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ALPHA-TOCOPHEROL ALTERS ENDOGENOUS OXIDATIVE DEFENSE SYSTEM IN MUNG BEAN PLANTS UNDER WATER-DEFICIT CONDITIONS

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Abstract

Foliar spray of plant growth regulating compounds including antioxidants is an effective strategy to overcome the adverse effects of environmental constraints on different plants. A pot experiment was conducted to assess the influence of exogenously applied α-tocopherol (Toc) in up-regulating the oxidative defense system in two mung bean cultivars (Cyclone 7008 and Cyclone 8009) grown under normal and water deficit conditions. After 30-day of water deficit treatment, four levels of Toc [0 (non spray), 100, 200 and 300 mg L^{-1}] were applied as a foliage application (at vegetative growth stage). A significant reduction was observed in plant height and total soluble proteins, while an increase was observed in the levels of hydrogen peroxide (H_2O_2), ascorbic acid, total phenolics, malondialdehyde (MDA), total free amino acids and the activities of enzymatic (SOD, POD and CAT) antioxidants in both mung bean cultivars under drought conditions. Foliar spray of Toc was effective in improving plant height, AsA, total soluble proteins, total free amino acids, and activities of POD and CAT enzymes, but reduced MDA under water stress conditions. However, no prominent change was observed on the concentrations of H_2O_2, phenolics, and SOD enzyme due to foliar-applied Toc in both mung bean cultivars under both water regimes. Both mung bean cultivars were almost similar in all attributes measured except that cv. Cyclone 7008 was higher in the levels of H_2O_2 and TSP while cv. Cyclone 8009 in phenolics. So, from the results of this study we can suggest that exogenous application of Toc is effective in improving growth and antioxidative potential of mung bean plants under dry arid environment.

Keywords: Tocopherol, Antioxidant enzymes, Mung bean, Drought tolerance.

Introduction

Water deficiency in the root growing medium is one of the major constraints to plant development and yield production at global level (Thakur *et al*., 2010; Kaur *et al.,* 2011; Akram *et al*., 2013). However, plants have developed complex changes in their metabolic pathways due to water shortage. Of which stunted plant growth is the typical one (Sairam & Srivastava, 2001) which could be due to impairment in cell division and cell proliferation/expansion which lastly result in reduced growth and low harvest (Hussain *et al.,* 2008). Drought stress also results in the production of ROS in different membrane-bound organelles like mitochondria, golgi bodies and chloroplast.

Plants have different protective measures including accumulation of various antioxidants (Parida & Das, 2005). These antioxidants neutralize the adverse effects of stress-induced oxidative stress (Vranová *et al*., 2002). Some antioxidant scavengers are the enzymes viz., peroxidase, catalase and superoxide dismutase (Khan & Panda, 2008; Demiral & Turkan, 2005). Phenolic compounds also act as antioxidants (Evans & Al-Hamdani, 2015). Similarly, proline is also a commonly occurring osmolyte in plants exposed to moisture deficiency (Dawood *et al.,* 2014). The production of excess amounts of free amino acids in protoplast is a physiological indication of the onset of water stress and it is a metabolic adaptation against the stress (Caballero, 2005).

Now-a-days, it is economical to use osmoprotectants exogenously in order to avoid the harmful effects of abiotic stresses on plants (Ashraf & Foolad, 2007). Under intense oxidative phase, plants are genetically stimulated to develop various defensive mechanisms such as accumulation/over-synthesis of ascorbic acid and alpha-tocopherol in membrane bound organelles (Dat *et al.,* 2000; Alscher *et al.,* 2002; Ali *et al.,* 2015a,b). The amount of alpha tocopherol varies among leaves and seeds. As stress becomes more severe, ROS concentration increases in biological membranes, which ultimately reduces α-tocopherol contents in them (Munne-Bosch & Alegre, 2002). Tocopherols (Vitamin E) are important antioxidants as they can scavenge reactive oxygen species such as singlet molecular oxygen. Only one tocopherol molecule can inhibit about 120 singlet oxygen molecules ($^1O_2$) (Fahrenholtz *et al.,* 1974; Szarka *et al.,* 2012). Genetically, tocopherol synthesis is up regulated by stress conditions. El-Bassiouny *et al.* (2005) reported that *in vitro* induction/foliar spray of alpha-tocopherol enhanced growth and yield components in *Vicia faba.*

