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

Gabriel Amalan Rabert, Paramasivam Manivannan*, Ramamurthy Somasundaram and Rajaram Panneerselvam

Stress Physiology and Plant Growth Regulation Lab, Department of Botany, Annamalai University, Annamalainagar 608 002, Tamilnadu, India

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-spread 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_2O_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

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*Corresponding Author

Paramasivam Manivannan
Stress Physiology and Plant Growth Regulation Lab, Department of Botany, Annamalai University, Annamalainagar 608 002, Tamilnadu, India

Email: maniphd78@yahoo.co.in

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 Gibberellic acid (GA), Abscisic acid (ABA) and Cytokinins (Kamountsis and Chronopoulon-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 qualities 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 30^{th} to 60^{th} 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 40^{th}, 50^{th} and 60^{th} 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)

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).

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

DAS	Control	Drought	D + HEXA	D + TEBU	D+PROP
Root					
40	5.79±0.52	9.47±0.63	7.58±0.57	7.69±0.63	7.74±0.53
50	7.14±0.42	11.57±0.58	9.36±0.61	9.47±0.58	9.62±0.62
60	9.78±0.61	16.03±0.62	12.64±0.58	12.99±0.61	13.21±0.57
Stem					
40	4.37±0.46	7.14±0.58	5.62±0.61	5.84±0.53	5.99±0.62
50	6.65±0.58	10.88±0.46	8.63±0.58	8.85±0.57	8.97±0.58
60	8.8±0.511	14.48±0.57	11.64±0.52	11.89±0.58	11.94±0.53
Leaf					
40	6.81±0.53	11.25±0.57	9.04±0.47	9.24±0.52	9.32±0.58
50	9.25±0.48	15.20±0.56	12.11±0.52	12.53±0.56	12.73±0.58
60	11.08±0.52	18.35±0.49	14.56±0.57	14.78±0.53	14.94±0.52

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

The amino acid 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). 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

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	0.523±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

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

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).

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

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).

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

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).

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

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

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

Table 6. Effects of drought stress and drought with triazole combination on the super oxide dismutase content of *Helianthus annuus*. (U/mg protein).

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

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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