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Comparative response of drought tolerant and drought sensitive maize genotypes to water stress

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Abstract

Maize genotypes Giza 2 (drought tolerant) and Trihybrid 321 (drought sensitive) were sown in the small pots under laboratory condition. Water stress condition was created by irrigating the pots with polyethylene glycol (PEG) solutions of 0.0, -5, -10 and -20 bars and observations were made on 21-day-old seedlings. The tolerant genotype Giza 2 exhibited lower accumulation of malondialdehyde (MDA) and H_2O_2 content related to increasing activities of superoxide dismutase (SOD, EC 1.15.1.1), catalase (CAT, EC 1.11.1.6), and peroxidase compounds (POX, EC 1.11.1.7), under water stress conditions. The higher water retention capacity and lower membrane injury made Giza 2 more drought tolerant. Drought stress was resulted by accumulation of gylycinebetain (GB) and free proline (PRO) in both cultivars. The magnitude of increase in both omsolytes was higher in Giza 2 than in Trihybrid 321. We suggest that free proline and gylycinebetaine accumulation in the leaves can be used as the possible indicator for drought tolerance in maize genotypes. Water deficits induced an increased level of photosynthetic activity ($^{14}CO_2$-fixation) in Giza 2 than Trihybrid 321. Therefore, it can be concluded that the stress tolerance mechanism exists at seedling stage of maize genotypes. The Giza 2 is comparatively tolerant to water stress owing to the lower increase in H_2O_2 and MDA content along with higher increase in PRO, GB accumulation, photosynthetic efficiency, SOD, CAT and POX activities. The higher membrane stability index and high water retention capacity might have also imparted water stress tolerance in Giza 2. Further, Trihybrid 321 was also able to resist water stress to some extent via the above adjustments.

Keywords : Photosynthesis; gylycinebetain; antioxidant enzyme; *Zea mays.*

Abbreviations: GB- gylycinebetain; MDA- malondialdehyde; PEG- polyethylene glycol; POX- peroxidase compounds; PRO- proline; SOD- superoxide dismutase

Introduction

Drought occurs in many parts of the world every year, often with devastating effects on crop production (Ludlow and Muchow, 1990). The environmental stresses such as drought, temperature, salinity, air pollution, heavy metals, pesticides and soil pH are major limiting factors in crop production because, they affects almost all plant functions (Lawlor, 2002 and Hernandez et al., 2001). 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). 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). In higher plants the oxygen toxicity is more serious under condition of water-deficit conditions. Water stress causes stomatal closure, which reduces the CO_2/O_2 ratio in

leaves and inhibits photosynthesis (Jason et at., 2004 and Moussa, 2006). These conditions increase the rate of reactive oxygen species (ROS) like superoxide radical ($O_2^{\cdot -}$) hydrogen peroxide (H_2O_2), and hydroxyl radical ($OH^{\cdot}$) particularly in chloroplast and mitochondria (Mittler, 2002 and Neill et al., 2002), via enhanced leakage of electrons to oxygen. The superoxide radicals and their dismutation product, hydrogen peroxide, can directly attack membrane lipid and inactivate SH-containing enzymes (Sairam et al., 2000). The hydroxyl radical, one of the most reactive oxygen species, is responsible for oxygen toxicity *in vivo*, causing damage to DNA, protein, lipids, chlorophyll and almost every other organic constituent of the living cell (Bacana et al., 1998). Plants protect cellular and sub-cellular system from the cytotoxic effects of active oxygen radicals with anti-oxidative enzymes such as SOD, POX and CAT as well as metabolites like glutathione, ascorbic acid, tocopherol and carotenoids (Alscher et al., 2002).

It has been reported which membranes are subject to damage rapidly with increasing water stress. This leakiness of membranes is caused by an uncontrolled increase in free radicals, which cause lipid peroxidation (Smirnoff, 1993). The stress-induced burst in free radicals could also be partially related to the activity of lipoxygenase, which convert C18:2 and C18:3 to the corresponding hydroxyl peroxides (Bell and Mullet, 1991). Further damage to fatty acid could then produce small hydrocarbon fragments including malondialdehyde (MDA) (Alscher et al., 2002). It hypothesized that modulation of the activities of these enzymes at early growth stage may be important in imparting resistance to a plant against environmental stresses. Therefore, in the present investigation the relative significance of antioxidative enzymes, MDA, H_2O_2 content, PRO, GB accumulation, photosynthetic activity and membrane permeability has been examined at seedling stage in drought-tolerant and susceptible maize genotypes.

