VII. CONCLUSION

Based on the present investigation, the following things can be concluded.

1. Drought stress decreased the growth, pigment contents and yield components of the crop studied.

2. Non-enzymatic antioxidants like ascorbic acid, α-tocopherol and reduced glutathione content were increased in the study crop under drought stress.

3. Enzymatic antioxidants like ascorbate peroxidase, superoxide dismutase, catalase and peroxidase activities were increased under drought condition.

4. The treatment with triazoles like hexaconazole, tebuconazole and propiconazole to the drought stressed plants increased the growth, yield and pigment contents.

5. The treatment with triazoles to the drought stressed plants decreased the amino acid, proline, glycine betaine and total sugar contents in sunflower when compared to drought stressed plants.

6. The activities of antioxidant enzymes like ascorbate peroxidase, superoxide dismutase, catalase and peroxidase deviated when compared to drought stressed plants.
7. The triazoles treatment significantly inhibited the leaf area and shoot length whereas, they increased root growth, fresh and dry weight, pigment content and yield of study crop.

8. Triazoles with drought treatment significantly decreased the enzymatic antioxidants and non-enzymatic antioxidants when compare to drought stressed plants.

9. Among the triazoles tried, the hexaconazole had significant level of ameliorate effect on drought stress followed by tebuconazole and propiconazole in sunflower.

10. The RAPD analyses of the present study on control, drought and drought with triazoles treated plants showed high level of DNA monomorphism than DNA polymorphism.

    Among the treatments, the present findings revealed that the growth regulator treatments to the drought stressed plants have great impact on the morphology and physiological status of sunflower. Triazoles can be used as potential ameliorative chemicals to increase the drought tolerance, yield productivity and antioxidant defense mechanisms of sunflower plants.
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EFFECT OF TRIAZOLE FUNGICIDE ON BIOCHEMICAL AND ANTIOXIDANT ENZYMES ACTIVITY IN OKRA (ABELMOSCHUS ESCULENTUS L.) PLANT UNDER DROUGHT STRESS

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Abstract

Abelmoschus esculentus L. was selected for present study under pot culture in Completely Randomized Block Design (CRBD) experiment, hexaconazole and tebuconazole are a triazole derivative, which have both fungicidal and plant growth regulator (PGR) properties and also protect plants from several types of abiotic stresses. The plants were subjected to 4 days interval drought stress and drought stress with hexaconazole (15mg l-1) and tebuconazole (10mg l-1). One-day-interval irrigation was kept as control. The plant samples were collected and separated into root, stem and leaf for estimating the amino acid (AA), proline (PRO) and glycine betaine (GB) contents and the activities of antioxidant enzymes. Drought stress and triazole treatments increased AA, PRO and GB contents, superoxide dismutase (SOD), ascorbate peroxidase (APX) and catalase (CAT) activities when compared to control. Triazole treatment modified biochemical and antioxidant enzyme content. From the result of this investigation it can be concluded that the application of triazole caused a partial recovery of the damaging effect of drought stress by its influence on antioxidant system.

Key words: Abelmoschus esculentus L., Drought, Hexaconazole, Tebuconazole, Biochemical, Antioxidant etc

1. INTRODUCTION

Abelmoschus esculentus L. was selected for present study for its economic importance, mainly for the tropical zone of developing and non-developed countries. It is cultivated especially in India Africa and Brazil, where it is also commonly known as Okra, Quiabo and lady’s finger, respectively. The tender pods are used as vegetable, ripe seeds, which are rich in protein (18-26%) are roasted and can be used as substitute for coffee. Immature pods are emollient, demulcent and diuretic and are employed in the form of decoction in catarrhal affections, dysuria and gonorrhrea. Seeds are stimulant, cordial and antispasmodic. Fatty fraction of fresh watery extract of seeds impaired cancerous cell growth in vitro (CSIR, 1985).

Water stress is a major problem in agriculture and the ability to withstand such stress is of immense economic importance. Plants are subjected to several environmental stresses that adversely affect growth, metabolism and yield (Lawlor, 2002). Drought, one of the environmental stresses, is the most significant factor restricting plant growth and crop productivity in the majority of agricultural fields of the world (Tas and Tas, 2007). Water deficit affects crop growth, depending on the stage of growth and the degree or intensity of water stress (Clavel et al., 2005).

Triazoles are a group of growth inhibitor chemical compounds that widely used as fungicides. The compounds contain three nitrogen atoms and a pentagonal ring. Triazole makes changes in plant by preventing the enzyme activity of CytP450 (Zhu, 2004). Application of PBZ partially alleviated the detrimental effects of rice senescence by modulating the activity of enzymatic antioxidants, and improving antioxidant system, which helped in sustaining plant growth. Therefore, spraying PBZ with 50 mg L-1 or 6-BA with 30 mg L-1 at the heading stage could increase grain yields and improve grain qualities in the two super hybrid rice. (Shenggang Pan et al., 2013). The application of TDM caused a partial recovery of the damaging effect of drought stress by its influence on antioxidant system. (Neda Mohamadi et al., 2013).

2. MATERIALS AND METHODS

Hybrid seeds Abelmoschus esculentus L. from Syngenta F1 Hybrid Okra variety OH-102 were used for this investigation. Plastic pots of 40 cm diameter and 45 cm
height size were used for the pot culture study. The pots were filled with 10 kg of soil mixture containing red soil; sand and farm yard manure at 1:1:1 ratio. 120 pots were arranged in Completely Randomized Design (CRBD). One set of 30 pots were kept as a control, two sets of 60 pots were used for four days interval of drought with triazole treatment and other one set was kept as four days interval drought treatment in order to impose drought stress. Hexaconazole (15mgl-1) and tebuconazole (10mgl-1) were used to determine the effect of these triazole compounds on *Abelmoschus esculentus* L.. The treatments were given as soil drenching, 30 days after planting (DAP). The plants were allowed to grow up to 30 DAS on 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 4 days interval. After the drought treatment all the pots were irrigated on alternate day were irrigated up to harvest. Plants were uprooted randomly on 40th, 50th and 60th DAS, washed with water and separated into root, stem and leaves for estimating biochemical, antioxidant enzyme activities.

2.1 BIOCHEMICAL ANALYSIS

2.1.1 ESTIMATION OF TOTAL FREE AMINO ACID CONTENT

Total free amino acids were extracted and estimated by following the method of Moore and Stein (1948).

Extraction

Five hundred milligrams of fresh plant material was homogenized in a mortar and pestle with 10 ml of 80% boiled ethanol. The extract was centrifuged at 800 g for 15 minutes and the supernatant was made upto 10 ml with 80% ethanol and used for the estimation.

Estimation

In 25 ml test tube, one millilitre of ethanol extract was taken and neutralized with 0.1 N NaOH using methyl red indicators. To which, 1 ml of ninhydrin reagent was added. The contents were boiled in a boiling water bath for 20 minutes, and then 5ml of diluting solution was added, cooled and made up to 25 ml with distilled water. The absorbance was read at 570 nm in a Spectrophotometer (U-2001–Hitachi) against an appropriate blank. The standard graph was prepared by using leucine as standard and the amino acid content was calculated using the standard graph and the results are expressed in milligram per gram dry weight.

*Ninhydrin Reagent preparation*

Solution I: 80 mg of stannous chloride in 50 ml citrate buffer at pH 5.0.

Solution II: 2 grams of ninhydrin in 50 ml methyl cellosolve, both solutions were mixed freshly.

* Diluting reagent*

Distilled water and n-propanol mixed in equal volume (1:1 v/v).

2.1.2 DETERMINATION OF PROLINE CONTENT

Proline was extracted and estimated following the method of Bates et al. (1973).

Extraction

Five hundred milligrams of fresh plant material was homogenized in a mortar and pestle with 10 ml of 3% aqueous sulfosalicylic acid. Then the homogenate was filtered through Whatman No.1 filter paper. The residue was re-extracted and pooled and the filtrates were made upto 20 ml with aqueous sulfosalicylic acid and this extract was used for the estimation of proline.

Estimation

To 2 ml of proline extract, 2 ml of acid ninhydrin and 2 ml of glacial acetic acid were added. The mixture was incubated for an hour at 100 °C in a boiling water bath. Then the test tubes were transferred to an ice bath to terminate the reaction. Then 4 ml of toluene was added and mixed vigorously using a test tube stirrer for 20 seconds and the toluene containing the chromophore was separated from the aqueous phase with the help of a separating funnel and the absorbance was measured at 520 nm in a Spectrophotometer using a reagent blank. The proline content was determined from a standard curve with proline and the results are expressed in milligrams per gram dry weight.

