Protective role of glycinebetaine in maize against drought-induced lipid peroxidation by enhancing capacity of antioxidative system

Shakeel Ahmad Anjum[1], Muhammad Farrukh Saleem[2], Long-chang Wang[1]*, Muhammad Faisal Bilal[2], Asif Saeed[3]

[1]College of Agronomy and Biotechnology, Southwest University, Engineering Research Center of South Upland Agriculture, Ministry of Education, P. R. China
[2]Department of Agronomy, University of Agriculture, Faisalabad, Pakistan
[3]Department of Plant Breeding and Genetics, University of Agriculture, Faisalabad, Pakistan

*Corresponding author: wanglc2003@163.com

Abstract

A pot-culture study was performed to investigate the efficacy of glycinebetaine (GB) for drought tolerance in two contrasting maize cultivars. Progressive drought stress noticeably reduced the relative leaf water content (RLWC) but increased protein and proline concentrations in both cultivars. The reduction in RLWC in DD-60 was lower than ND-95, whereas, the accumulation of protein and proline was substantially higher in DD-60 over ND-95 during experimental period. Nonetheless, GB-treatment led to increase in RLWC, protein and proline accumulation in DD-60 than ND-95. Prolonged drought stress induced the membrane lipid peroxidation, which was more severe in ND-95 than DD-60. GB-treatment substantially ameliorated the lipid peroxidation in DD-60 over ND-95 under drought stress. The activity of superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) were substantially enhanced in DD-60 than ND-95 with the prolongation of drought stress at beginning and then declined subsequently. These results possibly suggest that the DD-60 showed a better protection mechanism against water-induced lipid peroxidation by maintaining higher constitutive activities of antioxidant enzymes than ND-95. Nevertheless, GB-treated plants maintained higher antioxidant enzymes activity during drought stress than non-GB treated plants, which ultimately enhanced the growth, yield and yield components. The foliar application of glycinebetaine only considered advantageous when applied under drought and exhibit pronounced effects when applied at flowering. Drought tolerance can be improved in maize by the foliar GB-treatment through enhancing the antioxidants capacity.

Keywords: Antioxidant enzymes, Drought stress, Glycinebetaine, Lipid peroxidation, Maize, Proline.
Abbreviations: CAT- catalase, DD-60- dong dan-60, EL- electrolyte leakage, MDA- malondialdehyde, ND-95- nong da-95, POD-peroxidase, ROS- reactive oxygen species, SOD- superoxide dismutase.

Introduction

Environmental stresses provoke numerous plant responses, varying from altered gene expression to metabolic processes. Maintaining higher plant productivity under environmental stresses is plausibly the main challenge facing modern agriculture (Gill and Tuteja, 2010). Drought stress is major constraint which reduces the productivity of crop plants worldwide (Khamssi et al. 2011). Complete understanding of physio-biochemical responses of plants to drought is vital for improving plant tolerance mechanisms to drought stress (Jaleel et al., 2006). Generally, plants experience drought stress either when the water supply to roots becomes hard or when the transpiration rate becomes very high (Manivannan et al., 2007). Plants can avoid drought stress by improving water absorption or decreasing transpiration (Ruiz-Sánchez et al., 2007). The responses of plants to water deficit are observed in forms of phenological responses, morphological changes, physiological alterations, and biochemical adaptations, such as changes in plant structure, growth rate, tissue osmotic potential and antioxidant defenses (Duan et al., 2007). Osmolytes accumulation is mandatory in plants for osmotic adjustment under water limiting conditions. But osmolyte accumulation mainly depends upon water status, crop growth stage and cultivar (Shao et al., 2006).