Mung bean is more sensitive to drought than other grain legumes (Nezhad *et al*., 2014). Mung bean seeds are fully loaded with protein and different amino acids, thus it serves as an important protein source for human utilization. Fresh green pods and sprouts of mung bean are also served as vegetable and cheap source of vitamin and minerals. The present experiment was performed with a major objective to assess the influence of exogenously applied α-tocopherol on mung bean to cope drought stress, particularly by examining protein and amino acid metabolism, the concentration of hydrogen peroxide, ascorbic acid, phenolics, malondialdehyde and the activities of major antioxidant enzymes.

Materials and Methods

In the current study, two cultivars of mung bean (Cyclone 7008 and Cyclone 8009) were sown in plastic pots (depth 24.5 cm and radius 10.5 cm). The experiment was set-up in a completely randomized design with four replicates. Before sowing, about 250 healthy seeds of each of two mung bean cultivars were soaked in distilled water for one hour. The soil used for pots contained sand, 65%; clay, 7.5% and silt, 27.5%. Ten seeds of each cultivar were sown in a pot by gentle hand drilling. After one week, germination initiated and five seedlings of uniform size were maintained in each pot. Drought stress (60% field capacity) was started 21-day after seed germination. After three weeks of drought stress maintenance, alpha-tocopherol (100, 200 and 300 mg L^{-1}) with 0.1% T-20 was sprayed to the leaves of all mung bean seedlings. After two weeks of foliar spray of Toc, two plants were used for recording the plant height. The remaining plants were used for the following biochemical analyses:

Ascorbic acid (AsA) contents: Fresh leaf material (0.5 g) was homogenised in 10 mL trichloroacetic acid (6% w/v). The mixture was centrifuged and to 4 mL of the extract, 2 ml diphenyl hydrazine (2%) along with one drop of thiourea were added. The mixture was boiled for 15 min. in a water bath, and then cooled slowly. Finally 5 mL of 80% H_2SO_4 were added and optical density was recorded at 530 nm following Mukherjee & Choudhuri (1983).

Total phenolics: Fresh leaf material (0.1 g) was triturated in 5 mL of 80% acetone. This mixture was centrifuged and samples were treated as suggested by Julkunen-Titto (1985). The absorbance of the colored mixture was recorded at 750 nm.

Malondialdehyde (MDA) contents: Using the Cakmak & Horst (1991) protocol, absorbance of the reactants was read at 532 and 600 nm and MDA contents were calculated.

Hydrogen peroxide contents: The analysis of hydrogen peroxide was carried out following Velikova *et al.* (2000).

Total soluble proteins: Fresh leaf (0.25 g) was chopped and finally ground using 10 mL of 50 mM K buffer. The samples were centrifuged at 10,000 × *g* at 4°C for 15 minutes and following Bradford (1976), total soluble proteins were determined.

Total free amino acids: The Hamilton and Van Slyke (1943) protocol was used for the estimation of free amino acids.

Extraction of antioxidant enzymes: For determining antioxidant enzymes, fresh leaf material (0.5 g) was ground in 10 mL of potassium phosphate buffer (50 mM; pH 7.8) in a pre-chilled pestle and mortar. Then, the extract was centrifuged at 10,000 × *g* at 4 °C for 15 minutes. The supernatant was used to determine the activities of following enzymes:

Activity of CAT enzyme: The leaf extract (0.1 mL) was mixed with 1 mL of H_2O_2 (5.9 mM) and 1.9 mL of 50 mM phosphate buffer (pH 7.0) according to the method of Luck (1963). Then the absorbance was read at 240 nm for 3 minutes using a spectrophotometer.

Activity of POD enzyme: Following the method of Chance & Maehly (1955), the absorbance of the mixture was read at 400 nm for 3 min using a spectrophotometer.