Reagents

*Acid-ninhydrin reagent*

To 1.25 gms of ninhydrin, 30 ml warm glacial acetic acid, 20 ml of 6 M phosphoric acid were added with agitation.

2.1.3 ESTIMATION OF GLYCINE BETAINE CONTENT

The samples were extracted and estimated following the method of Grieve and Grattan (1983).

Extraction

Five hundred milligrams of finely ground dry plant sample were mechanically shaken with 20 ml of distilled water for 24 hours at 25 °C. Time required for this step was determined by extracting the plant samples for 4, 8, 16, 24 and 48 hours. The samples were then filtered through Whatmann No.1 filter paper and the filtrates were made upto 20 ml with deionized water and used for estimation immediately.

Estimation

One millilitre of the extract was diluted with one millilitre of 2 N H2SO4 and 0.5 ml of this acidified extract was cooled in ice water for 1 hour. Later 0.2 ml of cold potassium tri iodide solution was added and mixed gently with a Vortex mixture and the tubes were stored at 4°C for 15 minutes and then centrifuged at 10,000 g for 15 minutes. The supernatant was aspirated with a fine tipped glass tube. The per iodide crystals were dissolved in 9.0 ml of 1, 2-dichloroethane with vigorous vortexing. After 2.5 hours the absorbance was measured at 365 nm in a Spectrophotometer. Reference standard of glycine betaine was prepared in 1 N H2SO4 and used for estimating the glycine betaine content and the results are expressed in micrograms per gram dry weight.

Preparation of Reagent

*Potassium tri iodide reagent*

15.7 grams of iodine and 20 grams of potassium iodide were dissolved in 100ml of distilled water and gently stirred in a vortex mixture.

2.2 ANTIOXIDANT ENZYMES

2.2.1 ASCORBATE PEROXIDASE (APX) (EC: 1.11.1.11)
Ascorbate peroxidase was extracted and estimated by the method of Asada and Takahashi (1987).

**Extraction**

Five hundred milligrams of fresh plant tissue was ground in a pestle and mortar under liquid nitrogen and 10 ml of 50 mM potassium phosphate buffer (pH 7.0) containing 1 mM EDTA, 1 per cent PVP and 1 mM ascorbic acid. The homogenate was filtered through a double layered cheese cloth and centrifuged at 15,000 rpm for 20 minutes at 4 °C. The supernatant was used as source of enzymes.

**Estimation**

One ml of reaction mixture contained 50 mM potassium phosphate buffer (pH 7.0), 0.5 mM ascorbic acid, 0.1 mM H₂O₂ and 200 µl of enzyme extract. The absorbance was read as decrease at 290 nm against the blank, correction was done for the low, non-enzymatic oxidation of ascorbic acid by H₂O₂ (extinction coefficient 2.9 mM⁻¹ cm⁻¹). The enzyme activity was expressed in µg per gram dry weight.

**2.2.2 SUPEROXIDE DISMUTASE (SOD) (EC: 1.15.1.1)**

Crude enzyme extract was prepared, for the assay of superoxide dismutase by the method (Hwang et al., 1999).

**Extraction**

One gram of fresh tissue was homogenized with 10 ml of ice-cold 50 mM sodium phosphate buffer containing 1 mM PMSF. The extract was filtered through double-layered cheesecloth. The extract was centrifuged at 12, 5000 rpm for 20 minutes at 4°C. The supernatant was saved and made up to 10 ml with extraction buffer and used for estimation of the SOD enzyme activity.

**Estimation**

Superoxide dismutase activity was assayed by the method of Beauchamp and Fridovich (1971). The reaction medium was prepared and to 3 ml reaction medium, 1 ml of enzyme extract was added. The reaction mixture contained 1.17 x 10⁻⁶ M riboflavin, 0.1M methionine, 2x10⁻⁵ potassium cyanide and 5.6 x 10⁻⁵ M nitroblue tetrasodium salt (NBT), dissolved in 0.05 M sodium phosphate buffer (pH 7.8). The mixture was illuminated in glass test tubes by two sets of Philips 40 W fluorescent tubes. Illumination started to mixture was illuminated in glass test tubes by two sets of illuminated saving as blank and kept in dark. The absorbance was read at 560 nm in the spectrophotometer against the blank. Superoxide dismutase activity was expressed in units. One unit is defined as the amount of change in the absorbance by 0.1 per hour per milligram protein under the assay condition (Cherry, 1963).

**2.2.3 CATALASE (CAT, EC: 1.11.1.6)**

Catalase activity was assayed as described by Chandlee and Scandalios (1984).

**Extraction**

Five hundred milligrams of frozen material was homogenized in 5 ml of ice cold 50 mM sodium phosphate buffer (pH 7.5) containing 1 mM PMSF. The extract was centrifuged at 4°C for 20 minutes at 12,500 rpm. The supernatant was used for enzyme assay. The enzyme protein was determined by Bradford (1976) method.

**Assay**

The activity of enzyme catalase was measured using the method of Chandlee and Scandalios (1984) with modification. The assay mixture contained 2.6 ml of 50 ml of 50 mM potassium phosphate buffer (pH 7.0) 0.4 ml, 15 mM H₂O₂ and 0.04 ml of enzyme extract. The decomposition of H₂O₂ was followed by the decline in absorbance at 240 nm. The enzyme activity was expressed in units 1 mM of H₂O₂ reduction per minute per mg protein.

**3. STATISTICAL ANALYSIS**

The pot culture was carried out in completely randomized design (CRBD). The data are expressed as mean ± SD for seven samples in each group.

**4. RESULTS AND DISCUSSION**

**4.1 BIOCHEMICAL PARAMETERS**

**4.1.1 AMINO ACID**

Drought stress increased the amino acid content when compared to control in *Abelmoschus esculentus* L. The amino acid content increased under drought condition in sunflower (Manivannan et al., 2007a), in *Arachis hypogaea* (Asha and Rao, 2002); *Radix astragali* (Tan et al., 2006).

Accumulated amino acid may be occurring in response to the change in osmotic adjustment of their cellular contents (Shao et al., 2007). Amino acids accumulation plays a very important role in drought tolerance, probably through osmotic adjustment in different plant species, such as, *Radix astragali* (Tan et al., 2006). Amino acids 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 amino acids (Aspinall and Paling, 1981). Triazole compound Paclobutrazol treatment to the drought stressed peanut plants lowered the amino acid content when compared to drought stress but it was higher than that of control. Similar results were observed in paclobutrazol and triacontanol in olive varieties under water stress (Thakur et al., 1998) and paclobutrazol treated wheat seedlings under low temperature stress (Berova et al., 2002). Amino acids accumulation plays a very important role in drought tolerance, probably through osmotic adjustment in different plant species, such as, *Radix astragali* (Tan et al., 2006).

**4.1.2 PROLINE**

In *Abelmoschus esculentus* L. drought stress caused increased accumulation of proline content at all stages of growth. Water stress resulted in an increase in proline accumulation in sorghum (Yadav et al., 2005). The similar results were observed in wheat (Nayyar, 2006), soybean (Heerden and Kruger, 2002). Proline content increased in a large variety of plants under stress, up to 100 times the normal level, which makes up to 80 per cent of the total amino acid pool. Proline was known to accumulate in plants under water stress (Hsiao, 1973). Proline accumulation was maximum at flowering stage and minimum at vegetative stage. Proline content is effective in increasing osmotic stability of the plant. The accumulation of proline increased when water stress was followed by simultaneous increase in leaf water potential in chickpea (Gupta et al., 2000). Proline accumulation in plants might...
be a scavenger and acting as an osmolyte. The reduced proline oxidase may be the reason for increasing proline accumulation (Jaleel et al., 2008). Prolin content increased in both drought stress and with TDM treatments when compared to control. TDM treatments decreased prolin content in plants under drought stress compared with the plants had received only water stress treatment. (Neda Mohamadi et al., 2013).