Accumulation of osmolytes such as proline, helps maintaining cell water status, sub-cellular structures and protecting membranes and proteins from the denaturing effects of the osmotic stress (Ashraf and Foolad, 2007). The improved level of accumulated proline in crop plants is generally correlated with drought tolerance. Relative leaf water content (RLWC) is an integrative index of plant water status which is used to evaluate the tolerance to water stress. Reduction in RLWC under drought stress leads to stomatal closure (Gindaba et al., 2004), which further resulting in decreased CO_2 assimilation. Core consequence of drought stress is the loss of balance between the generation and scavenging of reactive oxygen species (Smirnoff, 1998). Drought stress mediates reactive oxygen species (ROS), such as superoxide radical ($O^{2\bullet-}$), hydroxy radical ($\bullet OH$), hydrogen peroxide (H_2O_2) and alkoxyradical ($RO\bullet$) (Munne-Bosch and Penuelas, 2003). ROS are considered immensely reactive and can drastically harm plants by lipid peroxidation, protein deterioration, DNA fragmentation and eventually cell death (Farooq et al., 2008). Drought-induced generation of ROS increase the contents of malondialdehyde (MDA), which is considered as a suitable marker for membrane lipid peroxidation. A decrease in membrane stability indicates the

degree of lipid peroxidation caused by ROS. Membrane lipid peroxidation can lead to the destruction of cellular integrity, cell dysfunction and ultimately survival of plants. In response to these drought-induced indicators that cross talk with each other, plants have developed a series of enzymatic and non-enzymatic antioxidant system to cope with drought stress (Ali et al., 2008). This dynamic mechanism comprise of various diverse antioxidant enzymes, such as superoxide dismutase (SOD), peroxidases (POD), ascorbate peroxidase (APX), glutathione reductase (GR) and catalase (CAT) and some low-molecular-weight non-enzyme antioxidants, such as ascorbic acid and reduced glutathione (GSH). The enzymatic antioxidants may directly dismutase ROS or may function in collaboration with a non-enzymatic antioxidants. Maintaining a relative higher antioxidants activity may lead to drought tolerance by improving the capacity to cope with ROS (Sharma and Dubey, 2005). Maize is one of the most important food crops in the world. During growing period, maize plants are extensively subjected to drought stress (Tai et al. 2011). Glycinebetaine (GB), a member of the compatible solutes, is found to be actively associated in plants as a defensive response to adverse conditions of salinity, drought, chilling or heat stress (Farooq et al., 2008). It occurs abundantly in many crop plants including spinach (Weigel et al., 1986), and bean (Gadallah, 1999). However, many important crop plants, like maize, potato, tomato, and eggplant lack the capability to synthesize GB in adequate amounts (Zwart et al., 2003), and its exogenous application becomes imperative to induce stress tolerance. Ali and Asharaf (2011) reported improved seed and seed oil quality of maize by exogenous GB under water deficit conditions. Nonetheless, to the best of our knowledge, no study have been undertaken to unravel the potential of GB in improving the drought tolerance in maize by improving growth, yield, photosynthesis, pigments, and antioxidants. Therefore, the present study was designed to investigate the protective role of GB in inducing the drought tolerance in two contrasting maize cultivars.

Results

Growth and yield

The growth and yield related traits of maize was drastically impaired under drought stress in both maize cultivars, however, this impairment was lower in DD-60 as compared to ND-95, while maximum growth and yield attributes were recorded in plants raised under well watered conditions. Exogenous application of GB under drought stress conditions in DD-60 improved the leaf area (7.32%), 100 kernels wt. (10.92%) biological yield/plant (15.41%), grain yield/plant (20.51%) and harvest index (5.44%), whereas the values for ND-95 were 5.53%, 6.81%, 9.20%, 13.73%, 4.83%, respectively. The growth and yield enhancement effect of GB was more pronounced in DD-60 under water stress conditions than ND-95 (Table 1).

Relative leaf water contents (RLWC)

Over the experimental period, the progressive drought stress caused subsequent reduction in RLWC of maize plants as compared to well water control. The RLWC of the DD-60 dropped from 89.14% at 4 d water stress to 50.36% at 20 d stress. Nonetheless, the same values for ND-95 were 87.08% at 4 d stress, and 44.63% at 20 d water stress (Fig 1). Moreover, drought-induced decrease in RLWC of maize plants was found to be increased by exogenous application of GB under drought as well as well-water conditions. However, the positive effect of GB on maize plants was more pronounced in water-stressed plants than non-stressed.

