Water Deficit Stress Tolerance Traits in Maize (*Zea mays* L.) and Identification of Tolerant Varieties

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Abstract

Water deficit (drought) during the summer season is one of the major production constraints for maize (*Zea mays* L.) in large areas of Southeast Asia. Identification and development of varieties capable of withstanding the stress conditions could be an ideal and affordable approach suitable for resource to poor maize-growing farmers of such areas. 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 six varieties of maize-30V92, BN 1133, Kaveri-Super 244, BN 101, Dhanya and Swarna, and for 3, 5 and 7 days. Assay of antioxidative enzymes revealed that peroxidase activities decreased on the 7th day of water stress in BN 1133, Dhanya, 30V92 and Swarna but in BN 101 and Super 244 the activity increased with period of stress. Ascorbate peroxidase and superoxide dismutase activities increased initially and then declined. Catalase activity declined at all periods of stress in BN 1133, Dhanya, 30V92 and Swarna while the other two varieties showed an increase during stress. Chlorophyll content showed a decline during the period of drought when compared to the control plants of all varieties. With the increase in the intensity of drought there was an increase in both proline and ascorbate content in all varieties. H_2O_2 accumulation and lipid peroxidation showed an increase during stress in BN 1133, Dhanya, 30V92 and Swarna but no increase was seen in the other two varieties. Water stress induced decrease in RWC was greater in BN 1133, Dhanya and Swarna. Thus enzymatic activity and biochemical tests showed that BN 1133, Dhanya, 30V92 and Swarna are more susceptible to drought stress than KS 244 and BN 101 which are the tolerant varieties.

1. Introduction

Plants in nature are continuously exposed to several biotic and abiotic stresses. Among these stresses, drought stress is one of the most adverse factors of plant growth and productivity and considered a severe threat for sustainable crop production in the conditions on changing climate (Anjum et al., 2011). Drought is characterized by reduction of water content, diminished leaf water potential and turgor loss, closure of stomata and decrease in cell enlargement and growth. Severe water stress may result in the arrest of photosynthesis, disturbance of metabolism and finally the death of plant (Ashraf, 2009). To overcome these, plants possess a complex antioxidant system, which consists of ascorbic acid, glutathione and enzymes that protect the plant against oxidative damage induced by environmental stresses (Kim et al., 2005).

Maize (*Zea mays* L.) is an important crop cultivated in most parts of the world and is one of the important crops of this region. Among various constraints responsible for low grain yield, inadequate supply of water at its critical development stage and high sensitivity of different maize cultivars to water stress are of immense importance (Shakhatreh et al.,2001). The present study was undertaken to determine the variability of different varieties to water stress in terms of tolerance and related antioxidant responses.

2. Materials and Methods

2.1. Plant material and growth conditions

Six varieties of maize (*Zea mays* L.) BN 1133, Swarna, Dhanya, BN 101, 30V92, KS 244 were used for the experiments. The seeds were soaked overnight and surface sterilized with 0.1% $HgCl_2$ and was then allowed to germinate in autoclaved petriplates. 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.

2.2. Relative water content (RWC)

Relative water content was determined by the method of Farooqui (2000). Following equation was used in this method.

RWC=(FW-DW) / (TW-DW)×100 .....(1)

FW:Fresh Weight, TW:Turgid Weight, DW:Dry Weight

2.3. Extraction and assay of enzyme activities

Leaf samples 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. Peroxidase (POX: EC. 1.11.17) activity was assayed spectrophotometrically in UV VIS spectrophotometer (Model 118 SYSTRONICS) at 460 (Chakraborty et al., 1993) activity was expressed as ΔA 460 mg protein^{-1} min^{-1}.Ascorbate peroxidase (APOX: EC.1.11.1.11) activity was assayed as decrease in absorbance by monitoring the oxidation of ascorbate at 290 nm according to the method of (Asada and Takahashi, 1987) and activity was expressed as ΔA 290 mg protein^{-1} min^{-1}. Catalase (CAT: EC.1.11.1.6) 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 and 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 nitrobluetetrazolium (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. Protein contents in each case were estimated following the method of Lowry et al. (1951).

2.4. Extraction and estimation of metabolites

For free proline estimation, the method of Bates et al. (1973) was followed andascorbate was extracted and estimated following the method of Mukherjee and Choudhuri (1983). Total chlorophyllcontent was estimated by following the method of Harborne (1973).

2.5. Lipid peroxidation

Lipid peroxidation was measured as MDA determined by the thiobarbituric acid (TBA) reaction. The absorbance was measured at 532 nm and 600 nm. The concentration of MDA was calculated using an extinction coefficient of 155 mM^{-1} and was quantified as μM MDA^{-1}(Heath and Packer, 1968).