4.1.3 GLYCINE BETAINE
Drought stressed Abelmoschus esculentus L. plants showed an increase in glycine betaine content when compared to control. The glycine betaine content increased under drought stress in Radix astragali (Tan et al., 2006), in barley (Nakamura, 2001) and in higher plants (Jun et al., 2000). Glycine betaine is considered to be one of the most abundant quaternary ammonium compounds produced in higher plants under stressful environment (Yang et al., 2003). Glycine betaine has been shown to protect enzymes and membranes and also to stabilize PSII protein pigment complexes under stressful conditions (Papageorgiou and Morata, 1995). Glycine betaine, an important quaternary ammonium compound, is considered to be one of the most predominant and effective osmoprotectants. It is well established that its exogenous application might have some advantages as it improves drought tolerance in plants (Iqbal et al., 2008). It has been also reported earlier that rate and timing of GB application significantly affects drought tolerance ability of sunflower (Iqbal et al., 2008 and 2009). Triazole compound Paclobutrazol treatment to the drought stressed plants decreased glycine betaine content but it was higher than that of control. Similar results were observed in Arachis hypogaea (Girija et al., 2002). The accumulation of glycine betaine might serve as an intercellular osmoticum and it can be closely correlated with the elevation of osmotic pressure (Kavikishore et al., 1995).

4.2 ANTIOXIDANT ENZYMES
4.2.1 ASCORBATE PEROXIDASE (APX)
APX activity increased in Abelmoschus esculentus L. under drought condition and in all the treatments. Increased APX activity was reported in Phaseolus acutifolius under drought stress (Turkan et al., 2005). APX found in organelles is believed to scavenge H2O2 produced from the organelles, whereas the function of cytosolic APX is probably to eliminate H2O2 that is produced in the cytosol or apoplast and that has diffused from organelles. In the chloroplast, H2O2 can be detoxified by the ASA–GSH–NADPH system, which has been catalyzed by APX (Jaleel et al., 2006). Drought stress with triazole treatment decreased APX activity in drought stressed plants, and increased it in control plants. 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). 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 (Reddy et al., 2004), also Kentucky bluegrass (Liu et al., 2008). Ascorbate peroxidase is one of the most important antioxidant enzymes of plants that detoxify hydrogen peroxide using ascorbate for reduction. APX reduces H2O2 to water by ascorbate as specific electron donor (Gara et al., 2003). In trifoliolate orange, under water stress, an increased APX activity was not significant. Variation in APX activity at mild water deficit in maize and wheat were observed (Nayyar and Gupta, 2006).

4.2.2 SUPEROXIDE DISMUTASE (SOD)
The activity of SOD increased under water stress in Abelmoschus esculentus L. Super oxide dismutase activity increased under drought stressed higher plants (Reddy et al., 2004). SOD activity increased under drought stress in Oryza sativa (Chandrashekara Reddy et al., 1998), maize (Jiang and Zhang, 2002), Euphorbia esula (Davis and Swanson, 2001), Cassia angustifolia (Agarwal and Pandey, 2003), wheat (Singh and Usha, 2003; Shao et al., 2005), rice (Wang et al., 2005), P. acutifolins (Turkan et al., 2005) and the SOD activity was higher under salinity stress in C. roseus (Misra and Gupta, 2006), while subjecting to water deficit stress, the SOD activity was increased in higher plants (Reddy et al., 2004). Triazole treatment decreased SOD activity when compared to drought-stress and increased it in the control. Triazoles increased the antioxidant potential in oxidative stressed plants under treatment when compared to control (Sankar et al., 2007). It was reported that SOD enhances water-stress tolerance in plants. The cytosolic Cu/Zn–SOD was induced strongly by stress, while Cu/Zn–SOD remained largely unaffected (Jaleel et al., 2008). TDM treatment increased the SOD activity in drought stressed as well as in control plants. (Neda Mohamadi et al., 2013). Furthermore, it was observed that spraying PBZ or 6-BA could increase super oxide dismutase (SOD). (Shenggang Pan et al., 2013). SOD activity increased in drought treatment when compared to control.

4.2.3 CATALASE (CAT)
Drought stress has increased the catalase activity in all the parts of the plants to a larger extent under all the treatments in Abelmoschus esculentus L. Similar results were observed in wheat (Lin and Wang, 2002; Gong et al., 2005). Catalase activity increased under drought stress in Oryza sativa (Chandrashekara Reddy et al., 1998), maize (Pastori et al., 2000), Zea mays (Jiang and Zhang, 2002), Allium schoenoprasum (Egert and Tovini, 2002), wheat (Dalmia and Sawhney, 2004; Shao et al., 2005) and P. acutifolius (Turkan et al., 2005). An increase in catalase activity was reported in higher plants under drought stress (Reddy et al., 2004). Similar results were found in Lotus corniculatus (Borsani et al., 2001) and rice (Wang et al., 2005). Catalase 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. (Neda Mohamadi et al., 2013). Triazole treatment decreased catalase activity when compared to drought stress and increased it in controls. This result is in accordance with the findings in Catharanthus roseus (Jaleel et al., 2006). The combined action of CAT and SOD converts the toxic O−2, H2O2 into water and molecular oxygen, averting the cellular damage under unfavourable conditions like water.
stress (Manivannan et al., 2007b).

**BIOCHEMICAL PARAMETERS**

Fig. 1-3. Effects of drought and drought with Triazole combination on the amino acid (AA) content of *Abelmoschus esculentus* L. Values are mean ± SE of seven replicates.

Fig. 4-6. Effects of drought and drought with Triazole combination on the Proline (PRO) content of *Abelmoschus esculentus* L. Values are mean ± SE of seven replicates.

Fig. 7-9. Effects of drought and drought with Triazole combination on the Glycine betaine content of *Abelmoschus esculentus* L. Values are mean ± SE of seven replicates.

**ANTIOXIDANT ENZYMES**

Fig. 10. Effects of drought and drought with Triazole combination on the Ascorbate peroxidase content of *Abelmoschus esculentus* L. Values are mean ± SE of seven replicates.
5. CONCLUSION

Plants are highly regulated by triazole compounds, drought stressed plants under triazole treatment maintain a balance between formation and detoxification of activated oxygen species, leading to partial improvement of their response to drought-induced oxidative stress. It can be concluded that triazole such as hexaconazole and tebuconazole may be useful to trigger drought avoidance mechanisms. The triazole treatment mitigated the adverse effects of drought stress by modifying biochemical and antioxidants and there by paved the way for overcoming drought stress in Okra (Abelmoschus esculentus L.).

REFERENCE


TRIAZOLE INDUCED MODIFICATION OF BIOCHEMICAL AND ANTIOXIDANT METABOLISM IN Capiscum annuum L. UNDER DROUGHT STRESS

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INTRODUCTION

Currently, drought study has been one of the main directions in global plant biology and biological breeding. So anti-drought physiology study is of importance to production and biological breeding for the sake of coping with abiotic and biotic conditions (Shao et al., 2005). Water is one of the most important ecological factors determining crop growth and development; water deficit plays a very important role in inhibiting the yields of crops. Drought stress occurs when the available water in the soil is reduced and atmospheric conditions cause continuous loss of water by transpiration or evaporation (Shao et al., 2007). There are many reports in the literature that underline the intimate relationship between enhanced constitutive antioxidant enzyme acivities and increased resistance to environmental stresses (V ranova et al., 2002; Bor et al., 2003). Plant experiences drought stress either when the water supply to roots becomes difficult or when the transpiration rate becomes very high. These two conditions often coincide under arid and semiarid climates. Water stress tolerance is seen in almost all plant species but its extent varies from species to species (Lin et al., 2006). Triazol e compounds such as triadimefon (TDM), Hexaconaz ole (HEX), propiconazole (PCZ), tebuconazole (TBZ) and p aclobutrazole (PBZ) etc. are widely used as fungicides and the y also posses varying degrees of plant growth regulating properties (Fletcher et al., 2000). Triazoles have been called plant multiprotectants because of their ability to induce tolerance in plants to environmental and chemical stresses (Kraus and Fletcher, 1994). Protection of plants from appeated unrelated stress by triazole is mediated by a reduction in free radical damage and increase in antioxidant potential (Kraus et al., 1995). Triazoles affect the isoprenoid pathway and alter the levels of certain plant hormones by inhibiting gibberellin synthesis, reducing ethylene evolution and increasing cytokinin levels (Kamounis and Chronopoulou-Serel i, 1999). Triazole treated plants have a more efficient f ree-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 e t al., 2006). The drought stress amelioration by triazole compounds is of major research interest, because, these compounds have innate potentiality for increasing antioxidant enzymes and molecules in oxidative stressed plants (Fletcher et al., 2000). So in the present investigation, an attempt has been made to evaluate the drought stress ameliorating ability of triazole fungicide, with special emphasis to antioxidant molecules and antioxidant enzymes in drought stressed C. annuum L. plants.

