Indian J. Plant Physiol., Vol. 12, No. 2, (N.S.) pp. 133-137 (April-June, 2007)

CHANGES IN ANTIOXIDANT METABOLISM UNDER DROUGHT STRESS IN *VIGNA UNGUICULATA* (L.) WALP.

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Received on 25 July, 2006, Revised 24 May, 2007

SUMMARY

A pot culture experiment was conducted to estimate the effects of drought stress in cowpea (*Vigna unguiculata* (L.) Walp.) plants. From 30 days after sowing (DAS), the plants were subjected to 3, 6 and 9 days interval drought (DID) stress and one day interval irrigation was kept as control. The plant samples were collected on 34 DAS (3 DID), 37 DAS (6 DID) and 40 DAS (9 DID). The plants were separated into root, stem and leaf for estimating the antioxidant contents and activities of antioxidant enzymes. Ascorbic acid (AA), α-tocopherol (α-toc) contents, superoxide dismutase (SOD), ascorbate peroxidase (APX), polyphenol oxidase (PPO) and catalase (CAT) activities were highly influenced under drought stress when compared to control.

Keywords: **Antioxidants, drought, *Vigna unguiculata.***

INTRODUCTION

Oxidative damage in the plant tissue is alleviated by a concerted action of both enzymatic and non-enzymatic antioxidant metabolisms (Hasegawa *et al.* 2000). These mechanisms include β-carotenes, ascorbic acid (AA), α-tocopherol (α-toc), reduced glutathione (GSH) and enzymes including superoxide dismutase (SOD), peroxidase (POX), ascorbate peroxidase (APX), catalase (CAT), polyphenol oxidase (PPO) and glutathione reductase (GR) (Prochazkova *et al.* 2001). There are many reports in the literature that underline the intimate relationship between enhanced antioxidant enzyme activities and increased resistance to environmental stresses (Vranova *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). Although the general effects of drought on plants are fairly well known, the primary effects of water deficit at the biochemical and molecular levels are not well understood (Chaitanya *et al.* 2003, Chaves *et al.* 2002). AA and a-toc are two important antioxidants in higher plants (Foyer 1993). They are concentrated in the chloroplasts and the cytosol and protect the photosynthetic apparatus under stress by scavenging excess ROS (Smirnoff 1995).

Water is one of the most important environmental factors that regulate plant growth and development. Among the diverse consequences of drought effect on plant development, restricted nutrient and water acquisition are commonly recognized (Agnew and Warren 1996). Increasing evidences suggests that drought induces oxidative stress in various plants, in which reactive oxygen species (ROS), such as superoxide radical ($O_2^{\cdot-}$), hydroxy radical ($^{\cdot}OH$), hydrogen peroxide (H_2O_2) and alkoxy radical ($RO^{\cdot}$) are produced (Munne-Bosch and

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Penuelas 2003). The toxic superoxide radical has a half-life of less than one second and is usually rapidly dismutated by SOD to H_2O_2, a product which is relatively stable and can be detoxified by catalase and peroxidases. These metalloenzymes constitute an important primary defense of cells against superoxide free radicals generated under stress conditions and thereby increased SOD activity is known to confer oxidative stress tolerance (Slooten *et al.* 1995).

Pulses gained importance in the global agriculture for their high protein content and also for their inherent capability of fixing atmospheric nitrogen through symbiotic bacteria (Ravichandran and Pathmanabhan 2004). In the post green revolution period, water stress problem is a major concern affecting the agriculture production. Generally pulses are very susceptible to water stress (Agele *et al.* 2006). *Vigna unguiculata* (L.) Walp. is one of the important crop species and belong to the subfamily Papilionaceae of the family Leguminosae. Comparatively, a little work has been reported on water stress problems and drought stress injuries in this plant. So in the present investigation, an attempt has been made to evaluate the drought stress affects on antioxidant molecules like AA, α-toc and antioxidant enzymes like SOD, APX, PPO and CAT in drought stressed *V. unguiculata* plants in pot culture.

