Journal of Environmental Biology
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September 2008, 29(5) 689-691 (2008)
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Studies on the changes in lipid peroxidation and antioxidants in drought stress induced cowpea (*Vigna unguiculata* L.) varieties

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(Received: April 07, 2006; Revised received: March 07, 2007; Accepted: April 02, 2007)

Abstract: *Cowpea (Vigna unguiculata L.) is one of the major vegetable crops cultivated in tropical conditions. Two varieties of cowpea, i.e., Vigna unguiculata L.cv Kanakamony and cv Pusakomal were selected for the present study. The changes in lipid peroxidation, ascorbic acid content and activities of enzymatic antioxidants associated with drought stress were determined. A high level of lipid peroxidation was observed in test plants subjected to water stress. The Pusakomal leaves with severe stress showed 2.7 fold increase in TBARS compared to control and 1.2 fold to that of Kanakamony. The drought tolerant variety showed significant increase in the activities of peroxidase and catalase on exposure to drought stress. Kanakamony leaves in severe stress possess 1.09 times increase in peroxidase and 1.8 times increase in catalase activity than Pusakomal. The concentration of ascorbic acid in test plants depleted with increased drought stress in both varieties. While the leaves of tolerant variety in severe stress showed 1.13 times higher ascorbic acid content than Pusakomal. Among the two varieties, Kanakamony possess the best antioxidant system to tolerate drought stress. This could limit cellular damage caused by active oxygen species, during water deficit. The variety Pusakomal was relatively poor in these adaptations.*

Key words: *Antioxidants, Catalase, Drought stress, Lipid peroxidation, Peroxidase, Cowpea*
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Introduction

Drought stress induced free radicals cause lipid peroxidation and membrane deterioration in plants. Drought stress leads to an imbalance between antioxidant defenses and the amount of active oxygen species (AOS) resulting in oxidative stress. AOS are necessary for inter and intracellular signaling, but at high concentration can cause damage at various levels of organization including chloroplasts (Smirnoff, 1993). These AOS have the capacity to initiate lipid peroxidation and degrade proteins, lipids and nucleic acids (Hendry, 1993). Mechanism of retardation of lipid peroxidation consists of free radical scavenging enzymes such as catalase, peroxidase and superoxide dismutase (Fridovich *et al.*, 2000). A number of enzymatic and non-enzymatic antioxidants are present in chloroplasts that serve to prevent AOS accumulation (Srivalli *et al.,* 2003). This study was conducted to assess the extent of lipid peroxidation, the changes in the activities of enzymatic antioxidants and the concentration of non enzymatic antioxidant, ascorbic acid in cowpea varieties under water stress.

Materials and Methods

The seeds of cowpea varieties *Vigna unguiculata* L.cv Pusakomal (PL) and cv Kanakamony (KY) were collected from National Seeds Corporation, Trivandrum and College of Agriculture, Vellayani, Trivandrum respectively. Earthen pots (20x30x40 cm) were filled with soil mixture containing garden soil, sand and cow dung in the ratio 1:1:1. Six seeds of uniform size were sown in each pot. Fourteen days after sowing, plants with same height and number of leaves were divided into three groups (Group I, II and III). The mature plants in the flowering stage in each group were divided into test and control plants. The control plants in each group were maintained with proper irrigation (zero stress); while the test groups were subjected to mild (3 days), moderate (6 days) and severe (9 days) drought stress. The pots were covered with aluminum foil and plastic sheets to avoid evaporation during the stressed period.

Lipid peroxidation was measured as the amount of thiobarbituric acid reactive substance (TBARS) and it was determined by the method of Buege and Aust (1978). The activities of antioxidant enzymes – peroxidase (EC1.11.1.7) (Chance and Maehly, 1955), catalase (EC1.11.1.6) (Aebi, 1984) and the concentration of non-enzymatic antioxidant, ascorbic acid (Sadasivam and Manickam, 1996) were determined by standard methods using an UV-visible spectrophotometer (Systronics, India). One unit of catalase activity corresponded to the amount of enzyme that decomposes 1 μ mole of H_2O_2/min/g fresh wt. One unit of peroxidase is the amount of enzyme required to oxidize one μ mole of guaiacol by H_2O_2 at test condition.

Statistical analysis: All the data were statistically analyzed by ANOVA. Statistical significance ($p<0.05$) of the means were determined by Duncan's multiple range test using SPSS software (Windows, 2000).