Proline accumulation

Free proline content in leaves of both cultivars shown in Fig 1. Under progressive drought stress the proline concentration in the leaves of both maize varieties enhanced at beginning of drought stress up to 12 d stress and then declined sharply on 20 d stress, this decline was severe between 12-16 d stress, and slight between 16-20 d stress in both cultivars. However, constitutive level of proline was higher in DD-60 over ND-95. Proline content in leaves of both cultivars showed an increase with GB application from 4 to 20 d stress with higher promotion under drought stressed conditions.

Protein

Under drought stress total soluble protein content in the leaves of both maize varieties first increased slowly up to 8 d stress but rapidly between 8 to 12 d stress, and then decreased and recorded minimum on 20 d stress. There was a linear reduction in protein content with increased water deficit in both cultivars. DD-60 was able to maintain higher protein contents at all days of water deprivation against ND-95. The combination of GB along with drought stress led to pronounced production of protein in DD-60, but very small increase in ND-95 upon drought treatment (Fig 1).

Malondialdehyde (MDA) contents and electrolyte leakage (EL)

Drought-stressed soybean plants exhibited membrane lipid peroxidation, measured as MDA content, manifesting possible damage by ROS. Membrane damage was assessed by determining EL. Under water stress, the levels of MDA and EL content in both maize cultivars increased subsequently with the progression of water stress. MDA levels increased slightly in both varieties at mild stress levels upto 12 d stress and then rose with sharply in leaf tissues between 12-20 days. However, the MDA and EL content was higher in ND-95 over DD-60 under drought stress conditions. Exogenous GB application significantly decreased MDA and EL levels and led to drought amelioration (Fig 2).

Antioxidative enzyme activities

Drought stress induced different changes in the anti-oxidative enzyme activities. Water-stressed plants showed higher SOD activity in both cultivars as compared to well-water control. However, DD-60 exhibited higher accumulation of SOD at 4, 8, 12, 16, and 20 d stress over ND-95. Exogenous application of GB further enhanced the SOD activity under drought as well as well watered control (Fig 3). However, the GB effect in improving SOD activity was more pronounced under water-stress condition than control. The POD activity in drought stress group was higher than that of non-stress group in both cultivars. The POD activity in both cultivars increased at the onset of drought stress upto 16 d stress and then declined rapidly with the prolongation of drought stress as estimated on 20 d stress. However, the decline was severe in ND-95 than DD-95. GB-treatment further enhanced the POD activity under drought as well as well watered control (Fig 3). However, the GB effect in improving POD activity was more pronounced under water-stress condition than

Table 1. Growth and yield attributes of two maize varieties as affected by exogenous application of glycinebetaine (GB) under drought.

Cultivars	Treatments	Leaf area (cm^2)	100 kernels wt. (g)	Biological yield/plant(g)	Grain yield/plant (g)	Harvest index (%)
DD-60	W	234.48±3.08 a	29.48±0.87 b	314.33±4.35 b	171.11±3.05 b	54.92±0.91 ab
	WGB	243.92±2.00 a	31.14±0.62 a	336.31±2.90 a	187.33±3.27 a	55.98±0.55 a
	D	197.84±3.37 c	24.55±0.73 d	233.67±2.90 d	125.86±2.34 d	51.25±0.47 c
	DGB	212.33±1.76 b	27.23±0.31 c	269.69±4.01 c	151.67±2.71 c	54.04±0.44 bc
ND-95	W	231.00±5.03 a	29.17±0.44 a	206.31±1.66 b	158.02±2.43 b	52.51±0.62 ab
	WGB	243.73±2.55 a	30.17±0.73 a	316.39±0.96 a	171.11±1.45 a	53.91±0.58 a
	D	192.67±2.73 c	23.33±0.88 b	212.32±1.72 d	117.32±3.54 d	48.04±0.43 c
	DGB	203.33±2.85 b	24.92±0.62 b	231.85±3.22 c	133.43±1.23 c	50.32±0.19 bc

