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Polyethylene glycol induced water stress in maize seedlings and evaluation of antioxidant defense mechanisms

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Abstract
Maize is one such crop, the production of which is highly challenged due to water shortage and soil water losses. The present study was undertaken on artificially induced water stress of maize *in vitro*, where stress was applied with PEG-6000 on one week old seedlings of four varieties BN 10, Dhanya, Kaveri-Super 244, and Swarna for 3, 5 and 7 days . The activity of antioxidative enzyme like peroxidase, catalase, ascorbate peroxidase, glutathione reductase and superoxide dismutase was assayed in the stressed and control plants. Peroxidase activity decreased on the 7th day in Dhanya and Swarna but in BN 101 and Super 244 the activity decreased slightly on the 5th day and increased again on the 7th day. Ascorbate peroxidase and superoxide dismutase showed a similar trend where the activity decreased after a certain period of stress. Similar trend was seen for GR activity too in case of Dhanya and Swarna. But in BN 101 and Super 244 there was an increase in the activity with the increase in the period of stress. Catalase activity declined during stress in Dhanya and Swarna while the other two varieties showed an increase during stress. Other than enzymatic activities, various biochemical analyses like proline, ascorbate, chlorophyll was also carried out. With the increase in intensity of drought there was an increase in both proline and ascorbate content in all. A significant increase in the ascorbate content was observed in BN 101 and super 244. H_2O_2 accumulation and lipid peroxidation showed an increase during stress in Dhanya and Swarna but no increase was seen in the other two varieties. Chlorophyll content showed a decline during the period of drought when compared to the control plants of all varieties. Enzymatic activity and biochemical tests show that Dhanya and Swarna are susceptible to drought stress than super 244 and BN 101 which are the tolerant varieties.

Keywords: maize, drought, antioxidant, proline, lipid peroxidation, H_2O_2.

Maize (*Zea mays* L.) is a major world crop and its productivity is greatly constrained by drought (Zinselmeier *et al*., 2002). Water stress induces several physiological, biochem-ical and molecular responses in several crop plants, which would help them to adapt to such limiting environmental conditions (Bajaj *et al*.,1999; Arora *et al*., 2002). Drought is a worldwide problem constraining plant production (Chinnusamy *et al*., 2004) and is prone to acute periods due to little rainfall or an imbalanced distribution of rainfall in growing seasons as the environment deteriorates. Water deficit (commonly known as drought) can be defined as the absence of adequate moisture necessary for normal plant grow and to complete the life cycle (Zhu 2002). Plants can respond and adapt to water stress by altering their cellular metabolism and invoking various defense mechanisms (Zhu 2002, Boudsocq and Laurie're 2005).The lack of adequate moisture leading to water stress is common occurrence in rain fed areas, brought about by infrequent rains and poor irrigation (Wang *et al*., 2005).Proline and quaternary ammonium compounds, e.g. glycinebetaine, choline, prolinebetaine are key osmolytes contributing towards osmotic adjustment (Huang *et al*., 2000 and Kavikishore *et al*., 2005). One of the most important responses of plants to drought and other abiotic stresses is an overproduction of different types of compatible solutes (Ashraf and Harris, 2004; Serraj and Sinclair, 2002). Of these solutes, proline is widely distributed in plants and it accumulates in larger amounts than other amino acids in drought stressed plants (Ashraf, 2004; Irigoyen et al., 1992; Kohl *et al*., 1991). Free proline and sugar contents significantly increased in *Vigna radiata* nodules under drought, but nodules had more proline than leaves (Hooda *et al*., 1999).Great efforts have been made to decipher the molecular mechanisms of plant drought tolerance (Bartels and Nelson 1994 Bohnert 2000 Munns 2002 Ciais *et al*., 2005 Mahajan and Tuteja 2005).It inhib-its the photosynthesis of plants, causes changes of chloro-phyll contents and components and damage to the photo-synthetic apparatus (Escuredo *et al*., 1998). When plants are subjected to drought stress, a variety of active oxygen species are generated, such as superoxide, H_2O_2 and hydroxyl radicals, which cause damage in plants. They are toxic to living organisms and, unless removed rapidly, they destroy or inactivate various cellular components (Smirnoff 1993, Trippi *et al*., 1989).