Activity of SOD enzyme: The reaction mixture was taken in a cuvette and placed under light for 15 min, and the absorbance was read at 560 nm following the method of Giannopolitis & Ries (1977).

Statistical Analysis: A three-way completely randomized design was employed and analysis of variance of data was carried-out using Cohort 3.60 Statistical program.

Results

Shoot and root lengths of the two mung bean cultivars Cyclone 7008 and Cyclone 8009 decreased significantly under drought stress (60% field capacity) compared with those of plants grown under control conditions (100% field capacity) (Fig. 1). External application of alpha-tocopherol significantly ($p \leq 0.01$; 0.05) improved the growth of both mung bean cultivars in terms of shoot and root lengths under both water regimes. Moreover, cv. Cyclone 7008 was higher in shoot length than the other cultivar particularly under 100% field capacity (Fig. 1).

Leaf H_2O_2 contents increased considerably in both mung bean cultivars under water deficit conditions. No prominent change in H_2O_2 contents was observed due to foliar-applied α-tocopherol in both mung bean cultivars under both water regimes. Cultivar cyclone 7008 showed relatively better response in hydrogen peroxide contents than cv. Cyclone 8009 under water stress conditions (Fig. 1).

A considerable rise in the concentration of ascorbic acid (AsA) was observed in both mung bean cultivars under water deficit conditions. Ascorbic acid concentration increased ($p \leq 0.05$) in both mung bean cultivars under water-deficit conditions when α-tocopherol was applied as a foliar spray (Fig. 1). Both mung bean cultivars had a uniform accumulation of AsA both under stress and non-stress conditions (Fig. 1).

No significant change was observed in the leaf total phenolics in both mung bean cultivars due to varying water regimes as well as exogenous application of tocopherol. Of both mung bean cultivars, cv. Cyclone 8009 was better in this secondary metabolite (Fig. 1).

Malondialdehyde (MDA) contents increased prominently ($p \leq 0.001$) in both mung bean cultivars under water stress (Fig. 1). However, exogenous application of α-tocopherol was effective ($p \leq 0.05$) in minimizing the levels of MDA in both mung bean cultivars under moisture deficit conditions. Both mung bean cultivars showed a similar response in this attribute.