W: well-water, WGB: glycinebetaine (GB) application in well water conditions, D: drought stress, DGB: glycinebetaine (GB) in drought conditions. Values in the table are mean ± SE. Values followed by the same letter within columns are not significantly different according to Newman–Keuls test (P < 0.05).
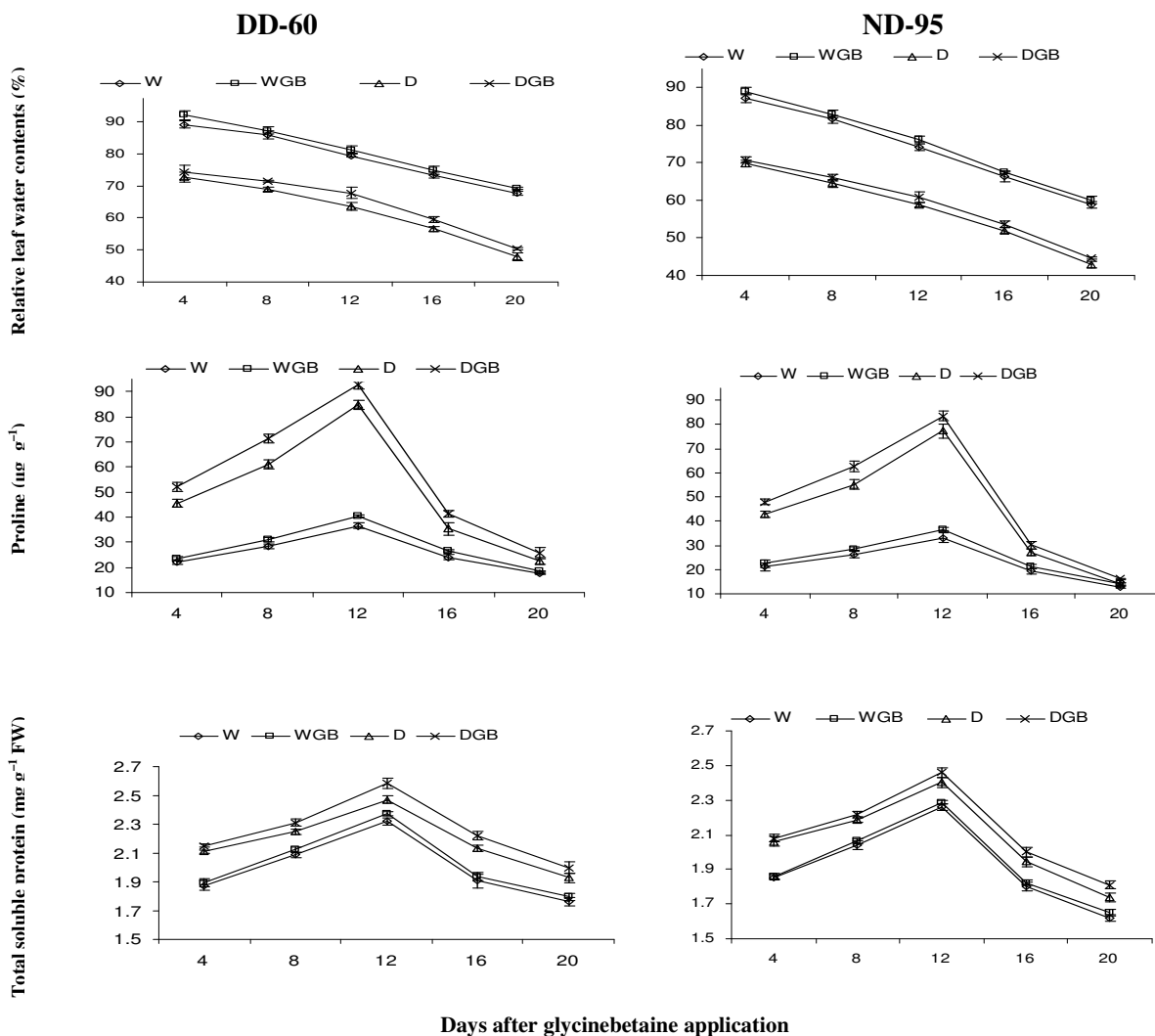

Fig 1. Influence of glycinebetaine (GB) application on relative leaf water contents, proline and total soluble protein of two maize cultivars (DD-60 and ND-95) under drought ± SE. W: well-water, WGB: glycinebetaine (GB) in well water conditions, D: drought stress, DGB: glycinebetaine (GB) in drought conditions.