Considering the importance of maize cultivation in this region, the present study was undertaken to determine

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how four varieties respond to water stress in terms of over expression of antioxidant enymes or other biomolecules.

Materials and Methods

Induction of water stress

Four maize varieties - BN 101, Kaveri-244 Super, Dhanya and Swarna were selected for experimental purposes. For induction of water stress, initially, seeds were soaked over night and surface sterilized with 0.1% $HgCl_2$ after which they were transferred to autoclaved petriplates in the laminar flow. The seeds were kept in petriplates and grown *in vitro* for a week. After a week the seedlings were subjected to drought stress by application of PEG 6000 and various biochemical tests were performed on the 3rd, 5th and 7th day of stress along with the control plants.

Preparation of enzyme extract

The leaves collected from treated and control plants were ground to fine powder with a mortar and pestle under liquid nitrogen in cold 50 mM sodium phosphate buffer, pH 7.5, containing 1% (w/v) polyvinylpolypyrrolidone. The homogenate was then centrifuged at 10,000 rpm for 20 min at 4°C. The supernatant was directly used as crude extract for enzyme assays.

Assay of activities

Peroxidase (POX: EC. 1.11.17)

Peroxidase activity was assayed spectrophotometrically in UV VIS spectrophotometer (Model 118 SYSTRONICS) at 460 nm by monitoring the oxidation of O-dianisidine in presence of H_2O_2 (Chakraborty *et.al.*, 1993). Specific activity was expressed as ΔA_{460} mg protein $^{-1}$ min $^{-1}$.

Ascorbate peroxidase (APOX: EC.1.11.1.11)

Activity of ascorbate peroxidase was assayed as decrease in absorbance by monitoring the oxidation of ascorbate at 290 nm according to the method of Asada and Takahashi (1987) with some modification. Enzyme activity was expressed as ΔA_{290} mg protein $^{-1}$ min $^{-1}$.

Catalase (CAT: EC.1.11.1.6)

Catalase activity was assayed as described by Beers and Sizer (1952) by estimating the breakdown of H_2O_2 which was measured at 240 nm in a spectrophotometer. The enzyme activity was expressed as ΔA_{245} mg protein $^{-1}$ min $^{-1}$.

Superoxide dismutase (SOD: EC 1.15.1.1)

Activity was assayed by monitoring the inhibition of the photochemical reduction of NBT according to the method of Dhindsa *et al.*, (1981) with some modification. The absorbance of samples was measured at 560 nm and 1 unit of activity was defined as the amount of enzyme required to inhibit 50% of the NBT reduction rate in the controls containing no enzymes.

Glutathione reductase (GR: EC 1.6.4.2)

Glutathione reductase activity was determined by the oxidation of NADPH at 340 nm as described by Lee and Lee (2000). Enzyme activity was expressed as µM NADPH oxidized mg protein^{-1} min^{-1}.

Protein contents in each case were determined by Lowry's method.

Lipid peroxidation

Lipid peroxidation was measured as MDA determined by the thiobarbituric acid (TBA) reaction. Cells (0.25 g) were homogenized in 2 ml of 0.1% (w/v) trichloroacetic acid (TCA). The homogenate was centrifuged at at 10,000 rpm for 10 min. To 0.5 ml of the aliquot of the supernatant, 2ml of 20% TCA containing 0.5% (w/v) TBA were added. The mixture was heated at 95 °C for

Table1: H_2O_2 accumulation and lipid peroxidation in maize seedling following water stress

Stress period (days)	Varieties	H_2O_2[a]	Lipid[b]
0	DHANYA	02.19	0.007
3		06.44	0.013
5		10.59	0.020
7		17.90	0.028
0	SWARNA	02.67	0.014
3		06.47	0.018
5		12.15	0.020
7		13.44	0.035
0	BN 101	01.87	0.008
3		04.19	0.006
5		06.24	0.012
7		05.25	0.006
0	SUPER244	02.89	0.009
3		06.20	0.011
5		07.55	0.011
7		05.43	0.009

[a]H_2O_2 content (µM/g tissue); [b]Lipid peroxidation (µM MDA/g tissue). All values are average of 3 replicates

Table 2: Proline content in leaf and root of four maize varieties following water stress