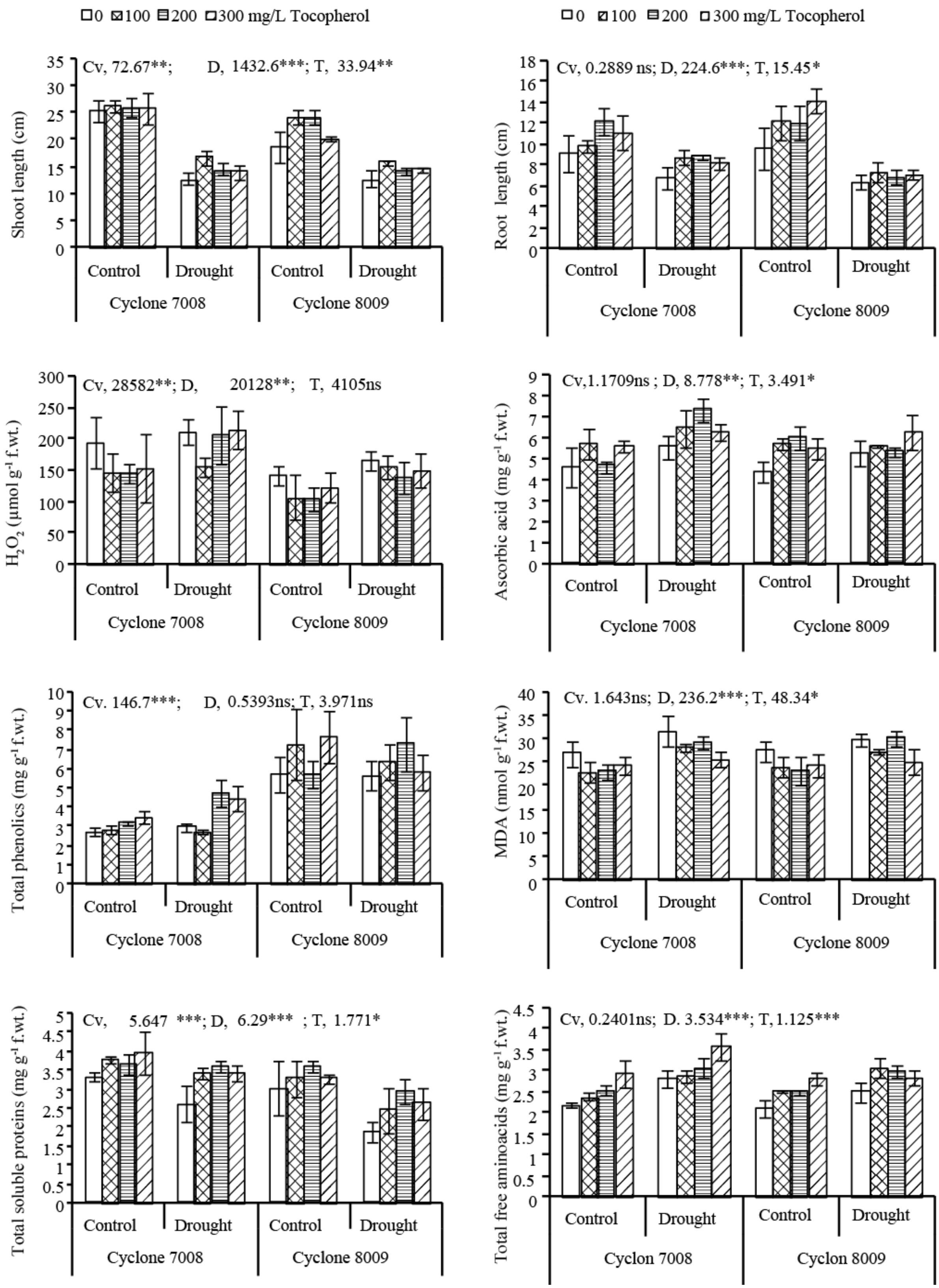

Fig. 1. Shoot and root lengths, leaf hydrogen peroxide (H_2O_2), ascorbic acid (AsA), total phenolics, malondialdehyde (MDA), total soluble proteins, total free amino acids of two cultivars of mung bean subjected to foliar-applied varying levels of tocopherol under stress and non-stress conditions (Mean ± S.E.), *, **, ***; significant at 0.05, 0.01 and 0.001 levels, respectively; ns, no significant; Cv, Cultivars; D, Drought; T, tocopherol.