control. Consequently, the drought stress in combination with GB-treatment resulted in highest POD activity over control. CAT activity improved in both maize varieties exposed to drought stress. The prolongation of drought stress was accompanied first by the increase and then decline of CAT activity, the CAT activity rose until 12 d stress in both cultivars then declined sharply as recorded on 20 d stress. GB application increased the activity of CAT under drought stress in both cultivars (Fig 3). Consequently, the CAT activity was higher in water stress but highest when GB was applied in water stress. The transient increase in CAT activity during initial periods of water stress might has protected the maize plants from oxidative injury but the scavenging function of CAT was hampered with prolonged drought stress.

Discussion

The improvement in drought tolerance capacity of plants is imperative to alleviate future threats to foods security in the world. This is important from agricultural point of view since the occurrence of stress is erratic and plants may be exposed to drought stress at any time during their growth period. In our study, the leaf area was severely suppressed by drought stress, which is in agreement with Shahenshah and Isoda (2010) who reported 63% and 45% reduction in leaf area for cotton and peanut, respectively. This reduced lead area was improved by foliar GB-treatment along with biological yield and grain yield. In our study, harvest index decreased under drought stress which is an important trait associated with crop yield which reflects the partitioning of photosynthesis (Pirzad et al. 2011). Moreover, the RLWC in leaves of both maize cultivars decreased with the exacerbation of water deprivation. Nevertheless, the reduction was considerably less in DD-60 against ND-95, suggesting that the high RLWC could help the tolerant genotype to perform physio-biochemical processes more efficiently under water stress conditions than susceptible genotype. Drought-induced reduction in RLWC was improved by GB application which is in accordance with Lv et al. (2007) who opined that GB may not only protect the integrity of the cell membrane from drought stress damage, but also involved in osmotic adjustment. GB-treated plants maintained high RLWC possibly due to the improved osmotic adjustment. The change in protein synthesis or degradation is one of the main metabolic mechanisms, which may substantially affect drought tolerance of plants (Jiang and Huang, 2002). The initial increase in total soluble proteins during drought stress could be due to the expression of new stress proteins, but the decrease could be due to a severe decrease in photosynthesis (data not shown). In present investigation, the subsequent improved soluble protein concentration in both maize cultivars under drought stress is in accordance with earlier findings of drought-induced accumulation of proteins to drought stress (Jiang and Huang, 2002). However, drought-induced decrease in soluble protein has also been reported in cotton (Parida et al., 2007). Proline is thought to play adaptive roles in inducing osmotic adjustment and protecting subcellular structures in stressed plants.

In response to water stress, proline accumulation generally occurs in the cytosol where it plays significant role in cytoplasmic osmotic adjustment (Anjum et al. 2011a). In our study, higher level of proline accumulation in DD-60 over ND-95 enabled the water-stressed plants to maintain low water potentials. By decreasing water potentials, proline accumulation involved in osmoregulation appeared to allow additional water to be taken up from the environment, thus counteracting the influence of drought stress on the plant tissues (Kumar et al., 2003). Moreover, the accumulation of proline under drought stress by GB treatment is consistent with earlier findings of Hussain et al. (2009). Cell membrane integrity undergoes diverse changes such as increase in penetrability and decrease in sustainability under drought stress (Blokhina et al., 2003). Hence, electrolyte leakage was determined to have a deep insight into the membrane stability. The increased MDA content under drought stress is consistent with Wang et al. (2008), is an indicator of oxidative damage to membrane lipids (Ozkur et al., 2009). In the present investigation, the lower values of MDA in DD-60 against ND-95 revealed that at cellular level this genotype was better equipped with efficient free radical scavenging system that offers protection against lipid peroxidation. The GB-treatment substantially reduced the MDA contents in DD-60 as compared to ND-95, which led to the cell membrane stability by reducing ROS (Farooq et al., 2010). GB-mediated reduction of MDA content, provide evidence that exogenous GB-treatment substantially ameliorate the impacts of drought on membrane integrity and stability in the maize plants. Plant water deficit tolerance requires the activation of complex anti-oxidative pathways within the cells which can contribute to continued plant growth under water stress. Once plants are exposed to drought stress, the generation and quenching of ROS becomes unsteady.