Materials and Methods

Plant materials

A homogenous lot of maize seeds *(Zea mays L.)* of two genotypes, Giza 2 (drought tolerant) and Trihybrid 321 (drought sensitive -but adapted) were obtained from the National Agriculture Research Center, Giza, Egypt. The caryopses of both cultivars were kept at 4 ^{o}C, the maize caryopses of both cultivars were surface sterilized in 0.1 % (w/v) sodium dodecyl sulphate solution and then thoroughly rinsed with sterile deionised water. The seeds were sown in small pots filled with loamy sand soil having bulk density 1.48 gCm^{-3}, pH 8.4, field capacity 11.8 % and permanent wilting point 2.8 %. Seedlings were grown up to 21 days by irrigating the pots with polyethylene glycol (PEG) solution of 0.0, -5, -10 and -20 bars for creating the different stress levels (Singh and Kumar, 1994). Each treatment was replicated three times and each replicate consisted of three pots. The plants were grown in a controlled growth chamber under the following growth conditions: 15-h photoperiod; 65-75% relative humidity; day and night temperature of 22°C and 20°C, respectively. The photosynthetic photon flux density (PPFD) at maximum plant height was about 440 µmol $m^{-2}s^{-1}$.

Relative water content and membrane injury

Leaf discs (5g, 1cm diameter) were collected from three weeks old maize seedlings for the estimation of relative water content (Barrs and Weatherly, 1992) and membrane injury (Deshmukh et al., 1991).

Enzyme activity, lipid peroxidation and H_2O_2 content

The activity of superoxide dismutase (SOD, EC.1.15.1.1), was assayed as given by Dhindsa et al. (1981), peroxidase (POX, EC 1.11.1.7), according to Macheix and Quessada (1984) and catalase (CAT, EC.1.11.1.6), was determined as described by Siminis et al. (1994). Lipid peroxidation level was determined as the content of malondialdehyde (MDA) using the thiobarbituric acid reaction as described by Madhava Rao and Sresty (2000). The contents of hydrogen peroxide in maize leaves were measured according to Patterson et al. (1984).

Free proline and gylycinebetaine determination

PRO content was quantified according to the method of Bates et al., (1973). GB was determined according to Grieve and Maas (1984).

Photosynthetic efficiency ($^{14}CO_2$-fixation)

Photosynthetic activity was measured in the Atomic Energy Authority, Radioisotope Department, Cairo, Egypt, according to Moussa (2006). One pot from each treatment was placed under a Bell jar, which was used as a photosynthetic chamber.

Table 1. **Water stress-induced changes in antioxidants and lipid peroxidation in maize seedlings**

Parameter	Giza 2 (tolerant)			Trihybrid 321 (sensitive)			LSD (0.05)
	0 bar	-10 bar	-20 bar	0 bar	-10 bar	-20 bar	
Superoxide dismutases activity (unit mg protein^{-1})	73.9	85.8	91.1	76.2	84.3	88.5	1.72
Peroxidase activity (unit mg protein^{-1})	40.7	42.1	60.8	33.5	39.9	40.8	1.19
Catalase activity (µmol H_2O_2 reduced g FW^{-1})	5.10	5.96	6.17	4.51	5.61	5.57	0.10
Malondialdehyde content (µmol g FW^{-1})	769	847	902	962	1069	1171	41
Hydrogen peroxide content (µmol g FW^{-1})	4.25	4.45	4.78	4.65	4.77	5.09	0.18

Radioactive $^{14}CO_2$ was generated inside the chamber by a reaction between 10% HCl and 50 µCi (1.87×10^6 Bq) $NaH^{14}CO_3$ + 100 mg Na_2CO_3 as carrier. Then the samples were illuminated with a tungsten lamp. After 30 min exposure time, the leaves were quickly detached from the stem, weighed and frozen for 5 min to stop the biochemical reactions, then subjected to extraction by 80% hot ethanol. The ^{14}C was assayed from the ethanol extracts in soluble compounds using a Bray Cocktail (Bray, 1960) and a Liquid Scintillation Counter (LSC2-Scaler Ratemeter SR7, Nuclear Enterprises).