Stress period (days)	Varieties	Proline[a]	Proline[b]
0	DHANYA	0.09	0.44
3		0.28	0.28
5		0.38	0.56
7		0.84	0.84
0	SWARNA	0.23	0.23
3		0.25	0.25
5		0.30	0.34
7		0.70	0.60
0	BN 101	0.88	0.75
3		1.50	1.25
5		1.88	1.63
7		2.63	2.50
0	SUPER244	0.75	0.35
3		1.25	1.40
5		1.60	1.58
7		2.48	2.03

[a]Proline content (mg/g tissue) in leaf; [b]Proline content (mg/g tissue) in root. All values are average of 3 replicates

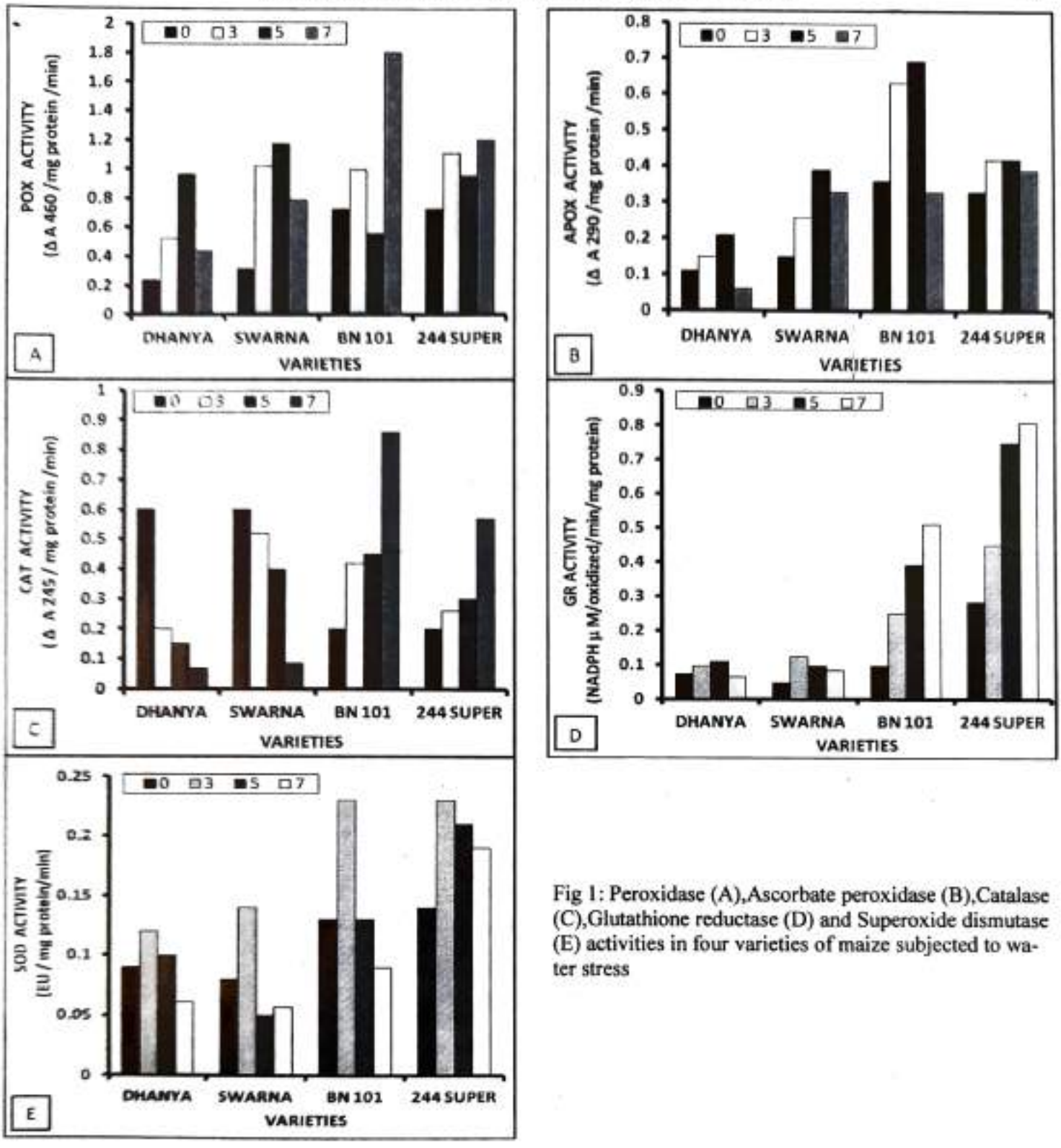

Fig 1: Peroxidase (A),Ascorbate peroxidase (B),Catalase (C),Glutathione reductase (D) and Superoxide dismutase (E) activities in four varieties of maize subjected to water stress