In order to cope with ROS, the plants have evolved a battery of antioxidants, such as SOD, POD, and CAT which function as an extremely efficient cooperative system (Anjum et al., 2011b). It is conspicuous that higher le of antioxidants are related to plant drought tolerance (Tahi et al., 2008). SOD is considered to form the first line of protection against ROS, which catalyzed the superoxide radical (O_2^-) to O_2 and H_2O_2 which are further quenched by diverse antioxidant enzymes (Wang et al., 2009), the most important being POD and CAT (Gong et al., 2006). POD is found in cytosol, vacuole as well as in extracellular space dismutated the H_2O_2 by oxidation of various substrates. POD can act as ROS scavenger and play multiple functions owing its higher number of iso-forms (Passardi et al., 2005). CAT is abundant in the glyoxysomes of lipid storing tissues. CAT eliminated the H_2O_2 by breaking it down to H_2O and O_2. SOD activity subsequently increased which indicated that it could clean more ROS and decrease the degree of membrane lipid peroxidation. Moreover, it has been reported that POD, and CAT activities showed an increase or maintenance in the early phase of drought and then a decrease with progression of water stress (Zhang and Kirkham, 1994). Same was true for present findings as with increasing severity of water stress, magnitude of POD and CAT activity also decreased. DD-60 by maintaining higher antioxidants (SOD, POD, and CAT) activity in leaves under drought stress may also have higher water retention and subsequent stress tolerance. Furthermore, our results suggest that GB-treated plants exhibit increased SOD, POD and CAT activities, indicating a more efficient quenching of ROS which is in accordance with Farooq et al. (2010). In sum, the GB-induced drought tolerance likely to be closely associated with efficient accumulation of the protein and proline as well as the increased capacity of the antioxidative system to scavenge reactive oxygen species (ROS) by suppressing MDA and EL under drought conditions. The protective role of GB was more pronounced in the DD-60 than ND-95. Exogenous GB application might be useful strategy to improve growth and productivity of maize plants under water-stressed conditions.
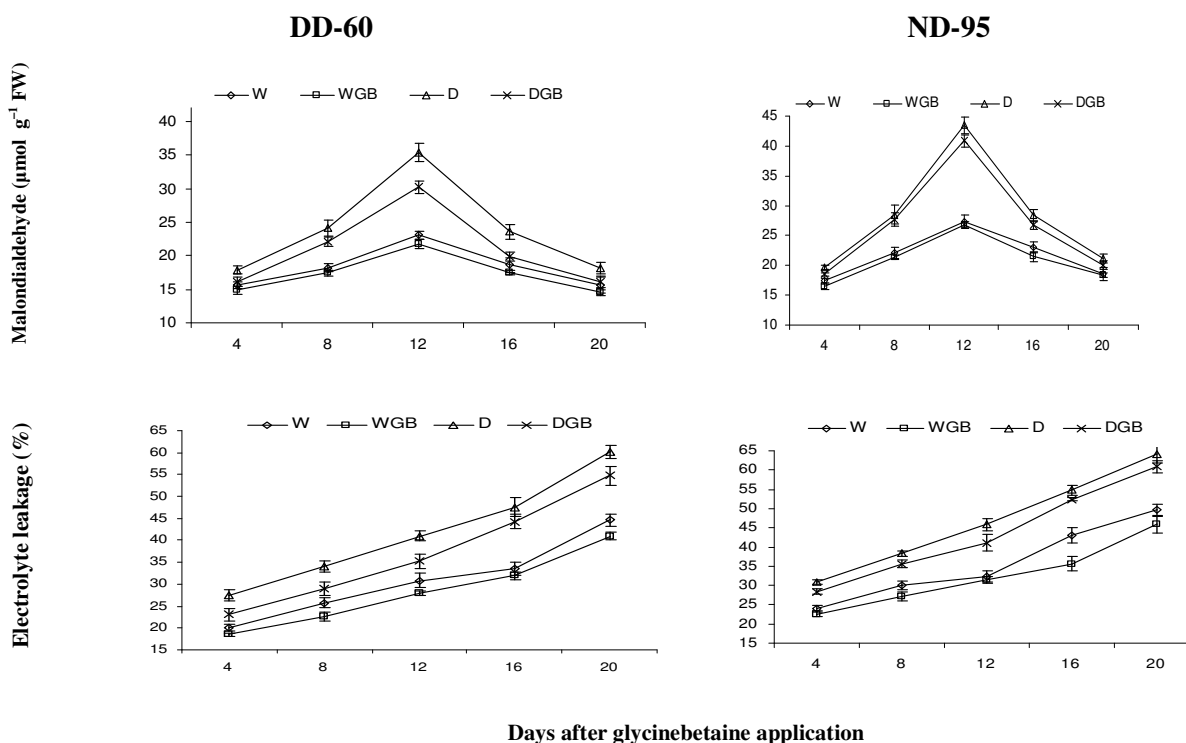
DD-60