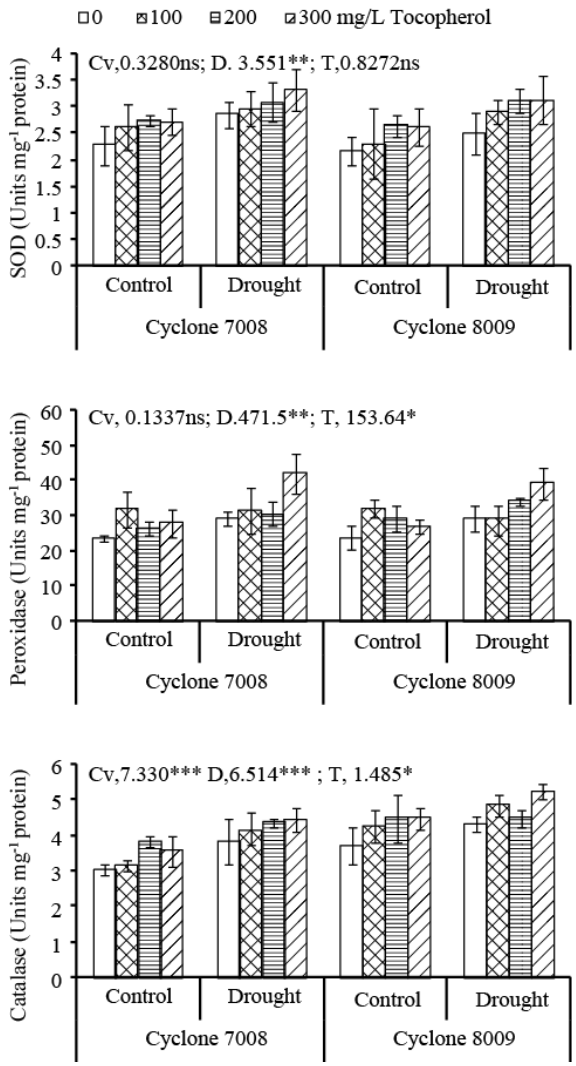

Fig. 2. Activities of superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) of two cultivars of mung bean subjected to foliar-applied varying levels of tocopherol under stress and non-stress conditions (Mean ± S.E.), *, **, ***; significant at 0.05, 0.01 and 0.001 levels, respectively; ns, no significant; Cv, Cultivars; D, Drought; T, tocopherol.

Total soluble proteins declined in both mung bean cultivars under water limited conditions. Alpha-tocopherol application (foliar) significantly ($p \leq 0.05$) improved the TSP particularly under drought stress conditions (Fig. 1). Cultivar Cyclone 7008 was superior to Cyclone 8009 in TSP under both water regimes.

Drought stress significantly ($p \leq 0.001$) increased the accumulation of total free amino acids in both mung bean cultivars. Foliage application of α-tocopherol significantly ($p \leq 0.001$) increased the accumulation of total free amino acids in both mung bean cultivars. The trend of total amino acids accumulation in both mung bean cultivars was similar under water stress conditions (Fig. 1).

A significant water-stress induced increase in the activities of superoxide dismutase, POD and CAT was observed in both mung bean cultivars (Fig. 2). Foliar application of α-tocopherol was effective in enhancing the activities of only POD and CAT enzymes in both mung bean cultivars under both water regimes. Both mung bean cultivars varied significantly in CAT enzyme activity and of both mung bean cultivars, cv. Cyclone 7008 was inferior to the other cultivar in CAT activity under water deficit conditions.

Discussion

In the present study, drought stress significantly suppressed the shoot and root lengths of both mung bean cultivars, whereas external application of alpha-tocopherol (Toc) was significantly effective in improving plant height of both mung bean cultivars under both non-stress and stress conditions (Fig. 1). Similarly, Toc-induced growth improvement was reported in *Vicia faba* (Orabi & Abdelhamid, 2014; Semida *et al.,* 2014), geranium (Ayad *et al.,* 2009) and *Hibiscus rosasineses* (El-Aziz *et al.,* 2009) under stress conditions. They all attributed Toc-induced improvement in growth attributes to better water potential, high accumulation of antioxidants, less oxidative damage and better cross-talk among different growth regulators.

Among different ROS, hydrogen peroxide is known to remain potent and stable even within biological membranes, where it disrupts various metabolic activities like Calvin cycle. Moreover, it can also act as a mutagen (Akram *et al*., 2012). In the present study, application of α-tocopherol via foliar spray had no effect upon hydrogen peroxide in both mung bean cultivars under drought stress conditions, although in some other crops it is otherwise. For example, Farouk (2011) reported that sodium chloride stress up to 11.5 dS m^{-1} induced the accumulation of hydrogen peroxide in the flag leaf of wheat plants and 100 mg L^{-1} α-tocopherol significantly decreased hydrogen peroxide content in the flag leaf of wheat. Kostopoulou *et al.* (2014) while studying the response of citrus seedlings to NaCl treatments, reported that H_2O_2 concentration was higher in the stressed seedlings than that in non-stressed ones, however, H_2O_2 level decreased in the leaves supplied with α-tocopherol.

