± standard error of seven samples in each group. Bar values are not sharing a common letters (a, b, c, d, e) differ significantly at $P \leq 0.05$ (Duncan's multiple range test)

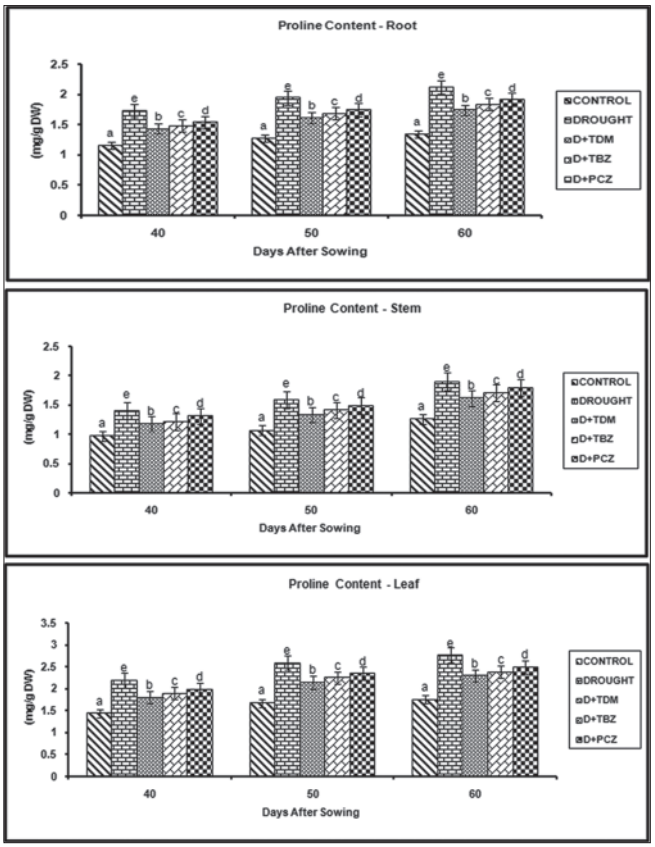

Figure 2: Effect of drought and drought with triazole combination on the proline content of *Zea mays.* Values are given as mean ± standard error of seven samples in each group. Bar values are not sharing a common letters (a, b, c, d, e) differ significantly at $P \leq 0.05$ (Duncan's multiple range test)

and wheat (Hamada, 2000). Triazole treatment to the drought stressed maize plants lowered the AA content when compared to drought stress, but it was higher than that of control. Similar results were observed in olive varieties under water stress (Thakur *et al.,* 1998), wheat seedlings (Berova *et al.*, 2002), *Abelmoschus esculentus* (Amalan Rabert *et al.*, 2013).

Effect of drought and drought with triazole combination on PRO content

Drought stress caused a higher accumulation of PRO content in all parts of the maize plants [Figure 2]. The results coincide with the reports of previous works. Increased PRO accumulation was reported in water stressed *Gossypium hirsutum* (Ronde *et al.*, 1999), bell pepper (Nath *et al.*, 2005) and wheat (Vendruscolo *et al.*, 2007). PRO accumulated under stressed conditions supplies energy for growth and survival and thereby helps the plant to tolerate stress (Jaleel *et al.*, 2007b). PRO content increased in a large variety of plants under stress, up to 100 times the normal level, which makes upto 80% of the total AA pool. PRO was known to accumulate in plants under water stress (Hsiao, 1973). PRO accumulation was maximum at flowering stage and minimum at vegetative stage.

Accumulated PRO may supply energy to increase salinity tolerance (Misra and Gupta, 2006), PRO as an osmoprotectant compound, plays a major role in osmoregulation and osmotolerance (Demir, 2000). The development of root system increases the water uptake and maintains requisite osmotic pressure through higher PRO levels in *Phoenix dactylifera* (Djibril *et al.*, 2005).

Antioxidant Enzyme Activities

Effect of drought and drought with triazole combination on SOD activity

The drought stress increased the SOD activity in all the maize plant when compared to control [Figure 3]. Triazole treatment decreased SOD activity when compared to drought stress and increased it in the control. SOD activity increased under drought stressed higher plants (Ramachandra Reddy *et al.*, 2004). An increase in SOD