2.6. Estimation of H_2O_2

H_2O_2 was extracted and quantified according to the method of Jena and Chowdhuri (1981). H_2O_2 content was measured in μM g^{-1} fresh wt.

3. Results and Discussion

Six 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 wilting was seen on the 7th day of stress.

Relative water content (Table 1) was minimum in BN 1133, Dhanya and Swarna on the 7th day of stress which is almost two times lesser than in varieties KS 244 and BN 101 indicating that these threevarieties may be drought susceptible.

Experiments performed on bread wheat by Keyvan (2010) showed cultivar and drought stress made an effect on the changes of flag leaf RWC and it was very significant statistically. A close relationship between antioxidant activity and stress tolerance has been identified in several crops (Zhang et al., 2003; Zhang and Ervin, 2004; Misra and Gupta, 2006). Assay of antioxidative enzyme activities showed that the POX activity increased in all varieties in early stages of stress. In BN 1133, Dhanya, Swarna and 30V92 activity decreased on the 7th day but BN 101 and KS 244 showed a different trend where there was a slight decrease in POX activity on the 5th day and again increases on the 7th day. Maximum decrease in POX activity was seen in Dhanyaand BN 1133 (Figure 1A). APOX activity was seen to decrease on the 7th day of stress in all six varieties of maize (Figure 1B). CAT activity showed a dramatic decrease in activity during drought in case of BN 1133, Dhanya, Swarna and 30V92 when compared to control but unlike these varieties KS 244 and BN101 showed a significant increase in CAT activity during drought (Figure 1C). SOD activity showed an increase in activity on the 3rd day of stress but on the 5th and 7th day there was a decrease in

Table 1: Relative water content in drought stress and control of five varieties of maize

Varieties	0 day	3 days	5 days	7 days
BN 1133	82.83	72.38	62.80	33.52
30V92	82.83	72.69	65.70	61.20
BN 101	85.86	83.86	77.69	72.33
DHANYA	76.25	72.38	54.05	32.87
SWARNA	75.37	71.90	57.51	33.00
KS 244	90.71	85.86	83.86	77.69

RWC in % (All values are average of three replicates)

SOD activity in all varieties of maize except for Swarna where SOD activity again showed a slight increase on the 7th day of stress after a decrease on the 5th day (Figure 1D). SOD is the first enzyme involved in the antioxidative process (Rubio et al., 2002) it is a class of enzymes that catalyze the dismutation of superoxide into oxygen and hydrogen peroxide (Corpas et al.,2006) and plays a key role in the reduction of oxidative damages (Zhang et al., 2005).

Proline accumulation both in leaves and root was more in case of drought stress than compared to the control in all varieties of maize (Figure 3A and 3B).

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).

H_2O_2 accumulation was seen to be very high in BN 1133, Dhanya, Swarna and 30V92 during drought stress but not much increase was seen in KS 244 and BN 101 in fact there was a decrease on the 7th day of stress in these two varieties of maize (Table 2). Lipid peroxidation was also higher in Dhanya, Swarna and 30V92 as these varieties could be drought sensitive resulting in more membrane destruction during drought (Table 3). Yang and Miao (2010) noted the increments of the MDA and H_2O_2 concentrations in the water-stressed cuttings were 88.9 and 99.7% in *Poplar cathayana,* respectively, whereas they were only 44 and 63.6% in *Poplar kangdingensis*.

Ascorbate content was seen to increase in all the six varieties of maize during drought (Figure 2). Tolerant variety BN 101 and KS 244 showed a maximum increase during drought stress but there was very little increase in the susceptible variety.