As an antioxidant, ascorbic acid (AsA) in small concentration is believed to minimize the adverse effects of free oxidative radicals in stressed plants (Farouk, 2011). In our present study, a prominent increase was observed in the AsA concentrations in both mung bean cultivars under water-deficit conditions. Ascorbic acid contents remained non-responsive in both mung bean cultivars under water-deficit conditions when different concentrations of α-tocopherol were sprayed to leaves. A previous study showed that AsA decreased during leaf abscission in the leaves of maize plant (Prochazkova *et al.,* 2001), a phenomenon which commonly occurs under different stresses. Kostopoulou *et al.* (2014) reported that total ascorbic acid concentration in the leaves and roots of citrus seedlings decreased following α-Toc application under saline medium as compared to that in control. Under drought stress, a non-significant response of phenolics was observed in both mung bean cultivars. Externally applied α-tocopherol had no effect on phenolics in both mung bean cultivars under drought conditions. However, cv. Cyclone 8009 showed better response under drought as well as 100% field capacity (Fig. 1). In contrast, Farouk (2011) showed enhanced accumulation of phenolics due to exogenously applied alpha-tocopherol in wheat plants. Furthermore, Rivero *et al.* (2001) reported enhanced accumulation of phenolics in sunflower plants under abiotic stresses. Rady *et al.* (2011) reported that total phenolics have a significant role in controlling plant metabolic reactions and general plant developmental processes.

Malondialdehyde (MDA) is produced during degradation of polyunsaturated lipids and is considered as a symptom of lipid peroxidation due to severe abiotic stresses (Seckin *et al.,* 2009). During the current study, under water deficit conditions a marked increase was observed in the MDA contents in both mung bean cultivars showing that considerable lipid peroxidation of biological membranes took place. A significant reduction was observed in the levels of MDA because of α-Toc foliage application in both mung bean cultivars under drought and normal watering conditions are related to Jie *et al.* (2008) in *Leymus chinensis* seedlings pretreated with vitamin E, although MDA contents increased under moisture stress, application of vitamin E reduced MDA contents. In another study, Ayad *et al.* (2009) observed that at all levels of stigmasterol and alpha-tocopherol as well as peroxidation of lipids decreased in geranium. These reductions were obvious at 100 or 150 mg L^{-1} of alpha-tocopherol or stigmasterol in all cuttings of geranium.

Total soluble proteins (TSP) contents decreased significantly ($p \leq 0.001$) by drought stress in both mung bean cultivars. Alpha-tocopherol application (foliar) was found to be effective in improving soluble proteins under drought stress conditions. Both cultivars showed a similar response in terms of TSP and results are in good harmony with those of Al-Qubaie (2012) who applied three antioxidants, vitamin E (50 ppm), citric acid (500 ppm) and amino acids (methionine, cystein and tryptophan) at 500 ppm either single/or in different compositions to sunflower plants. Single as well as combined applications of these antioxidants were very effective in improving soluble proteins relative to the check treatment. Similar types of results were reported by Sadak *et al.* (2010) in sunflower plants. They concluded that application of α-tocopherol being antioxidant mitigates the harmful effects of oxidative stress caused under stresses. In the present study, drought stress significantly raised the levels of total free amino acids in both mung bean cultivars and exogenous application of α-tocopherol considerably improved the total free amino acids (Fig. 1). These results are in agreement with the findings of Rady *et al.* (2011) in sunflower, and Sadak *et al.* (2013) in flax plant, where they concluded that abiotic stress acts as an activator for the accumulation of free amino acids. Thus, it can be explained that abiotic stress causes hydrolysis of proteins into free amino acids, and foliar treatment of antioxidants enhances the biosynthesis of free amino acids and their utilization into protein.

Activities of different enzymatic antioxidants such as CAT, superoxide dismutase and POD increased significantly in both mung bean cultivars under water-deficit conditions (Fig. 2). Foliar applied α-tocopherol affected positively the activities of POD and CAT, but did not affect that of SOD antioxidant in both mung bean cultivars under stress and non-stress conditions (Fig. 2). There is very little literature available on the role of alpha-tocopherol in the regulation of different antioxidants in plants under drought stress, except a few reported below, e.g., Jie *et al.* (2008) pretreated *Leymus chinensis* seedlings with vitamin E and subjected them to drought stress and found non-significant effect on the activities of SOD and POD antioxidant enzymes. These results are also in agreement with those of Kostopoulou *et al.* (2014) in citrus seedlings, Orabi & Abdelhamid (2014) in *Vicia faba* under saline stress.

In conclusion, the findings of the current study suggest a beneficial role of exogenously applied TOC particularly in up regulation of the antioxidative potential of mung bean plants which resulted in increased growth under drought conditions.

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