Results and Discussion

The changes in TBARS content, the activities of peroxidase, catalase, and the concentration of ascorbic acid in the leaves and roots of control and water stressed cowpea varieties studied were shown in Fig. 1 to 4 respectively. Lipid peroxidation increased with drought stress in both varieties; but the Kanakamony accumulates less TBARS compared to Pusakomal. Under water stress, the formation of AOS increased and the antioxidant system protects the

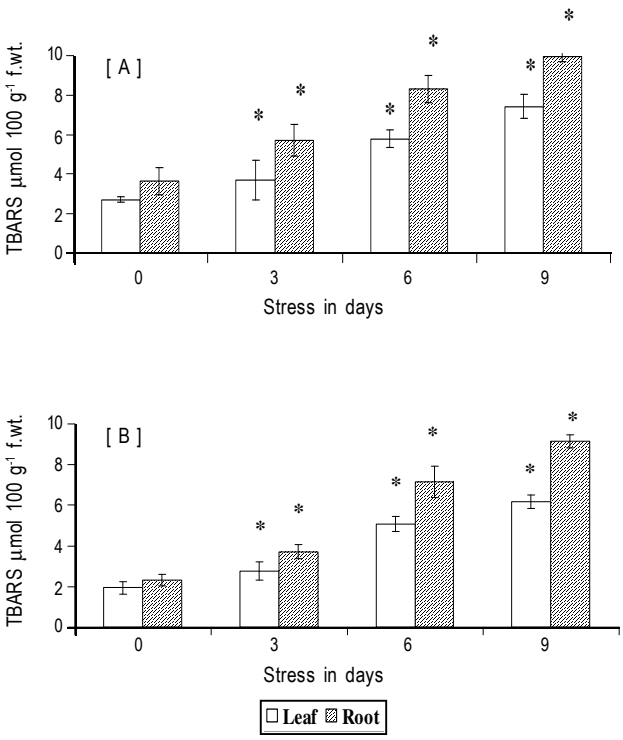

Fig. 1: Changes in TBARS content in leaves and roots of (a) Pusakomal (b) Kanakamony. Values are mean of four replicates ± SD and if significantly different from control groups, were indicated with asterisks (*)

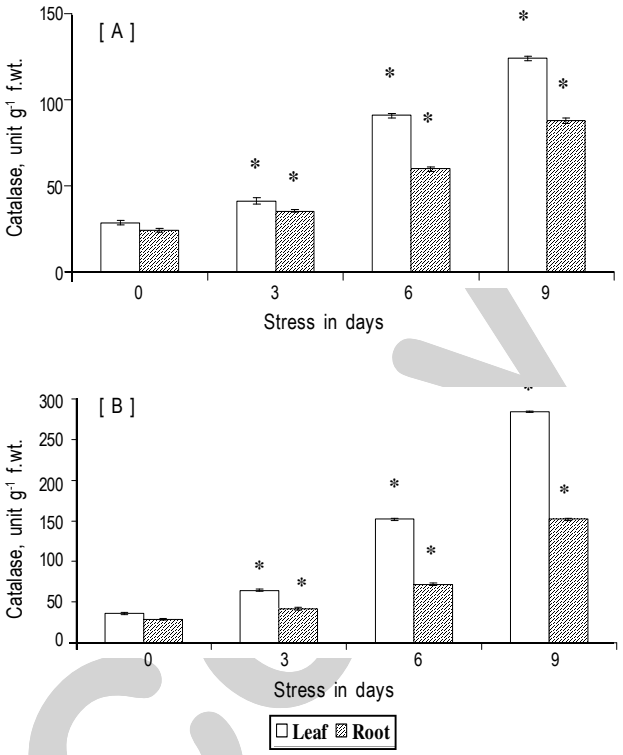

Fig. 3: Changes in the activity of catalase in leaves and roots of (a) Pusakomal (b) Kanakamony. Values are mean of four replicates ± SD and if significantly different from control groups, were indicated with asterisks (*)

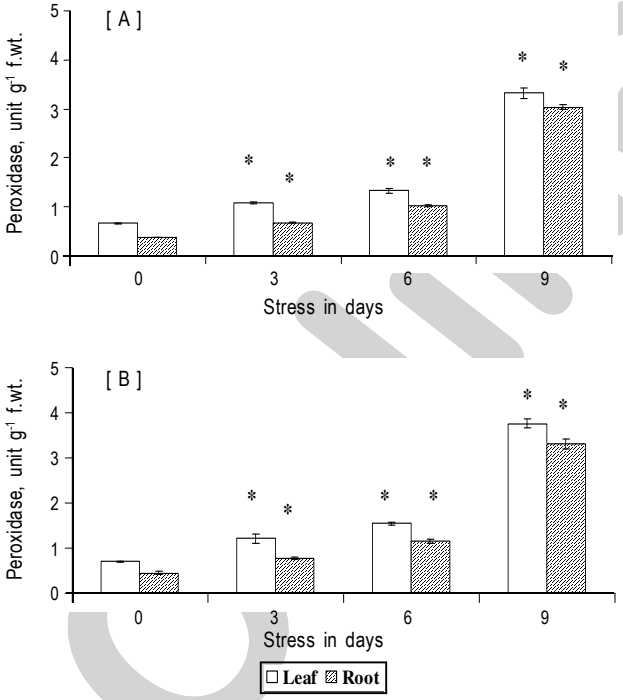

Fig. 2: Changes in the activity of peroxidase in leaves and roots of (a) Pusakomal (b) Kanakamony. Values are mean of four replicates ± SD and if significantly different from control groups, were indicated with asterisks (*)