Statistical analysis

Means were compared between treatments from the error of mean square by LSD (Least Significant Difference) at the p= 0.05 level using Tuckey's test (Tuckeys, 1953).

Results and Discussion

Experimental findings on antioxidant system indicate that two maize genotypes responded differently under normal and water stress conditions. The superoxide dismutase activity enhanced continuously with increasing water stress in both the genotypes. However, the percent enhancement was higher in Giza 2. Under non-stress conditions, the SOD activity was higher in Trihybrid 321 (Table 1). It is known that plants have a well-organized defense system against ROS under stress conditions and SOD constitutes the first line of defense via detoxification of superoxide radicals (Sairam et al., 2000). Comparatively higher increment of SOD activity in Giza 2 might have decreased the possible toxic concentration of $O_2^{\cdot-}$ radicals more efficiently than Trihybrid 321 (Table 1). Peroxidase, another important H_2O_2 scavenging enzyme, also followed the similar trend (Table 1). The percent increment in its activity under water stress condition was also higher in Giza 2. Enhancement in POX activity under various stress conditions has been linked with protection from oxidative damage, lignifications and cross-linking of cell wall to prevent from such adverse conditions (Dalal and Khanna-Chopra, 2001).

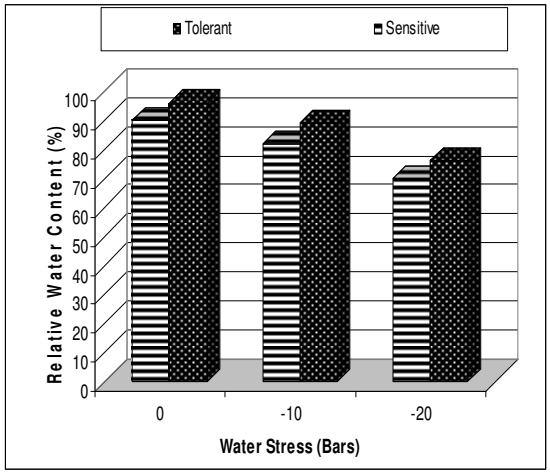

Figure 1. **Effects of different levels of water stress on relative water content in maize seedlings (LSD 0.05= 2.98).**

Catalase activity increased under water stress conditions in both tolerant and susceptible genotypes (Table 1). It is suggested that the higher

concentrations of catalase and ascorbate peroxidase might have removed the $O_2^{\cdot -}$ radicals and its product H_2O_2 induced by water stress (Sairam et al., 2000; Gupta and Gupta, 2005). Nayar and Kaushal (2002) also reported that the increased activity of CAT and POX enzymes constitute potential defense mechanism against chilling induced oxidative damage in germinating wheat grains.

The MDA content was significantly higher in Trihybrid 321 both under nonstress and water-stress conditions over Giza 2. The rise in MDA content under stress conditions suggests that water stress could induce membrane lipid peroxidation by means of ROS (Sairam et al., 2000). The hydrogen peroxide content enhanced linearly with increasing level of water stress in both the genotypes. It was always significantly higher in Trihybrid 321 than Giza 2 (Table 1). It is known that H_2O_2 is a toxic compound that is produced as a result of scavenging of superoxide radicals. Its higher concentration is injurious to plant via lipid peroxidation and membrane injury (Nayar and Kaushal, 2002). In the present investigation, the lower values of MDA and H_2O_2 in Giza 2 indicate that at cellular level this genotype is better equipped with efficient free radical quenching system that offers protection against oxidative stress.

The increased accumulation of PRO and GB (Table 2) in Giza 2 more than Trihybrid 321 is in accordance with previous data (Abdul Jaleel et al., 2007 and Manivannan et al., 2007), which is suggested to be associated with drought tolerance. The GB content increased under drought stress in barley (Nakamura et al., 2001) and in higher plants (Jun et al., 2000). High levels of proline enabled the plant to maintain low water potentials. By lowering water potentials, the accumulation of compatible osmolytes, involved in osmoregulation allows additional water to be taken up from the environment, thus buffering the immediate effect of water shortages within the organism (Kumar et al., 2003).