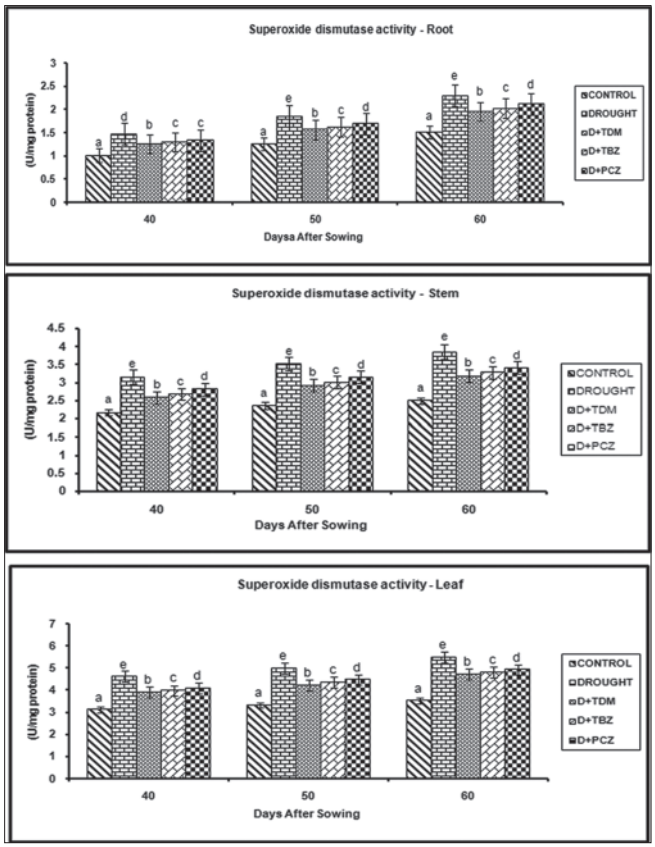

Figure 3: Effect of drought and drought with triazole combination on the superoxide dismutase activity of *Zea mays.* Values are given as mean ± standard error of seven in each group. Bar values are not sharing a common letters (a, b, c, d, e) differ significantly at $P \leq 0.05$ (Duncan's multiple range test)

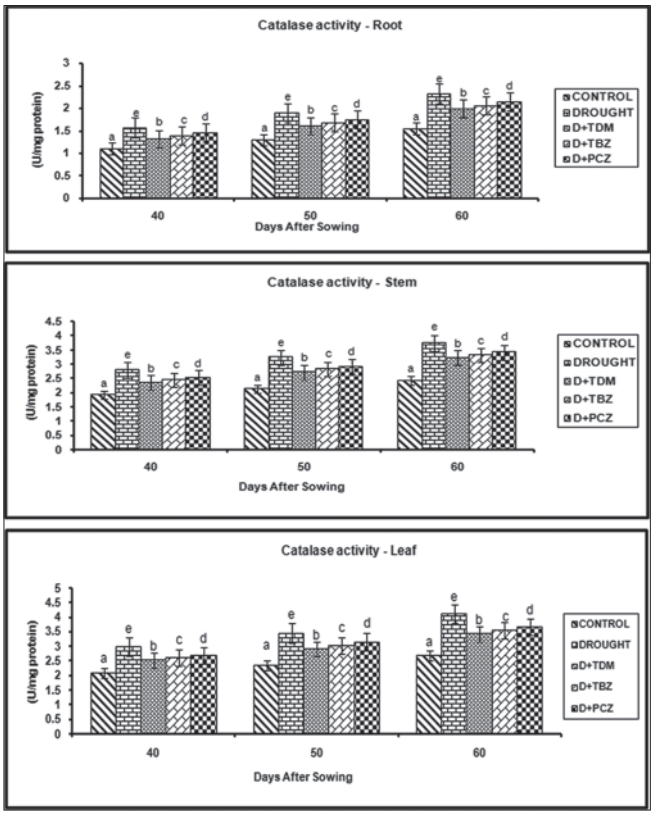

Figure 4: Effect of drought and drought with triazole combination on the catalase activity of *Zea mays*. Values are given as mean ± standard error of seven in each group. Bar values are not sharing a common letters (a, b, c, d, e) differ significantly at $P \leq 0.05$ (Duncan's multiple range test)

activity was reported in *Vigna* plants under water deficit stress and PCZ application (Manivannan *et al*., 2007) and rice (Wang *et al*., 2005). Spraying PBZ at the heading stage could increase the number of spikelets per panicle, seed setting rate and grain yields in Peizataifeng and Huayou86 in both seasons. PBZ treatment significantly improved head rice rate and amylose content in Peizataifeng and Huayou86 in the early season. Furthermore, it was observed that spraying PBZ or 6-BA could increase SOD (Pan *et al*., 2013). Similar results reported under hexaconazole in *A. esculentus* (Amalan Rabert *et al*., 2013) TDM in *Catharanthus* plants (Jaleel *et al*., 2006).