MATERIALS AND METHODS

The seeds of *Vigna unguiculata* (L.) Walp. were obtained from Pulses Research Division, Rice Research Institute, Tamil Nadu, India. Seeds were surface sterilized with 0.2 per cent $HgCl_2$ solution for 5 minutes with frequent shaking and thoroughly washed many times with deionized water to remove $HgCl_2$. Six seeds were sown in each pot of 30 x 30 cm containing 3 kg of soil mixture composed of red soil, sand and the farmyard manure (FYM) at 1:1:1 ratio. All the pots were watered to the field capacity with ground water upto 30 days after sowing (DAS). The seedlings were thinned to 2 pot^{-1} on 20 DAS. Pots were irrigated with ground water at one day interval and kept as control and 3, 6 and 9 days interval drought (DID) was given to other plants up to 30 DAS. Plants were uprooted randomly on 34, 37 and 40 DAS, washed carefully and separated into root, stem and leaf for estimating antioxidant contents and antioxidant enzyme activities.

Ascorbic acid (AA) content was assayed as described by Omaye *et al.* (1979) and expressed in mg g^{-1} dry weight (DW). α-Tocopherol (α-toc) activity was assayed as described by Backer *et al.* (1980) and expressed in mg g^{-1} fresh weight (FW).

Crude enzyme extract was prepared for assay of SOD by the method of Hwang *et al.* (1999). The enzyme protein was determined (Bradford 1976) for all the three enzymes for expressing the specific activity of enzymes. SOD activity was assayed according to Beauchamp and Fridovich (1971). SOD activity was expressed in units (U mg^{-1} protein). One U is defined as the amount of change in the absorbance by 0.1 hr^{-1} mg^{-1} protein. Ascorbate peroxidase (APX) activity was determined according to Asada and Takahashi (1987). The enzyme activity was expressed in U mg^{-1} protein (U = change in 0.1 absorbance min^{-1} mg^{-1} protein). Polyphenol oxidase (PPO) activity was assayed by the method of Kumar and Khan (1982). PPO activity is expressed in U mg^{-1} protein (U = change in 0.1 absorbance min^{-1} mg^{-1} protein). Catalase (CAT) was measured according to Chandlee and Scandalios (1984). The enzyme activity was expressed in U mg^{-1} protein (U = 1 mM of H_2O_2 reduction min^{-1} mg^{-1} protein).

The experiment culture was carried out in Completely Randomized Design (CRD). Each treatment was analysed with atleast seven replicates and a standard deviation (SD) was calculated. The data were expressed in mean ± SD of 7 replicates.

RESULTS AND DISCUSSION

The drought stress increased the antioxidant AA (Fig. 1a) and α-toc (Fig. 1b) content in all parts of the cowpea when compared to control. Efficient destruction of O_2^{1-} plant cells requires the concerted action of antioxidants. Among the non-enzymatic antioxidants, AA is found to be one of the best characterized compounds, required for many key metabolic functions in plant cells (Smirnoff and Wheeler 2000). AA acts as an antioxidant, protecting cells against oxidative stress. AA has the capacity to