30 min and then quickly cooled on ice. The absorbance was measured at 532 nm and 600. The concentration of MDA was calculated using an extinction coefficient of 155 mM^{-1}. (Heath and Packer, 1968).

Estimation of H_2O_2

H_2O_2 was extracted and quantified content was measured according to the method of Jena and Chowdhuri (1981).

Ascorbate

Ascorbate was extracted and estimated by following the method of Mukherjee and Choudhuri (1983). The concentration of ascorbate was calculated from a standard curve plotted with known concentration of ascorbic acid.

Chlorophyll

Total chlorophyll content was estimated by following the method of Harborne (1973). Extraction was done in 80% acetone and the extract was filtered. Absorbance of the filtrate was noted at 663nm and 645nm in a VIS spectrophotometer and the chlorophyll content was calculated using the standard formula.

Results & Discussion

Four varieties of one week old seedlings of maize were subjected to drought stress *in vitro* by application of PEG-6000 (Polyethylene glycol).On the 3rd, 5th and 7th days of drought stress plants were sampled for various biochemical assays along with the controls. No significant morphological changes were observed in the test pants during initial stages of drought but slight

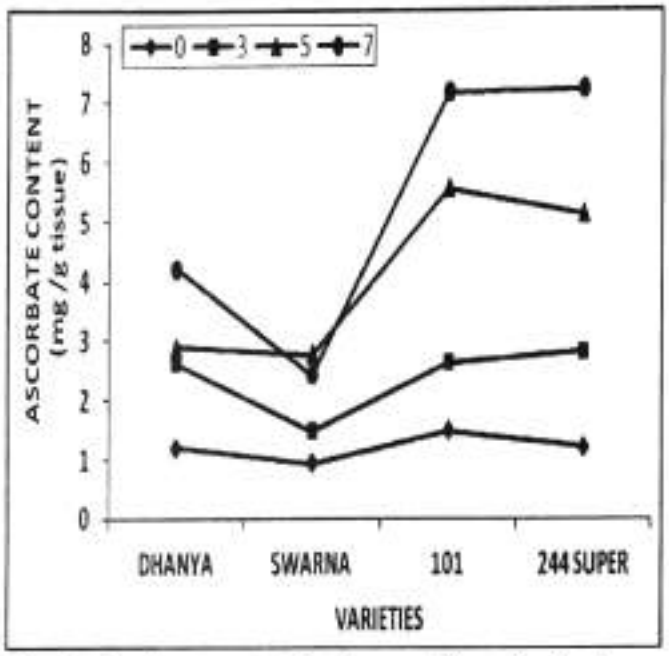

Fig. 2: Ascorbate content in four varieties of maize during water stress *invitro*