MATERIALS AND METHODS

Plant material and drought stress applications

The seeds of Capiscum annuum L. Varity bomby F1 hybrid were obtained from syngenta pvt ltd. Seeds are surface...
sterilized with 0.2 per cent HgCl₂ solution for 5 minutes with frequent shaking and thoroughly washed many times with deionized water to remove HgCl₂. A pot culture experiment was conducted to estimate the drought stress modification and ameliorating effect of combination of drought with triazole compounds, five seeds were sown in each pot of 40 × 45 cm containing 10 kg of soil mixture composed of red soil, sand and the farmyard manure (FYM) at 1:1:1 ratio. The seedlings were thinned to 2 pot⁻¹ on 20 DAS. Plants pots were arranged in Completely Randomized Block Design (CRBD). Pots were irrigated with ground water one day interval as a control and other treatment is 3 days interval drought and drought with triazole compounds TDM @ 10 mg l⁻¹, HEX @ 10 mg l⁻¹ and PCZ @ 15 mg l⁻¹ treatments through drenching method on 30 DAS. Plants were uprooted randomly on 40, 50, 60 DAS, washed carefully and separated into root, stem and leaf for estimating antioxidant contents and antioxidant enzyme activities.

Biochemical Constituents

Amino acid (AA)

Extraction and estimation of Amino acid (AA) content was 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, 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.

Proline (PRO)

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. 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 1 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 Content Estimations

Ascorbic acid

Ascorbic acid content was assayed as described by Omaye et al. (1979). The extract was prepared by grinding 1 g of fresh material with 5 ml of 10% TCA, centrifuged at 3500 rpm for 20 minutes, reextracted twice and supernatant made up to 10 ml and used for assay. To 0.5 ml of extract, 1 ml of DTC reagent (2,4-dinitrophenyl hydrazine-thiourea-CuSO₄ reagent) was added and incubated at 37°C for 3 hrs and 0.75 ml of ice-cold 65% H₂SO₄ was added, allowed to stand at 30°C for 30 minutes, resulting color was read at 520 nm in spectrophotometer (U-2001-Hitachi). The ascorbic acid content was determined using a standard curve prepared with Ascorbic acid and the results were expressed in mg g⁻¹ dry weight (DW).

α- Tocopherol

α-Tocopherol content was assayed as described by Backer et al. (1980). Five hundred milligrams of fresh tissue was homogenized with 10 ml of a mixture of petroleum ether and ethanol (2:1.6 v/v) and the extract was centrifuged at 10,000 rpm for 20 minutes and the supernatant was used for estimation of α-Tocopherol. To one ml of extract, 0.2 ml of 2 per cent 2,2-dipyridyl in ethanol was added and mixed thoroughly and kept in dark for 5 minutes. The resulting red colour was diluted with 4 ml of distilled water and mixed well. The resulting colour in the aqueous layer was measured at 520 nm. The α-Tocopherol content was calculated using a standard graph made with known amount of α-Tocopherol and expressed in mg g⁻¹ fresh weight (FW).

Enzyme Extractions and Assays

Ascorbate peroxidase (APX)

Ascorbate peroxidase (APX) (EC 1.11.1.1) activity was determined according to Asada and Takahashi (1987). The reaction mixture (1 ml) contained 50 mM potassium phosphate buffer (pH 7.0), 0.5 mM ascorbic acid, 0.1 mM H₂O₂ and 200 μl of enzyme extract. The absorbance was read as decrease at 290 nm against the blank, correction was done for the low, non-enzymatic oxidation of ascorbic acid by H₂O₂ (extinction coefficient 2.9 mM⁻¹ cm⁻¹). The enzyme activity was expressed in U mg⁻¹ protein (U = change in 0.1 absorbance min⁻¹ mg⁻¹ protein).

Catalase (CAT)

Catalase (CAT) was measured according to Chandee 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 H₂O₂ and 0.04 ml of enzyme extract. The decomposition of H₂O₂ was followed by the decline in absorbance at 240 nm. The enzyme activity was expressed in U/mg protein.

Statistics

The pot culture was carried out in completely randomized design (CRBD). The data are expressed as mean ± SE for seven samples in each group.

RESULTS

Amino acid (AA) contents increased in all the parts of drought stressed C. annuum L. when compared to control and triazole treated plants partially ameliorated drought stress (Fig.1). Proline (PRO) contents were increased in all the parts of C. annuum L. when compared to control (Fig.2). The ascorbic acid content increased with the age in drought stressed plants (Fig.3). α- tocopherol content of the drought stressed plants significantly increased when compared to control plants (Fig.4). Ascorbate Peroxidase (APX) activity increased in Capsicum annuum L. (Fig.5) under drought condition and in all the treatments. Drought stress has increased the catalase activity in all the parts and the plants to a larger extent under all the treatments in C. annuum L. (Fig.6).
DISCUSSION

Amino acid (AA) contents increased in all the parts of drought stressed C. annuum L. when compared to control and triazole treated plants partially ameliorated drought stress (Fig.1).

Similar result recorded in sunflower (Manivannan et al., 2007) and Catharanthus roseus (Jaleel et al., 2007). The AA content increased under drought condition in Arachis hypogaea (Asha and Rao, 2002); sorghum (Yadav et al., 2005); pepper (Nath et al., 2005); Radix astragali (Tan et al., 2006); Molus domestica (Sircelj et al., 2005) and M. rsh grasses. Accumulated AA may be occurring in response to the change in osmotic adjustment of their cellular contents (Shao et al., 2007). Triazole treatments partially ameliorated drought stress in Capsicum annuum L. Similar results were observed in triadimefon treatment increased the AA content in radish (Muthukumarasamy et al., 2000) and soybean (Pa meerselvam et al., 1998). Uniconazole treated Phaseolus vulgaris (Mackay et al., 1990); penconazole induced a moderate increase in amino acid in higher plants (Radice and Pesci, 1991) and in tomato (Sharp et al., 2000; Sharp and Lenoble, 2002). Proline (PRO) contents were increased in all the parts of C. annuum L. when compared to control (Fig.2). Water stress resulted in an increase in PRO accumulation in sorghum (Yadav et al., 2005). The similar results were observed in wheat

![Fig:1a - Amino acid content (Root)](image1)

![Fig:1b - Amino acid content (Stem)](image2)

![Fig:1c - Amino acid content (Leaf)](image3)

Fig. 1. Effect of drought stress and drought with triazole combination on the Amino acid content of Capsicum annuum L. (expressed in mg/g dry weight)

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Fig. 1. Effect of drought stress and drought with triazole combination on the Amino acid content of Capsicum annuum L. (expressed in mg/g dry weight)

The ascorbic acid content increased with the age in drought stressed plants (Fig.3). Water stress resulted in significant increase in antioxidant ascorbic acid concentration in turf grass (Zhang and Schmidt, 2000). Similar results were observed in sorghum seedlings (Zhang and Kir kham, 1996); Triticum aestivum (Carlos et al., 1999); rice (Srivalli et al., 2003); apple tree (Sircelj et al., 2005) and in Poncirus trifoliata (Wu et al., 2006). Triazole treatment to the drought stressed capsicum
Fig. 2. Effects of drought stress and drought with triazole combination on the Proline content of Capsicum annuum L. (expressed in mg/g.\textsuperscript{-1} dry weight).

Fig. 3a - Ascorbic Acid Content (Root)
plants decreased the ascorbic acid content but, it was higher than that of control. Increase in ascorbic acid content was reported in the paclobutrazol treated grape fruit s (Fucik and Swietlik, 1990) and citrus limon (Jain et al., 2002). Uniconazole increased the level of antioxidants ascorbic acid and α-tocopherol like in tomato seedlings and protected the membrane by preventing or reducing oxidative damage (Singh, 1996). α- tocopherol content of the drought stressed plants significantly increased when compared to control plants (Fig.4).

Water stress resulted in significant increases in antioxidant α-tocopherol concentration in turf grass (Boggess et al., 1976; Zhang and Schmidt, 2000). Similar results were observed in maize (Pastori and Trippi, 1993); sorghum (Zhang and Kirkham, 1996); Triticum aestivum (Carlos et al., 1999) and (Srivalli et al., 2003). grasses (Fu and Huang, 2001); Wheat (Baisak et al., 1994; Loggini et al., 1999); apple tree (Sircelj et al., 2005); Poncirus trifoliata (Wu et al., 2006). Triazole treatment to the drought stressed Capsicum annuum L. plants
Fig. 4. Effects of drought stress and drought with triazole combination on the α-Tocopherol content of Capsicum annuum L. (expressed in µg g⁻¹ fresh weight)

Fig: 5a - Ascorbate peroxidase (Root)

Fig: 5b - Ascorbate peroxidase (Stem)
Fig. 5. Effects of drought stress and drought with triazole combination on the Ascorbate peroxidase activity of Capsicum annuum L. (u/mg protein).

