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SHORT COMMUNICATION

OXIDATIVE STRESS AND THE ACTIVITY OF ANTIOXIDANT ENZYMES IN MUNGBEAN SEEDLING SUBJECTED TO WATER STRESS

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The effect of PEG induced moisture stress during seedling development on oxidative stress and antioxidant enzymes activities were studied in mungbean (*Vigna radiata* L. Wilczek) using two cultivars namely tolerant K 851 and susceptible PDM 84-139 respectively. Water stress increased H_2O_2 content and TBARS content (lipid peroxidation) as well as superoxide dismutase and peroxidase activities in both the cultivars compared to control. However, catalase activity decreased under water stress in both the cultivars tested. Tolerant genotype K 851 showed lower H_2O_2 and TBARS content and higher activity of antioxidant enzymes like SOD, peroxidase and catalase than susceptible PDM 84-139 in response to water stress.

Key words: **Antioxidant, mungbean seedling, oxidative stress, water stress.**

Mungbean (*Vigna radiata* L. Wilczek) is an important grain legume, which is predominantly cultivated as rainfed crop under Indian scenario. In India, rainfall during monsoon is unpredicted, as a result this crop suffers from drought stress at different growth stages. Drought stress affects many physiological processes of plants leading to accumulation of reactive oxygen species (ROS) like superoxide radical ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2), hydroxyl radical ($OH^{\cdot}$) etc. (Morgan 1984, Thompson *et al*. 1987). These ROS inhibit protein synthesis by oxidation of mRNA (Corcuera *et al*. 1989). Therefore, plant must adapt certain mechanism to scavenge these reactive oxygen species. Tolerance to drought stress in plants has been reported to be associated with an increase in antioxidant activity (Badiani *et al*. 1990, Sairam *et al*. 2001). Therefore, the present experiment was conducted with two mungbean cultivars to study the effect of moisture stress on antioxidant systems and to analyse the significance of these systems in imparting drought tolerance to mungbean cultivars during seedling development.

Mungbean (*Vigna radiata* L Wilczek) cultivars K 851 (tolerant to drought) and PDM 84-139 (susceptible to drought) were selected from a laboratory screening with sixteen mungbean cultivars collected from the Project Coordinator, All India Coordinated Research Project on MULLaRP, Indian Institute of Pulses Research, Kalyanpur, Kanpur, UP, India. Seeds of uniform size were surface sterilized with 0.1% (w/v) $HgCl_2$ for two minutes and then washed thoroughly with glass distilled water. A range of four external water potentials (*viz.* –1.0, –2.0, –3.0 and –4.0 bars) were prepared by using polyethylene glycol (PEG) 6000 as per method of Michael and Kaufmann (1973). Surface sterilized seeds of mungbean cultivars were soaked in different PEG solutions separately for 5 hours. Pre-soaked seeds were then allowed to develop seedlings in

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respective PEG solutions for 6 days under indoor laboratory conditions following standard glass plate technique (Nandi and Bera 1995). Surface sterilized seeds treated similarly with glass distilled water served as control (0.0). After 2, 4 and 6 days seedlings were removed from glass plate and biochemical analyses were done on respective days.

Hydrogen peroxide was measured according to Teranishi *et al*. (1974). Lipid peroxidation was measured in terms of thiobarbituric acid reactive substances (TBARS) content following the method of Heath and Packer (1968). Enzyme (superoxide dismutase and catalase) extract was prepared by homogenizing plant material (0.2 g) with 5 ml of ice-cold phosphate buffer (0.1 M, pH 7.5) containing 0.5 mM EDTA followed by centrifugation at 4^0C for 15 min at 15,000 g in a refrigerated centrifuge. The supernatant was referred to as the source of enzyme. Superoxide dismutase (SOD) activity was estimated according to the method of Dhindsa *et al.* (1981). Catalase (CAT) activity was assayed according to Teranishi *et al.* (1974). Peroxidase (POX) extraction was done by homogenizing freshly harvested plant samples (0.2 g) with 3 ml 0.05 Tris- HCl buffer (pH 8.0) followed by centrifugation at 4^0C for 15 min at 10,000 g. The peroxidase activity was assayed according to the method of Lowenstein and Linsey (1961). All the observations are means of three replications repeated twice. Data were analysed by analysis of variance to determine the significance of treatments and cultivars.

Water stress at all the three stages (2, 4 and 6 days) significantly increased hydrogen peroxide content (Fig.1A) in both parts of the seedlings of the two cultivars studied. Hydrogen peroxide content was found to increase with the progress of time in both mungbean cultivars. Out of two cultivars tested, K 851 accumulated less H_2O_2 than PDM 84-139. The increase in H_2O_2 content under water stress might be due to a shift of cellular environment from a reductive to oxidative state under such conditions. Many studies in recent years established that water stress and other stresses induce generation of superoxide radical, hydrogen peroxide and hydroxyl radical as the major cause of stress induced injury experienced at cellular and at crop level (Baisak *et al.* 1994).