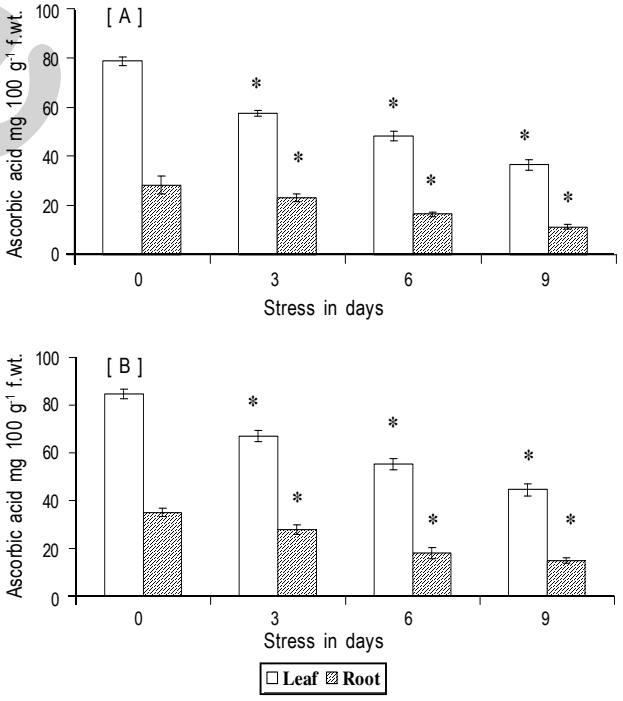

Fig. 4: Changes in ascorbic acid content in leaves and roots of (a) Pusakomal (b) Kanakamony. Values are mean of four replicates ± SD and if significantly different from control groups, were indicated with asterisks (*)

cell by controlling the intracellular AOS concentration. One of the expected consequences of water stress induced cellular build up of AOS is an increase in lipid peroxidation.The assay of cellular accumulation of lipid peroxidation products in the form of TBARS can provide a comparative indication of such activity. The peroxidation of lipids in the cell membrane is one of the most damaging cellular responses observed in response to water stress (Thankamani *et al.,* 2003). The amount of lipid peroxidation has also long been considered as one of the factors, which indicate the severity of stress experienced by a plant (Chowdhury and Chowdhury, 1985). Drought induced changes in lipid peroxidation and the activity of antioxidant enzyme, catalase in drought tolerant moss *Tortula ruralis* showed lower level of lipid peroxidation together with increased level of catalase enzyme than the drought sensitive *Cratoneuron filicinum* (Arora *et al.,* 2002).

Activity of the antioxidant enzymes like peroxidase and catalase increased significantly in both varieties with increased water stress. Drought tolerance or sensitivity of plants is well correlated with their antioxidant response. In general, tolerant varieties have a better capacity to protect themselves from drought induced oxidative stress by maintaining high antioxidant molecules and an enhancement in antioxidant enzyme activity under stress conditions. The peroxidase and catalase activity in Kanakamony is more than Pusakomal. The increase in activities of these enzymes, which are having protective roles, indicate the biochemical adaptability of this variety to tolerate water stress. Peroxidase activity increased significantly in the leaves of drought tolerant soybean cultivars exposed to drought stress, but decreased in case of drought sensitive cultivar; suggesting that peroxidase played an important role in drought stressed plants (Zheng and Han, 1997). Ground nut plants subjected to water stress showed an increased peroxidase activity (28%) than the corresponding controls (Reddy *et al*., 2003). Similar studies on the catalase activity were found to be much higher in leaves than in roots of lentil, under control and stress conditions (Ebru *et al.,* 2004). Catalase activity also has been found to increase under salt stress in wheat genotypes (Sairam *et al.,* 2002).

L-Ascorbic acid functions as an antioxidant, an enzyme cofactor, and a precursor for oxalate and tartarate synthesis in plants. It participates in a variety of processes including photosynthesis, cell cycle, cell wall growth, cell expansion, synthesis of ethylene, gibberellins, anthocyanins, hydroxyprolines and is resistant to environmental stresses (Noctor and Foyer, 1998). Ascorbic acid functions as an antioxidant by several mechanisms (Horemans *et al.,* 2000). In our present study, as the water stress level increases, the ascorbic acid content found significantly decreased in the leaves and roots of both varieties. Ascorbic acid is shown to inhibit peroxy radicals to produce H_2O_2 and the triketo derivative, dehydro ascorbic acid is formed (Halliwell, 1982). It was also found that leaves of both varieties have more ascorbic acid content than in roots. The ascorbic acid content in the leaves and roots of Kanakamony variety is high compared to that of Pusakomal.

The extent of lipid peroxidation in Pusakomal is much high compared to Kanakamony variety. The catalase (CAT) and peroxidase (POD) activities in Kanakamony variety increased significantly with increased levels of water stress, while in both the varieties of cowpea, the ascorbic acid content decreased significantly with increased levels of water stress. These results suggest that the drought stress caused critical changes in lipid peroxidation leading to modifications in the activities of enzymatic and content of non enzymatic antioxidants in both the varieties of cowpea plants.

Acknowledgments

The financial support as JRF to Ms Akhila S. Nair by Kerala State Council for Science, Technology and Environment is gratefully acknowledged.

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