Fig. 6. Effects of drought stress and drought with triazole combination on the Catalase activity of Capsicum annuum L. (u/mg protein)
decreased the α-tocopherol content but, it was higher than that of control. Triazole caused an enhancement in α-tocopherol content under drought as well as well-watered plants. A similar result was observed in bean (Simontacchi et al., 1993) under chilling stress. Singh (1996) reported an increase in α-tocopherol and β-carotene content in the fruit juice of paclobutrazol treated mango (Mangifera indica L). Similar results were observed in grape fruits (Fucik and Swietlik, 1990) and Citrus limon (Jain et al., 2002). Ascorbate Peroxidase (APX) activity increased in Capsicum annum L (Fig.5) under drought condition and in all the treatments. Increased APX activity was reported in Phaseolus acutifolius under drought stress (Turkan et al., 2005) and in soybean (Heerden and Kruger, 2002). Similar results were obtained by many workers under drought stress in many higher plants Pinus halepensis (Alonso et al., 2001); maize (Jiang and Zhang, 2002); Phaseolus acutifolius (Turkan et al., 2005), soybean (Heerden and Kruger, 2002); wheat (Baisak et al,1994; Lin and Wang, 2002; Gong et al., 2005) and Kentucky bluegrass (Liu et al., 2008). 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 (Reddy et al., 2004). Triazole treatment in combination with drought decreased the APX activity but, it was higher than that of control plants. Increased APX activity was reported in Phaseolus acutifolius under drought stress (Turkan et al., 2005) and in soybean (Heerden and Kruger, 2002). Triazoles increased the level of APX activity in Solenostemon rotundifolius (Kishorekumar et al., 2008). Paclobutrazol increased the APX activity in peanut plants under drought stress (Sankar et al., 2007) and C. roseus plants under salt stress (Jaleel et al., 2007a). Similar increase was also reported in Vigna plants under propiconazole treatments in combination with drought (Manivannan et al., 2007a) and ketoconazole in C. roseus (Jaleel et al., 2007).

Drought stress has increased the catalase (CAT) activity in all the parts of the plants to a larger extent under all the treatments in C. annum L. (Fig.6). The CAT activity increased under drought in Nicotiana plumbaginifolia (Nicolas smirnoff, 1998) and Pinus halepensis (Alonso et al., 2001) Similar results were observed in wheat (Sgherri et al., 2000; Lin and Wang, 2002; Gong et al., 2005; Shao et al., 2005), Phaseolus acutifolius (Turkan et al., 2005); Zea mays (Jiang and Zhang, 2002). Under salt stress the CAT activity increased in spinach (Ozturk and Demir, 2003). Triazole treatment to the stressed plants caused a decrease in CAT activity but, it was higher than that of control plants. Increased CAT activity was reported in Phaseolus acutifolius under drought stress (Turkan et al., 2005) and in soybean (Heerden and Kruger, 2002). Triazoles increased the level of CAT activity in Solenostemon rotundifolius (Kishorekumar et al., 2008). Paclobutrazol increased the CAT activity in peanut plants under drought stress (Sankar et al., 2007). Similar increase was also reported in Vigna plants under propiconazole treatments in combination with drought (Manivannan et al., 2007a) and ketoconazole in C. roseus (Jaleel et al., 2007). Thus, from these results, it is clear that plants are highly regulated by triazole compounds. Drought stressed plants under triazole treatment leading to partial improvement of their response to drought-induced stress. It can be concluded that triazole such as Triadimefon (TDM), Hexaconazole (HEX) and Propiconazole (PCZ) may be useful to trigger drought avoidance mechanisms in plants like Capsicum annum L. Further work is needed to understand the genetic mechanism behind triazole induced water stress tolerance in Capsicum annum L.

REFERENCES


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PLANT SCIENCE

Triazole compounds alters the antioxidant and osmoprotectant status in drought stressed *Helianthus annuus* L. plants

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Abstract

A pot-culture experiment was conducted to estimate the ameliorating effect of triazole compounds Hexaconazole, Tebuconazole and Propiconazole on drought stress in sunflower (*Helianthus annuus* L.). The plants were subjected to drought (DID) stress after four days of interval from the 30th day of sowing (DAS). One-day-interval irrigation was kept as control. The plant samples were collected and separated into root, stem and leaf for estimating the amino acid (AA), proline (PRO) and glycine betaine (GB) contents and the activities of antioxidant enzymes. Drought stress and triazole treatments increased AA, PRO and GB contents, superoxide dismutase (SOD), ascorbate peroxidase (APX) and catalase (CAT) activities when compared to control. From the results of this investigation, it can be concluded that the application of triazole compounds caused a partial amelioration of the adverse effects of drought stress by its influence on antioxidant potentials in *H. annuus*.

Key words: Drought, Hexaconazole, Tebuconazole, Propiconazole, Antioxidant

Introduction

Drought is a major abiotic factor and is a world-wide problem seriously influencing crop production and quality. Drought is a meteorological term, and is commonly defined as a period without significant rainfall. Drought is a major abiotic constraint limiting the chickpea yield up to a greater level. The yield level remain very low under prolong moisture deficit conditions (Muhammad Yaqoob et al., 2013). Water is one of the most important ecological factors determining crop growth and development; water deficit plays a very important role in inhibiting the yields of crops. Soil drought inhibits plant growth and development established dry matter reduction in wheat under water deficiency stress (Ahmad et al., 2007). Drought stress occurs when the available water in the soil is reduced and atmospheric conditions cause continuous loss of water by transpiration or evaporation (Shao et al., 2007). Plants are subjected to several environmental stresses that adversely affect growth, metabolism and yield. Water is one of the most important environmental factors that regulate plant growth and development. Drought is the major limiting factor in many parts of the world, which seriously affects plant growth and yield (Manivannan et al., 2007a). The toxic superoxide radical has a half-life of less than 1 s and is usually rapidly dismutated by superoxide dismutase (SOD) to H$_2$O$_2$, a product that is relatively stable and can be detoxified by catalase and peroxidases. Drought indices, derived over decades, have rainfall as a major parameter causing droughts (Mishra and Singh, 2010). Water deficit induces expression of particular genes and this is associated in most cases with adaptive responses of stressed plants. The functions of many of them are still not established. One of valuable approaches to understand drought resistance mechanisms is to identify the key metabolic steps that are most sensitive to drought (Zlatev and Lidon, 2012).

*Helianthus annuus* L. belongs to Asteraceae (=compositae) family, commonly known as sunflower. Sunflower is one of the major and most important non-conventional oilseed crop in the world due to its excellent oil quality (Baydar and Erbas, 2005). Although sunflower is known as a
drought tolerant crop or grown under dryland conditions, substantial yield increases are achieved with frequent irrigation. Sunflower is one of the important oilseed crops of India. The potential of the crop is far from being exploited and the yield levels of the country are the lower in the world due to several biotic and a biotic stresses.

Triazole compounds are systemic fungicides having plant growth regulating properties. The plant growth regulating properties of triazoles are mediated by their ability to alter the balance of important plant hormones including Gibberelllic acid (GA), Abscisic acid (ABA) and Cytokinins (Kamounstis and Chronopoulou-Sereli, 1999; Hajihashemi et al., 2007). Plant growth regulators play vital roles in coordination of many growth and behavioral processes in rice, which regulates the amount, type and direction of plant growth (Rajendra and Jones Jonathan, 2009; Anjum et al., 2011). Triazoles induce a variety of morphological and biochemical responses in plants, inhibited shoot elongation, stimulated root growth, increased cytokinin synthesis and a transient rise in ABA, as well as conferring protection from various environmental stresses (Fletcher et al., 2000; Gopi et al., 2007). The application of triadimefon (TDM) caused a partial recovery of the damaging effect of drought stress by its influence on antioxidant system (Neda and Rajaei, 2013). Triazoles protect plants against various stresses including drought, low and high temperatures, UV 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). The use of plant growth regulators, as GA3, PBZ, 6-BA or their compounds, is becoming popular to ensure efficient production. Remarkable accomplishments of plant growth regulators such as manipulating plant growth and crop yield have been actualized in recent years (Sarkar et al., 2002; Sakamoto et al., 2005; Morinaka et al., 2006; Yan et al., 2011; Zvi and Eduardo 2011). Plant growth regulators (GA3, PBZ and 6-BA) play important roles in plant growth, development, yield and quality formation (Ekamber and Kumar, 2007; Rajendra and Jones Jonathan, 2009; Zheng et al., 2011). Application of PBZ partially alleviated the detrimental effects of rice senescence by modulating the activity of enzymatic antioxidants, and improving antioxidant system, which helped in sustaining plant growth. Therefore, spraying PBZ with 50 mg L-1 or 6-BA with 30 mg L-1 at the heading stage could increase grain yields and improve grain qualities in the two super hybrid rice (Shenggang et al., 2013).