wilting was seen on the 7th day of stress. Assay of antioxidative enzyme activities showed that the POX activity increased in all varieties in the initial period of stress (Fig 1 A); in Dhanya and Swarna activity decreased on the 7th day but BN 101 and Super244 showed a different trend where there was a slight decrease in POX activity on the 5th day and again an increases on the 7th day. APOX (Fig 1 B) and SOD (Fig 1 E) in all the four varieties showed an increase in the initial period of stress but decreased on the later stages. In wheat, SOD activity increased or remained unchanged in the early phase of drought but decreased with prolonged water stress (Zhang and Kirkham 1995).CAT (Fig 1 C) activity decreased during drought in case of Dhanya and Swarna when compared to control but in the other two varieties there was a significant increase in the activity and the similar trend was also seen in GR activity too (Fig 1 D). When comparing antioxidative activity among the four varieties, BN 101 showed maximum increase in antioxidative activity during the period of drought stress. Maintaining a high level of antioxidative enzyme activities may contribute to drought tolerance by increasing the capacity of better protection mechanisms against oxidative damage (Sharma and Dubey, 2005; Tu" rkan et al., 2005). Among the four varieties Dhanya and Swarna showed greater accumulation of H_2O_2 during the stressed days and was maximum on the 7th day. But in BN 101 and Super 244 there was a slight decrease on the 7th day (Table1). Lipid peroxidation also increased in the same varieties (Table 1). During drought ascorbate content increased in all varieties but was maximum in BN 101 and Super 244 (Fig2). Ascorbate can act directly as a free radical scavenger (Bowler *et al.* 1992, Larson 1998). Results of present experiment showed that there was a decrease in chlorophyll content in all four varieties during drought stress (Fig 3). There are similar reports about decrease of chlorophyll in the drought stress conditions (Kuroda et al, 1990). Proline accumulation was more in case of drought stress than compared to the control in all the four varieties (Table 2). Maximum proline content was seen in BN 101 in both the leaves and root during drought condition. Accumulation of proline in plants under stress is a result of the reciprocal regulation of two pathways: increased expression of proline synthetic enzymes and repressed activity of proline degradation (Delauney *et al.*, 1993, Peng *et al.*, 1996). Accumulation of proline is an important indicator of drought stress tolerance in bacteria, algae, and higher plants. This amino acid has been reported to play multiple physiological functions in plants subjected to drought, such as osmoregulation, a sink for energy and nitrogen, and a signal of senescence (Aspinall and Paleg, 1981).

Results of the present study therefore indicate that water stress induces oxidative stress in all the four varieties but as antioxidative mechanism was much more pronounced in BN 101, this variety was the most tolerant to drought stress. Although laboratory conditions may not always reflect the behaviour of the plants exposed to water stress under field conditions, but such finding may help to understand the mechanism of drought stress

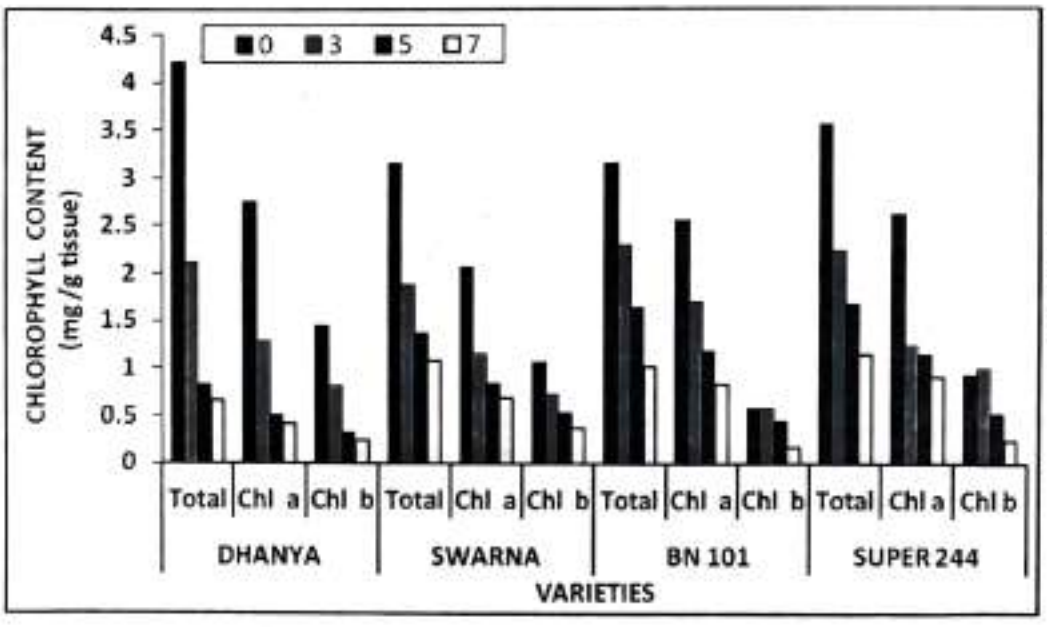

Fig.3: Changes in chlorophyll content of four varieties of maize following water stress

management and selection or development of maize genotypes resistant to drought stress.

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