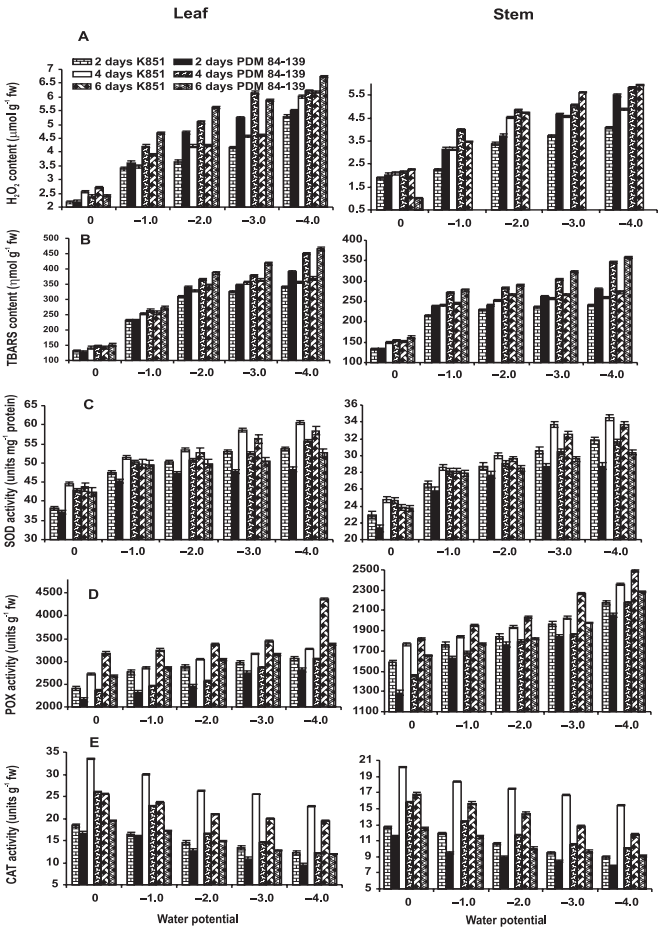

Fig. 1. Effect of different moisture stresses on H_2O_2 (A), TBARS (B) contents, SOD (C), POX (D) and CAT (E) activities in leaf and stem of 2, 4 and 6 days old seedlings of two mungbean cultivars. Vertical bars represent ±SE of mean of 3 replicates of 2 independent experiments

Lipid peroxidation as TBARS content increased under water stress in both parts of the seedlings and also with seedling age in both the cultivars studied (Fig.1B). PDM 84-139 showed higher TBARS content compared to K 851 in all water stress treatments. Lipid peroxidation is a consequence of generation of reactive oxygen species (ROS) or oxidative stress induced under water deficit conditions. Many workers reported that the increase in lipid peroxidation is associated with generation of reactive oxygen species (ROS) as a result of oxidative stress (Baisak *et al.* 1994, Sairam *et al.* 1998). The results of increasing TBARS content with the increase in age of the seedlings are in accordance with Sulochana *et al.* (2002). The lower accumulation of H_2O_2 in tolerant genotype K 851 is correlated with lower

TBARS content as compared to PDM 84-139. Sairam and Srivastava (2001) observed higher H_2O_2 accumulation along with greater lipid peroxidation in susceptible wheat genotypes than in tolerant ones.

An increase in superoxide dismutase (SOD) activity in both parts under water stress was noticed in both the genotypes studied upto 4th day and decreased thereafter on 6th day (Fig.1C). Peroxidase activity was also found to increase at all the three days and in both parts of the two genotypes studied under water stress (Fig.1D). Unlike superoxide dismutase (SOD) and peroxidase, catalase activity decreased significantly in both genotypes at all stages of study under increasing magnitude of water stress (Fig.1E). Peroxidase activity in both leaf and stem of two cultivars increased with the progress of time throughout the growing period but catalase activity was found to reach maximum value on 4th day in most of the cases for both cultivars and then decreased. All the three enzymes activity showed higher level in tolerant genotype K 851 than PDM 84-139. It was also found that varietal differences in all the three antioxidant enzymes were greater at later developmental stages particularly in leaves.

Superoxide dismutase (SOD) is associated with scavenging of superoxide radical ($O_2^{\cdot-}$) resulting in the formation of hydrogen peroxide (Fridovich 1986). Sairam *et al.* (2001) have reported that water stress tolerance is associated with the increase in SOD activity. The results of the increase in SOD activity during early period of drought and decrease thereafter are in agreement with Zhang and Kirkham (1994). Peroxidase and catalase scavenge the hydrogen peroxide, which accumulates in the plants under water stress. The increase in peroxidase activity might be due to formation of large amount of H_2O_2 during water stress whereas the decrease in catalase activity under water stress could be due to inhibition of protein synthesis. An increase in peroxidase activity under drought and with the progress of time of radical emergence was observed by Cakmak *et al*. (1993) and Zhang and Kirkham (1994). Sulochana *et al.* (2002) reported that catalase activity in both cotyledon and embryonic axis in groundnut decreased during seedling development under PEG induced moisture stress. However, higher levels of peroxidase as well as catalase in tolerant K 851 ensure better scavenging of H_2O_2 than PDM 84-139 under moisture stress. It seems that scavenging of H_2O_2 as represented by peroxidase and catalase is limiting in susceptible cultivar PDM 84-139, leading to higher accumulation of H_2O_2, increased lipid peroxidation and consequently more injury during the stress.

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