Materials and methods

Plant material and drought-stress induction

Hybrid Sunflower seeds, Sunbred – 275 PR (ARENA), obtained from Syngenta India limited were used for this investigation. Plastic pots of 40 cm diameter and 45 cm height size were used for the pot culture study. The pots were filled with 10 kg of soil mixture containing red soil; sand and farm yard manure at 1:1:1 ratio. 120 pots were arranged in Completely Randomized Block Design (CRBD). One set of 30 pots were kept as a control, two sets of 60 pots were used for four days interval of drought and drought with triazole treatment and other one set was kept as four days interval drought treatment in order to impose drought stress. 10mg/l of Hexaconazole, 15mg/l of Tebuconazole and 15mg/l of Propiconazole were used to determine the effect of these triazole compounds on Helianthus annuus L. The treatments were given as soil drenching, 30 days after planting (DAP). The plants were allowed to grow up to 30 DAS on 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 4 days interval. After the drought treatment all the pots were irrigated on alternate day were irrigated up to harvest. Plants were uprooted randomly on 40th, 50th and 60th DAS, washed with water and separated into root, stem and leaves for estimating biochemical, antioxidant enzyme activities. The plant growth is shown in Figures 1-4.

Osmolyte concentration

Amino acid (AA) content

Extraction and estimation of AA content was 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, 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.
Figure 1. (a) 7 days old sunflower seedlings (b) 30 days old sunflower plants before treatment (c) 40 days old sunflower after triazole treatment (d) 60 days old sunflower after triazole treatment.
Proline (PRO) concentration

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. 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 1 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.

Glycine betaine (GB) concentration

The amount of GB was estimated according to the method of Grieve and Grattan (1983). The plant tissue was finely ground, mechanically shaken with 20 ml of deionised water for 24 h at 25°C. The samples were then filtered and filtrates were diluted to 1:1 with 2 N H2SO4. Aliquots were kept in centrifuge tubes and cooled in ice water for 1 h. Cold KI–I2 reagent was added, and the reactants were gently stirred with vortex mixture. The tubes were stored at 4°C for 16 h and then centrifuged at 10,000 rpm for 15 min at 0°C. The supernatant was carefully aspirated with a fine glass tube. The periodide crystals were dissolved in 9 ml of 1, 2-dichloroethane. After 2 h, the absorbance was measured at 365 nm using GB as a standard and expressed in mg/g DW.

Enzyme extractions and assays

Ascorbate peroxidase (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 H2O2, 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, nonenzymatic oxidation of ascorbic acid by H2O2 (extinction coefficient: 2.9 mM−1 cm−1). The enzyme activity was expressed in U/mg protein.

Catalase (CAT)

Catalase (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 H2O2 and 0.04 ml of enzyme extract. The decomposition of H2O2 was followed by the decline in absorbance at 240 nm. The enzyme activity was expressed in U/mg protein.

Superoxide dismutase (SOD) assay

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 (KCN) and 5.6 × 10−5 M of nitroblue tetrazolium salt (NBT) dissolved in 3 ml of 0.05 M sodium phosphate buffer (pH 7.8). Three millilitres 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 1 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).

Statistical analysis

The pot culture was carried out in completely randomized design (CRBD). The data are expressed as mean ± SE for seven samples in each group.

Results and discussion

Effect of drought and drought with Triazole combination on Osmolite concentration Amino acid

Drought stress increased the amino acid (AA), contents in all the parts of the sunflower when compared to control and triazole treated plants partially ameliorated drought stress (Table 1). AA content has been shown to increase under drought condition in Vigna (Manivannan et al., 2007b). Similar results were obtained in Abelmoschus (Sankar et al., 2007) and Vigna unguiculata (Manivannan et al., 2008). The accumulation of AA content may be due to protein hydrolysis and also may occur in response to the change in the osmotic adjustment of their cellular contents (Sankar et al., 2007). It is shown that plants have evolved a great number of adaptive mechanisms that allow the biochemical systems to cope with increased water deficit. 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).
The amino acid content increased under drought condition in *Arachis hypogaea* (Asha and Rao, 2002); *sorghum* (Yadav et al., 2005); *Radix astragali* (Tan et al., 2006). Accumulated amino acid may be occurring in response to the change in osmotic adjustment of their cellular contents (Shao et al., 2007). Paclobutrazol treatment to the drought stressed peanut plants lowered the amino acid content when compared to drought stress but it was higher than that of control. Similar results were observed in paclobutrazol and triacontanol in olive varieties under water stress (Thakur et al., 1998) and paclobutrazol treated wheat seedlings under low temperature stress (Berova et al., 2002). Amino acids accumulation plays a very important role in drought tolerance, probably through osmotic adjustment in different plant species, such as, *Radix astragali* (Tan et al., 2006). Free amino acid accumulation is a more important account for most of the changes in osmotic potential. The accumulation of AA under stress at all the growth stages indicates the possibility of their involvement in osmotic adjustment (Sankar et al., 2008). Osmotic adjustment is one of the important mechanisms that alleviate some of the detrimental effects of water stress (Sankar et al., 2007). The extent of increase was higher in leaf, followed by root and stem.

**Proline**

Proline (PRO) contents were increased in all the parts of the sunflower when compared to control (Table 2). Drought stress with triazole treatment leads to an enhancement in biochemical contents when compared to drought-stressed and control plants. Increased proline in stressed plants may be an adaptation to overcome the stress conditions. Proline accumulated under stressed conditions supplies energy for growth and survival and thereby helps the plant to tolerate the stress. Proline accumulation in plants might be a scavenger and acting as an osmolyte. Increased proline in the stressed plants may be an adaptation to overcome the stress conditions. Water stress resulted in an increase in proline accumulation in *sorghum* (Yadav et al., 2005). The similar results were observed in *sorghum* (Zaifnejad et al., 1997; Al-Karaki et al., 1996) wheat (Nayyar, 2003; Zhu et al., 2005; Vendruscolo et al., 2007); soybean (Heerden and Kruger, 2002). Proline content increased in both drought stress and with TDM treatments when compared to control. TDM treatments decreased prolin content in plants under drought stress compared with the plants had received only water stress treatment (Neda and Rajaei, 2013).

**Glycine betaine**

Glycine betaine (GB) contents were increased in all the parts of the sunflower when compared to control (Table 3). Drought stress with triazole treatment leads to an enhancement in biochemical contents when compared to drought-stressed and control plants. Aliphatic quaternary ammonium compounds (QAC) such as GB, stachydrine, homostachydrine, trigonelline have been found to accumulate in a large number of plants exposed to salt and water stress. The glycine betaine content increased under drought stress in *Radix astragali* (Tan et al., 2006). The glycine betaine content increased under drought stress in barley (Nakamura et al., 2001) and in higher plants (Jun et al., 2000). Glycine betaine is considered to be one of the most abundant quaternary ammonium compounds.

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**Table 1. Effects of drought stress and drought with triazole combination on the amino acid content of *Helianthus annuus* (Expressed in mg/g dry weight).**

<table>
<thead>
<tr>
<th>DAS</th>
<th>Control</th>
<th>Drought</th>
<th>D + HEXA</th>
<th>D + TEBU</th>
<th>D+PROP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Root</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>40</td>
<td>5.79±0.52</td>
<td>9.47±0.63</td>
<td>7.58±0.57</td>
<td>7.69±0.63</td>
<td>7.74±0.53</td>
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<tr>
<td>50</td>
<td>7.14±0.42</td>
<td>11.57±0.58</td>
<td>9.36±0.61</td>
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<td>9.62±0.62</td>
</tr>
<tr>
<td>60</td>
<td>9.78±0.61</td>
<td>16.03±0.62</td>
<td>12.64±0.58</td>
<td>12.99±0.61</td>
<td>13.21±0.57</td>
</tr>
<tr>
<td>Stem</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>40</td>
<td>4.37±0.46</td>
<td>7.14±0.58</td>
<td>5.62±0.61</td>
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<td>50</td>
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<td>60</td>
<td>8.8±0.511</td>
<td>14.48±0.57</td>
<td>11.64±0.52</td>
<td>11.89±0.58</td>
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</tr>
<tr>
<td>Leaf</td>
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</tr>
<tr>
<td>40</td>
<td>6.81±0.53</td>
<td>11.25±0.57</td>
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<td>12.53±0.56</td>
<td>12.73±0.58</td>
</tr>
<tr>
<td>60</td>
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<td>18.35±0.49</td>
<td>14.56±0.57</td>
<td>14.78±0.53</td>
<td>14.94±0.52</td>
</tr>
</tbody>
</table>

**DAS: Days after sowing; Values are mean ± SE of seven replicates.**
produced in higher plants under stressful environment (Yang et al., 2003). Paclobutrazol treatment to the drought stressed plants decreased glycine betaine content but it was higher than that of control. Similar results were observed in Arachis hypogaea (Girija et al., 2002). The accumulation of glycine betaine might serve as an intercellular osmoticum and it can be closely correlated with the elevation of osmotic pressure (Kavikishore et al., 1995).