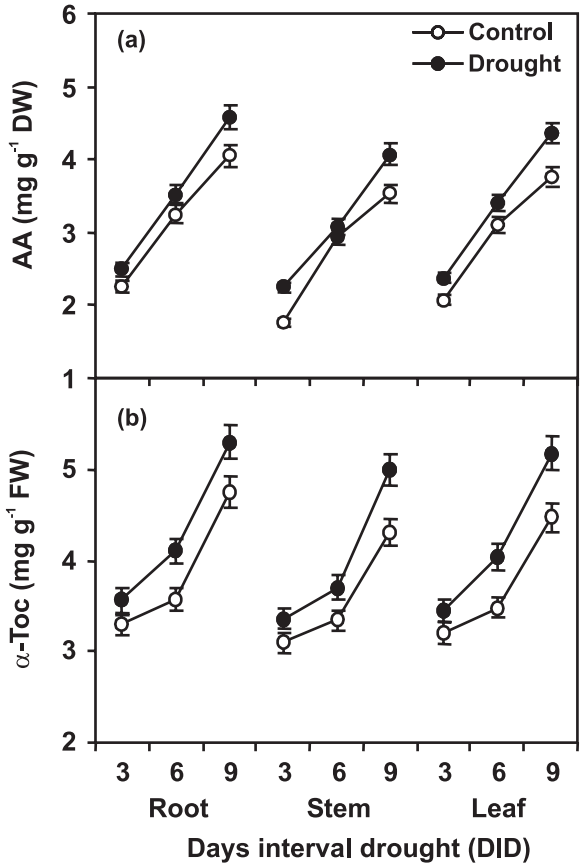

Fig. 1. Effects of drought on (a) AA and (b) α-toc contents of *V. unguiculata* plants. Values are mean ± SD of 7 replicates

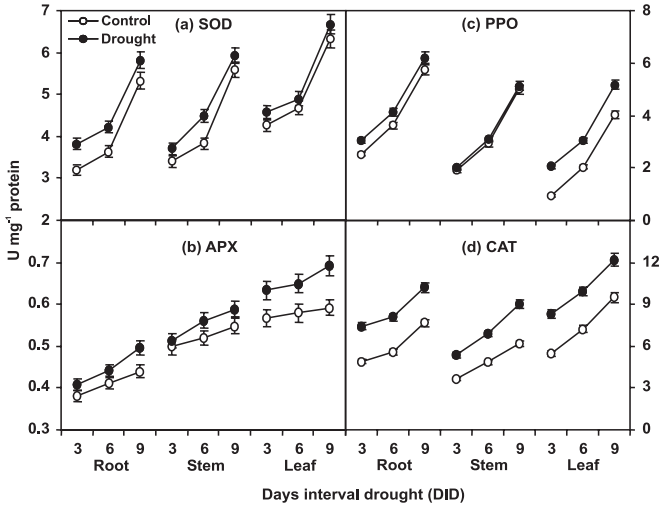

Fig. 2. Effects of drought on (a) SOD, (b) APX, (c) PPO and (d) CAT activities of *V. unguiculata* plants. Values are mean ± SE of 7 replicates

eliminate different AOS including singlet oxygen, superoxide and hydroxyl radicals (Foyer 2001). α-Toc is synthesized in the chloroplasts and closely associated with the thylakoid membrane of the chloroplasts. The thylakoid membrane, which contains substantial unsaturated lipids, is one of the major sites of oxidative damage through lipid peroxidation. Perl *et al.* (1993) noted that water stress causes an accumulation of ROS in the chloroplasts. This may result in an increase of α-toc which quenches oxygen radicals with in the membrane and terminates chain reaction that cause oxidative damage.

SOD activity increased in all the DID stress. It was reported that SOD enhance water stress tolerance to plants. In tomato cytosolic Cu/Zn-SOD was induced strongly by drought, while Cu/Zn-SOD remained largely unaffected (Bowler *et al.* 1992). APX activity increase in all the drought treatment when compared to control. Asada (1992) reported that the APX found in organelles is believed to scavenge H_2O_2 produced from the organelles, whereas the function of cytosolic APX is probably to eliminate H_2O_2 that is produced in the cytosol or apoplast and that which has diffused from organelles. In the chloroplast, H_2O_2 can be detoxified by the ASA-GSH-NAPDH system that has been catalyzed by APX (Mehlhorn *et al.* 1995). The CAT activity was increased in drought. This result is in accordance with the findings in wheat (Shao *et al.* 2005) and *Phaseolus acutifolius* (Tukan *et al.* 2005). The combined action of CAT and SOD converts the toxic O_2^{1-}, H_2O_2 to water and molecular oxygen, averting the cellular damage under unfavourable conditions like water stress (Reddy *et al.* 2004, Chaitanya *et al.* 2002). PPO activity increased in drought when compared to control. ROS scavenging is important in imparting tolerance against oxidative stress. It may be presumed that enhancement of the antioxidative system favours water stress resistance (Nocter *et al.* 2000). Thus from these results, it is clear that, plants under drought stress are highly regulated by components of the antioxidative system.

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