Effect of drought and drought with triazole combination on CAT activity

CAT activity was increased in all the parts of drought and drought with triazole treatment in maize plants when compared to control [Figure 4]. The CAT activity is increased under drought in *Pinus halepensis* (Alonso *et al*., 2001). The combined action of CAT and SOD converts the toxic $O_2^{\cdot-}$, H_2O_2 into the water and molecular oxygen, averting the cellular damage under unfavorable conditions such as water stress (Manivannan *et al*., 2007). Similar results were observed in wheat (Gong *et al*., 2005). CAT activity increased in drought stress and with TDM treatments compared with control. Enzyme activity in stressed plants treated with TDM showed no significant increase compared with control (Mohamadi and Rajaei, 2013). Under salt stress, the CAT activity increased in spinach (Ozturk and Demir, 2003). Increased CAT activity was reported in *Phaseolus acutifolius* under drought stress (Turkan *et al*., 2005) and in soybean (Heerden and Kruger, 2002).

Effect of drought and drought with triazole combination on APX activity

Drought stress has increased the APX activity in all the parts of plants to a larger extent under all the treatments in *Z. mays* L. [Figure 5]. Increased APX activity was reported in *P. acutifolius* under drought stress (Turkan *et al*., 2005). APX found in organelles is believed to scavenge H_2O_2 produced from the organelles, whereas the function of cytosolic APX is probably to eliminate H_2O_2 that is produced in the cytosol or apoplast and that

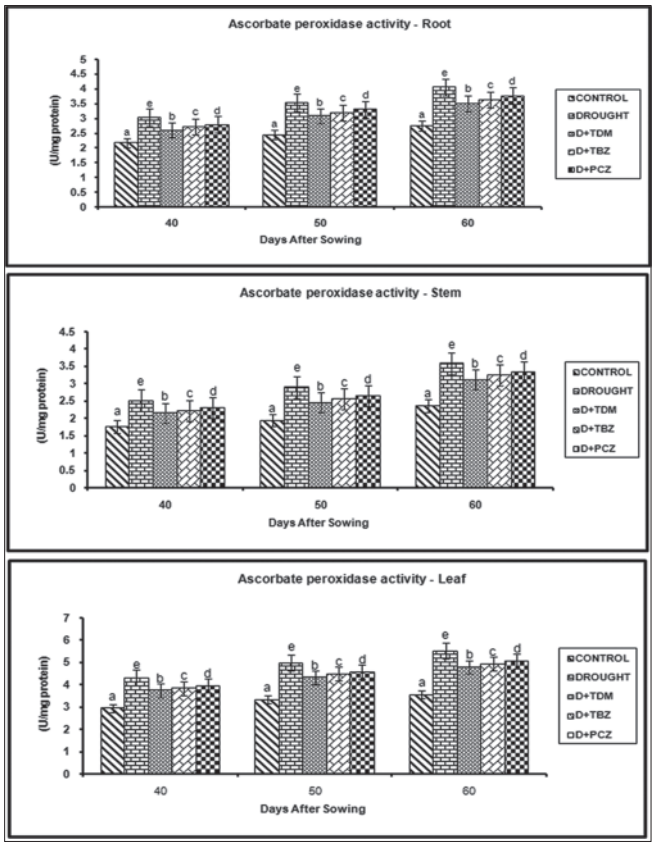

Figure 5: Effect of drought and drought with triazole combination on the ascorbate peroxidase activity of *Zea mays*. Values are given as mean ± standard error of seven in each group. Bar values are not sharing a common letters (a, b, c, d, e) differ significantly at $P \leq 0.05$ (Duncan's multiple range test)

has diffused from organelles. In the chloroplast, H_2O_2 can be detoxified by the ASA–GSH–NAPDH system, which has been catalyzed by APX (Jaleel *et al.*, 2006). Drought stress induced generation of active oxygen species is well recognized at the cellular level and is tightly controlled at both the production and consumption levels through increased antioxidant systems (Ramachandra Reddy *et al.*, 2004). Triazole treatment increased APX activity when compared to the case of control and drought-stressed plants. Similar results were obtained by many workers in many higher plants under drought stress (Manivannan *et al.*, 2007b). Paclobutrazol increased the APX activity in peanut plants under drought stress (Sankar *et al.*, 2007). A similar increase was also reported in under ketoconazole treatments in *C. roseus* (Jaleel *et al.*, 2007a), hexaconazole and TBZ in *A. esculentus* (Amalan Rabert *et al.*, 2013).

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