**Ascorbate peroxidase (APX) activity**

Ascorbate peroxidase (APX) activity was increased in all the drought treatments when compared to control (Table 4). APX found in organelles is believed to scavenge H2O2 produced from the organelles, whereas the function of cytosolic APX is probably to eliminate H2O2 that is produced in the cytosol or apoplast and that has diffused from organelles. In the chloroplast, H2O2 can be detoxified by the ASA–GSH–NAPDH system, which has been catalyzed by APX (Sharma et al., 2012). Drought stress with triazole treatment decreased APX activity in drought stressed plants, and increased it in control plants. 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., 2007a,b).

| Table 2. Effects of drought stress and drought with triazole combination on the proline content of Helianthus annuus. (Expressed in mg/g-1 dry weight). |
|-----------------|----------------|----------------|----------------|----------------|----------------|
| DAS             | Control        | Drought        | D + HEXA       | D + TEBU       | D+PROP         |
| Root            |                |                |                |                |                |
| 40              | 0.52±0.057     | 0.846±0.074    | 0.683±0.058    | 0.694±0.058    | 0.698±0.063    |
| 50              | 0.806±0.083    | 1.427±0.088    | 1.042±0.064    | 1.085±0.072    | 1.098±0.077    |
| 60              | 1.247±0.063    | 2.301±0.072    | 1.603±0.057    | 1.628±0.083    | 1.655±0.081    |
| Stem            |                |                |                |                |                |
| 40              | 0.453±0.045    | 0.754±0.071    | 0.594±0.055    | 0.613±0.048    | 0.624±0.074    |
| 50              | 0.791±0.063    | 1.385±0.048    | 1.042±0.073    | 1.075±0.051    | 1.086±0.068    |
| 60              | 0.925±0.055    | 1.698±0.066    | 1.215±0.079    | 1.236±0.063    | 1.243±0.046    |
| Leaf            |                |                |                |                |                |
| 40              | 1.257±0.089    | 2.005±0.098    | 1.625±0.099    | 1.663±0.084    | 1.696±0.097    |
| 50              | 1.703±0.086    | 2.951±0.089    | 2.256±0.122    | 2.302±0.105    | 2.327±0.1      |
| 60              | 1.986±0.082    | 3.675±0.086    | 2.584±0.086    | 2.608±0.122    | 2.622±0.097    |

**Table 3. Effects of drought stress and drought with triazole combination on the glycine betaine content of Helianthus annuus. (Expressed in mg g-1 dry weight).**

<table>
<thead>
<tr>
<th>DAS</th>
<th>Control</th>
<th>Drought</th>
<th>D + HEXA</th>
<th>D + TEBU</th>
<th>D+PROP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Root</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>40</td>
<td>23.71±2.7</td>
<td>37.89±2.63</td>
<td>31.34±2.36</td>
<td>31.89±2.39</td>
<td>32.07±2.51</td>
</tr>
<tr>
<td>50</td>
<td>37.65±2.43</td>
<td>61.22±2.56</td>
<td>49.22±2.88</td>
<td>50.35±2.64</td>
<td>50.98±2.25</td>
</tr>
<tr>
<td>60</td>
<td>42.19±2.58</td>
<td>69.73±2.21</td>
<td>55.73±2.17</td>
<td>56.31±2.26</td>
<td>56.85±2.34</td>
</tr>
<tr>
<td>Stem</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>40</td>
<td>19.73±2.11</td>
<td>32.74±2.25</td>
<td>26.13±2.14</td>
<td>26.47±1.98</td>
<td>26.85±2.31</td>
</tr>
<tr>
<td>50</td>
<td>29.46±2.07</td>
<td>47.86±2.14</td>
<td>38.11±2.26</td>
<td>38.58±2.31</td>
<td>39.77±2.23</td>
</tr>
<tr>
<td>60</td>
<td>38.14±2.18</td>
<td>62.59±2.22</td>
<td>50.28±2.1</td>
<td>50.83±2.28</td>
<td>51.55±2.29</td>
</tr>
<tr>
<td>Leaf</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>40</td>
<td>29.81±3.12</td>
<td>48.84±3.67</td>
<td>38.31±3.81</td>
<td>38.92±3.23</td>
<td>39.64±3.44</td>
</tr>
<tr>
<td>50</td>
<td>40.76±3.54</td>
<td>67.96±3.28</td>
<td>52.75±3.51</td>
<td>53.42±3.73</td>
<td>54.03±3.61</td>
</tr>
<tr>
<td>60</td>
<td>54.73±3.38</td>
<td>86.57±3.65</td>
<td>72.68±3.82</td>
<td>73.66±3.49</td>
<td>73.97±3.57</td>
</tr>
</tbody>
</table>

DAS: Days after sowing. Values are mean ± SE of seven replicates.
Table 4. Effects of drought stress and drought with triazole combination on the ascorbate peroxidase content of *Helianthus annuus*. (U/mg protein).

<table>
<thead>
<tr>
<th>DAS</th>
<th>Control</th>
<th>Drought</th>
<th>D + HEXA</th>
<th>D + TEBU</th>
<th>D+ PROP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Root</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>40</td>
<td>3.43±0.43</td>
<td>5.58±0.58</td>
<td>4.48±0.41</td>
<td>4.58±0.55</td>
<td>4.69±0.38</td>
</tr>
<tr>
<td>50</td>
<td>5.02±0.51</td>
<td>8.26±0.53</td>
<td>6.61±0.47</td>
<td>6.88±0.42</td>
<td>6.94±0.53</td>
</tr>
<tr>
<td>60</td>
<td>6.51±0.42</td>
<td>10.74±0.49</td>
<td>8.58±0.44</td>
<td>8.77±0.52</td>
<td>8.83±0.59</td>
</tr>
<tr>
<td>Stem</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>40</td>
<td>2.93±0.34</td>
<td>4.85±0.43</td>
<td>3.82±0.30</td>
<td>3.90±0.33</td>
<td>3.98±0.39</td>
</tr>
<tr>
<td>50</td>
<td>4.48±0.41</td>
<td>7.36±0.48</td>
<td>5.95±0.34</td>
<td>6.07±0.35</td>
<td>6.14±0.31</td>
</tr>
<tr>
<td>60</td>
<td>5.04±0.37</td>
<td>8.41±0.40</td>
<td>6.61±0.39</td>
<td>6.73±0.36</td>
<td>6.85±0.38</td>
</tr>
<tr>
<td>Leaf</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>40</td>
<td>4.94±0.52</td>
<td>7.96±0.64</td>
<td>6.56±0.53</td>
<td>6.69±0.49</td>
<td>6.78±0.62</td>
</tr>
<tr>
<td>50</td>
<td>6.01±0.61</td>
<td>9.84±0.68</td>
<td>8.07±0.55</td>
<td>8.17±0.58</td>
<td>8.23±0.83</td>
</tr>
<tr>
<td>60</td>
<td>8.29±0.58</td>
<td>13.58±0.65</td>
<td>10.89±0.57</td>
<td>10.95±0.51</td>
<td>11.27±0.55</td>
</tr>
</tbody>
</table>

DAS: Days after sowing; Values are mean ± SE of seven replicates.

Increased APX activity was reported in *Phaseolus acutifolius* under drought stress (Turkan et al., 2005) and in soybean (Heerden and Kruger, 2002). Similar results were obtained by many workers under drought stress in many higher plants (Reddy et al., 2004), *Pinus halepensis* (Alonso et al., 2001), *Phaseolus acutifolius* (Turkan et al., 2005), wheat (Baisak et al., 1994; Gong et al., 2005) and *Kentucky bluegrass* (Liu et al., 2008). 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 (Reddy et al., 2004). Paclobutrazol treatment in combination with drought decreased the APX activity but, it was higher than that of control plants. Increased APX activity was reported in *Phaseolus acutifolius* under drought stress (Turkan et al., 2005).