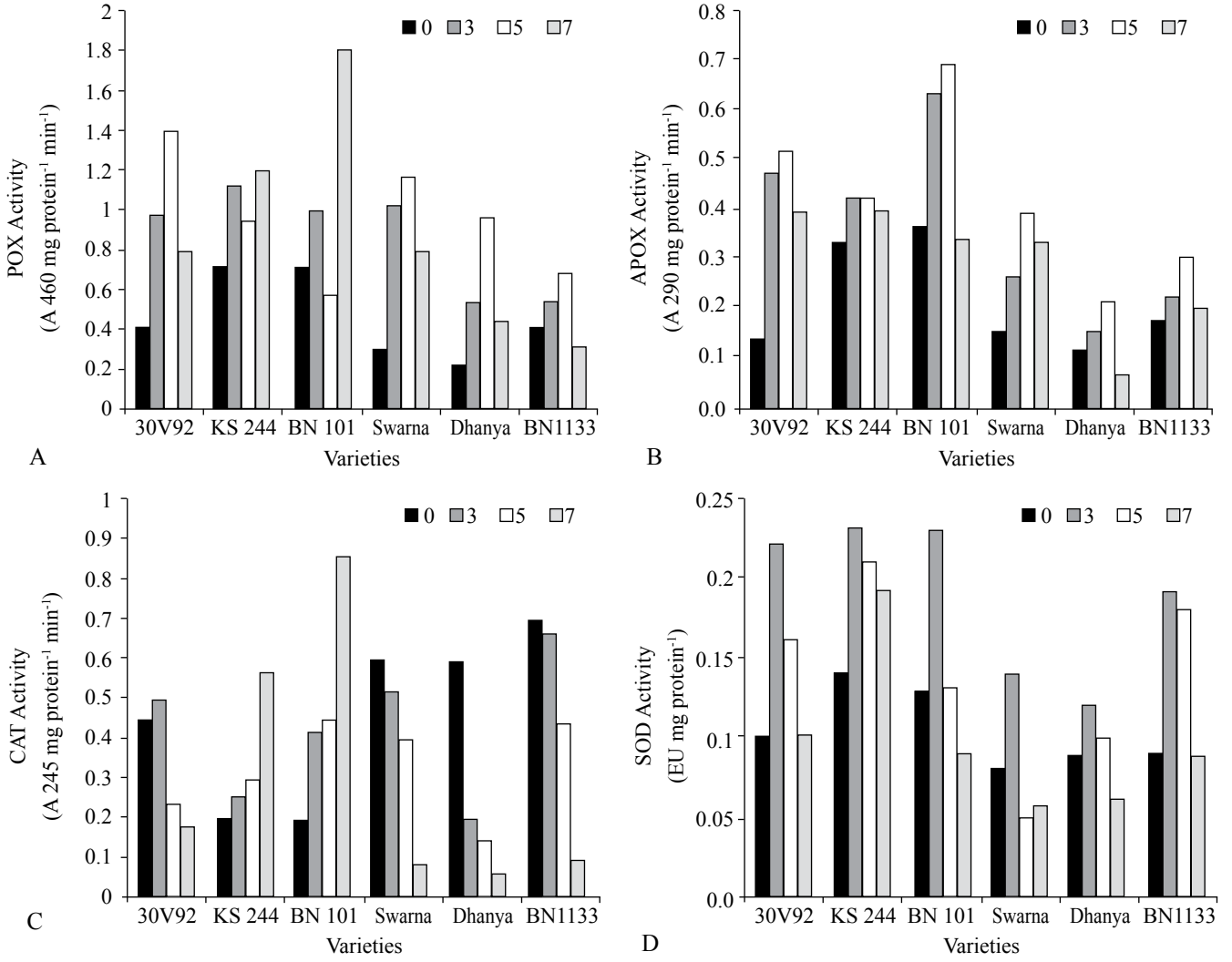

Figure 1: Peroxidase (A); Ascorbate peroxidase (B); Catalase (C); and Superoxide dismutase (D); activities in six varieties of maize subjected to water stress

Table 2: H_2O_2 accumulation in six varieties of maize during drought stress

Varieties	0 day	3 days	5 days	7 days
BN 1133	5.49	11.49	12.51	17.65
30V92	6.40	5.14	09.66	18.34
KS 244	2.89	6.20	07.55	05.43
BN 101	1.87	4.19	06.24	05.25
Dhanya	2.19	6.44	10.59	17.90
Swarna	2.67	6.47	12.15	13.44

H_2O_2 content: µM g^{-1}fresh wt. (All values are average of three replicates)

Table 3: Lipid peroxidation in six varieties of maize during drought stress

Varieties	0 day	3 days	5 days	7 days
BN 1133	0.014	0.022	0.023	0.025
30V92	0.005	0.009	0.013	0.017
KS 244	0.009	0.011	0.011	0.009
BN 101	0.008	0.006	0.012	0.006
Dhanya	0.007	0.013	0.020	0.028
Swarna	0.014	0.018	0.020	0.035

Lipid peroxidation: µM MDA^{-1} (All values are average of three replicates)