Relative water content decreased significantly in leaf discs of both the genotypes under different levels of water stress levels of water stress. It was significantly higher in Giza 2 both under non stress and water stress conditions (Figure 1). It is suggested that the high relative water content could help the tolerant genotype to perform physio-biochemical processes more efficiently under water stress conditions than susceptible genotype. Membranes are main loci affected under water-stress conditions. In the present investigation, tolerant maize genotype, Giza 2 maintained a higher membrane stability index under water-stress conditions (Figure 2).

The lower membrane stability index reflects the extent of lipid peroxidation, which in turn is a consequence of higher oxidative stress due to water stress conditions. Our data on H_2O_2 content also support these findings (Table 1). Enhanced water retention and membrane stability in tolerant wheat genotypes have also been observed in other studies (Deshmukh et al., 1991; Gupta and Gupta, 2005). Water deficits induced an increased level of photosynthetic activity ($^{14}CO_2$-fixation) in Giza 2 than Trihybrid 321. Reduced photosynthetic capacity (Figure 3) during drought can be indication of photo inhibition (Osonubi and Davies, 1980) or direct effects of dehydration on photosynthetic processes (Kaiser, 1987).

Thus, because of these results it can be inferred that the stress tolerance mechanism exists at seedling stage of maize genotypes. The Giza 2 is comparatively tolerant to water stress owing to the lower increase in H_2O_2 and MDA content along with higher increase in SOD, CAT and POX activities. The higher membrane stability index and high water retention capacity might have also imparted water stress tolerance in Giza 2. Also, photosynthetic activity was higher in Giza 2 than Trihybrid 321. Further, Trihybrid 321 was also able to resist water stress to some extent via the above adjustments.

Table 2. **Effects of different levels of water stress during the growth (for three weeks) on proline (mM g^{-1}FW) and gylycinebetaine (mM g^{-1}DW) concentrations of maize cultivars differing in drought tolerance. Each value is the mean ± S.D. of three replications.**

Treatment	Proline		Glycinebetaine	
	Giza 2 (tolerant)	Trihybrid 321 (sensitive)	Giza 2 (tolerant)	Trihybrid 321 (sensitive)
0 bar	1.80±0.180	2.24±0.089	137±11	157±3
-10 bar	2.40±0.144	2.81±0.112	210±22	195±9
-20 bar	5.82±0.523	3.96±0.396	386±47	365±10

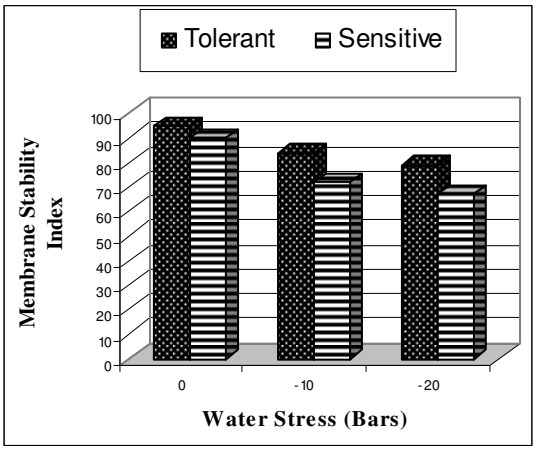

Figure 2. **Effect of different levels of water stress on membrane stability index in maize seedlings (LSD 0.05= 3.45).**

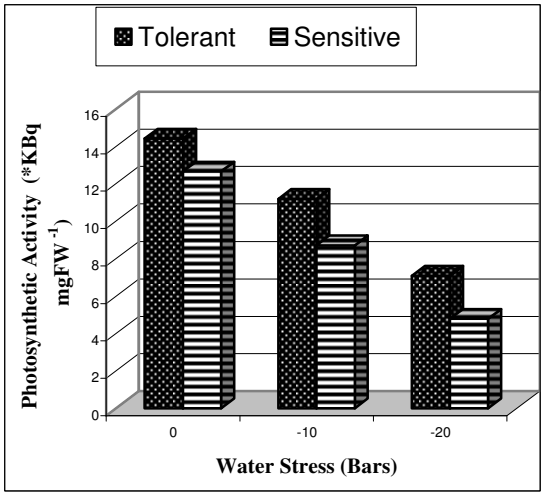

Figure 3. **Effects of different levels of water stress on photosynthetic activity (*kBq $mgFW^{-1}$) of maize cultivars. *kilo Becquerel (10^3 Bq), (LSD 0.05= 1022).**

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