Table 5. Effects of drought stress and drought with triazole combination on the catalase content of *Helianthus annuus*. (U/mg protein).

<table>
<thead>
<tr>
<th>DAS</th>
<th>Control</th>
<th>Drought</th>
<th>D + HEXA</th>
<th>D + TEBU</th>
<th>D+ PROPI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Root</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>40</td>
<td>2.61±0.26</td>
<td>4.28±0.32</td>
<td>3.39±0.35</td>
<td>3.46±0.38</td>
<td>3.58±0.29</td>
</tr>
<tr>
<td>50</td>
<td>3.8±0.22</td>
<td>6.33±0.37</td>
<td>5.02±0.30</td>
<td>5.14±0.37</td>
<td>5.23±0.35</td>
</tr>
<tr>
<td>60</td>
<td>4.53±0.29</td>
<td>7.49±0.36</td>
<td>5.98±0.23</td>
<td>6.17±0.34</td>
<td>6.25±0.32</td>
</tr>
<tr>
<td>Stem</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>40</td>
<td>3.19±0.32</td>
<td>5.38±0.42</td>
<td>4.25±0.36</td>
<td>4.39±0.42</td>
<td>4.42±0.42</td>
</tr>
<tr>
<td>50</td>
<td>4.64±0.36</td>
<td>7.62±0.39</td>
<td>6.08±0.35</td>
<td>6.23±0.37</td>
<td>6.34±0.45</td>
</tr>
<tr>
<td>60</td>
<td>5.97±0.29</td>
<td>9.59±0.33</td>
<td>7.83±0.43</td>
<td>7.99±0.34</td>
<td>8.17±0.41</td>
</tr>
<tr>
<td>Leaf</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>40</td>
<td>4.84±0.57</td>
<td>7.97±0.67</td>
<td>6.3±0.46</td>
<td>6.48±0.61</td>
<td>6.52±0.57</td>
</tr>
<tr>
<td>50</td>
<td>5.87±0.62</td>
<td>9.85±0.53</td>
<td>7.78±0.51</td>
<td>7.96±0.47</td>
<td>8.14±0.53</td>
</tr>
<tr>
<td>60</td>
<td>7.29±0.59</td>
<td>12.39±0.64</td>
<td>9.84±0.58</td>
<td>9.86±0.58</td>
<td>9.99±0.55</td>
</tr>
</tbody>
</table>

DAS: Days after sowing; Values are mean ± SE of seven replicates.

CAT activity

CAT activity was increased in all parts of drought-stressed sunflower plants and of that undergoing triazole treatment when compared to control (Table 5). The catalase activity increased under drought in *Nicotiana plumbaginifolia* (Nicholas Smirnoff, 1998) and *Pinus halepensis* (Alonso et al., 2001). Similar results were observed in wheat (Zhang and Kirkam, 1994; Lin and Wang, 2002; Gong et al., 2005; Shao et al., 2005), *Phaseolus acutifolius* (Turkan et al., 2005). Under salt stress the catalase activity increased in spinach (Ozturk and Demir, 2003). Paclobutrazol treatment to the stressed plants caused a decrease in CAT activity but, it was higher than that of control plants. Increased CAT activity was reported in *Phaseolus acutifolius* under drought stress (Turkan et al., 2005) and in soybean (Heerden and Kruger, 2002). Triazoles increased the level of CAT activity in *Solenostemon rotundifolius* (Kishorekumar et al., 2008).
Table 6. Effects of drought stress and drought with triazole combination on the super oxide dismutase content of *Helianthus annuus*. (U/mg protein).

<table>
<thead>
<tr>
<th>DAS</th>
<th>Control</th>
<th>Drought</th>
<th>D + HEXA</th>
<th>D + TEBU</th>
<th>D+ PROPI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Root</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>40</td>
<td>0.751±0.099</td>
<td>1.260±0.144</td>
<td>1.003±0.128</td>
<td>1.016±0.123</td>
<td>1.024±0.139</td>
</tr>
<tr>
<td>50</td>
<td>1.238±0.131</td>
<td>1.983±0.127</td>
<td>1.649±0.137</td>
<td>1.673±0.122</td>
<td>1.681±0.119</td>
</tr>
<tr>
<td>60</td>
<td>1.872±0.128</td>
<td>2.997±0.133</td>
<td>2.451±0.124</td>
<td>2.508±0.126</td>
<td>2.569±0.117</td>
</tr>
<tr>
<td>Stem</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>40</td>
<td>1.127±0.132</td>
<td>1.836±0.167</td>
<td>1.468±0.142</td>
<td>1.498±0.138</td>
<td>1.513±0.152</td>
</tr>
<tr>
<td>50</td>
<td>1.639±0.144</td>
<td>2.683±0.154</td>
<td>2.139±0.137</td>
<td>2.227±0.129</td>
<td>2.316±0.147</td>
</tr>
<tr>
<td>60</td>
<td>2.151±0.153</td>
<td>3.572±0.146</td>
<td>2.881±0.122</td>
<td>2.949±0.136</td>
<td>2.997±0.125</td>
</tr>
<tr>
<td>Leaf</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>40</td>
<td>1.938±0.216</td>
<td>3.214±0.266</td>
<td>2.518±0.225</td>
<td>2.622±0.263</td>
<td>2.673±0.255</td>
</tr>
<tr>
<td>50</td>
<td>2.515±0.237</td>
<td>4.152±0.253</td>
<td>3.279±0.274</td>
<td>3.341±0.277</td>
<td>3.409±0.264</td>
</tr>
<tr>
<td>60</td>
<td>3.236±0.284</td>
<td>5.317±0.231</td>
<td>4.326±0.255</td>
<td>4.378±0.247</td>
<td>4.418±0.288</td>
</tr>
</tbody>
</table>

DAS: Days after sowing; Values are mean ± SE of seven replicates.

Triazole treatment decreased catalase activity when compared to drought stress and increased it in controls. This result is in accordance with the findings in *Catharanthus roseus* (Jaleel et al., 2006). The combined action of CAT and SOD converts the toxic O·− 2, H2O2 into water and molecular oxygen, averting the cellular damage under unfavourable conditions like water stress (Bowler et al., 1992; Manivannan et al., 2007a). Catalase 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 (Neda and Rajaei, 2013).

**SOD activity**

SOD activity increased in all the DID stress and with triazole treatments when compared to control (Table 6). Triazole treatment decreased SOD activity when compared to drought-stress and increased it in the control. Super oxide dismutase activity increased under drought stressed higher plants (Reddy et al., 2004), rice (Wang et al., 2005), *Phaseolus acutifolius* (Turkan et al., 2005), wheat (Quartacci et al., 1994; Zhang and Kirkam, 1994; Gong et al., 2005; Shao et al., 2005). Drought stressed plants treated with paclobutrazol showed a decreased SOD activity but, it was higher than that of control. An increase in SOD activity was reported in *Vigna* plants under water deficit stress and propiconazole application (Manivannan et al., 2007a). The SOD activity increased under drought in *Phaseolus acutifolius* (Turkan et al., 2005). Triazoles increased the antioxidant potential in oxidative stressed plants under treatment when compared to control (Sankar et al., 2007). It was reported that SOD enhances water-stress tolerance in plants. The cytosolic Cu/Zn–SOD was induced strongly by stress, while Cu/Zn–SOD remained largely unaffected (Bowler et al., 1992). 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 early season. Furthermore, it was observed that spraying PBZ or 6-BA could increase super oxide dismutase (SOD) (Shenggang et al., 2013). SOD activity increased in drought treatment when compared to control. TDM treatment increased the SOD activity in drought stressed as well as in control plants (Neda and Rajaei, 2013).

**Conclusion**

Thus, from these results, it is clear that plants are highly regulated by triazole compounds, in terms of enhanced components of osmoprotectants under drought stress. Drought stressed plants under triazole treatment maintain a balance between formation and detoxification of activated oxygen species, leading to partial improvement of their response to drought-induced oxidative stress. It can be concluded that triazole such as hexaconazole, tebuconazole and propiconazole may be useful to trigger drought avoidance mechanisms in plants like *Helianthus annuus* L. Further work is needed to understand the genetic mechanism behind triazole induced water stress tolerance in sunflower.

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