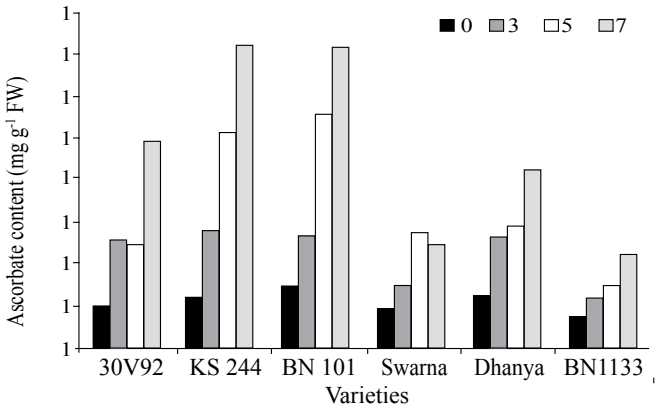

Figure 2: Ascorabte content in six varieties of maize during drought stress and control

Table 4: Total chlorophyll content in drought stress and control of five varieties of maize

Varieties	0 day	3 days	5 days	7 days
BN 1133	2.33	1.30	1.31	0.88
30V92	3.28	2.84	2.36	1.28
KS 244	3.56	2.25	1.68	1.15
BN 101	3.15	2.31	1.65	1.02
Dhanya	4.21	2.11	0.83	0.67
Swarna	3.16	1.89	1.38	1.09

Chlorophyll content : mg g^{-1}FW(All values are average of three replicates)

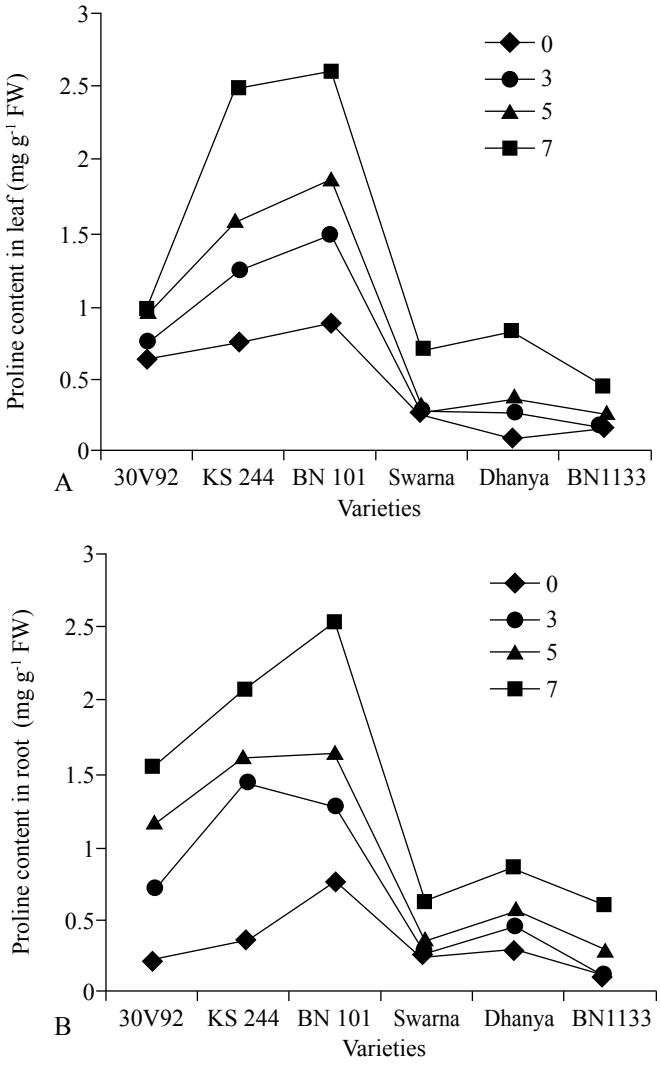

Figure 3: Proline content in leaf (A) and root (B) of six varieties of maize during drought stress.

In Swarna, ascorbate content decreased slightly on the 7th day of stress. There was a significant decrease in chlorophyll content in all six varieties of maize during drought stress (Table 3) maximum decrease was seen in Dhanya on the 7th day of drought. The reaction of plants to water stress differs significantly at various organizational levels depending upon intensity and duration of stress as well as plant species and its stage of development (Demiral and Turkan, 2005). Drought means water loss and dehydration at normal or even elevated temperatures. Shrinking of cells leads to loss of turgor, osmotic stress and a potential change of membrane potentials. Upon severe water loss from the cells, membrane disintegration and abolition of metabolic processes occur (Mahajan and Tuteja, 2005).

4. Conclusion

Water stress induces oxidative stress in all six varieties of maize

but as antioxidative mechanism was much more pronounced in BN 101 and KS 244 which indicated that these two varieties are much more tolerant to drought then the rest of the four varieties(BN 1133, 30V92, Dhanya and Swarna).

5. Acknowledgement

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