ND-95

Days after glycinebetaine application

Fig 2. Influence of glycinebetaine (GB) application on malondialdehyde contents and electrolyte leakage of two maize cultivars (DD-60 and ND-95) under drought ± SE. W: well-water, WGB: glycinebetaine (GB) in well water conditions, D: drought stress, DGB: glycinebetaine (GB) in drought conditions

Materials and methods

Plant materials, growth conditions and treatments

A pot study was performed during the February to July 2010 at College of Agronomy and Biotechnology, Southwest University, Chongqing, China. The seeds of two cultivars of maize (*Zea mays* L.), DD-60 and ND-95, were obtained from Liaoning Dongya Seed Company Ltd., Liaoning, China. The seeds were germinated in PVC nursery trays, five seeds were sown in each hill initially, and two uniform seedlings were kept when the third leaves were fully expanded. The nursery trays were placed in a greenhouse where temperature ranged 22-31°C and relative humidity 64-72%. Two weeks old seedlings were transplanted into plastic pots (34 cm in diameter, 24 cm in depth) filled with 13 kg soil composed of loam and organic fertilizer by the ratio in 7:3 and 500 g of compound fertilizer (N: 15%, P_2O_5: 5%, K_2O: 5%) provided by Jiuhe Gufen Youxiang Company, China was added. The pots were then shifted to rain-protected wire-house after transplanting the seedlings. The seedlings were grown with normal water supply till heading and then were divided into following four groups: (1) W = well-watered and non-GB treated, (2) WGB = well watered and GB treated, (3) D = drought stressed and non-GB treated and (4) DGB = drought stressed and GB treated. Well-watered and drought-stressed treatments were maintained at 80% and 35% soil field capacity, respectively. The Glycinebetaine (GB) provided by Shiying Chemical Plant, Changping, Beijing, was applied @ 100 mM on the leaves until runoff. The pots were weighed daily to maintain the desired soil water levels by adding appropriate volumes of water. The temperature in wire-house ranged 26.5-35.1°C and relative humidity 68.61-81.4% for the entire growth period.

Data Measurement

The chlorophyll contents, RLWC, protein, proline, MDA, and the activity of antioxidant enzymes were determined on 4, 8, 12, 16, and 20 days after onset of water deprivation. After washing, the leaves were frozen in liquid N_2 and stored at -75 °C until biochemical analysis. The remaining plants were harvested at maturity for assessing the growth, yield and yield components.

Relative leaf water contents (RLWC)

To determine RLWC, fresh leaves (1.5 g) were weighed, and then these leaved were placed in water for 20 h to regain full turgor then weighed the turgid weight. These leaves were dried in oven for 72 h at 70 °C to measure dry weight. RLWC was then assessed as: RLWC = [(fresh weight - dry weight)/ (turgid weight - dry weight)] x 100.

Free proline estimation

Free proline content was quantified by following the method of Bates et al. (1973). Fresh leaf samples (0.5 g) were homogenized in 3% (w/v) sulphosalicylic acid, and centrifuged at 4000 rpm for 10 min at 4 °C. The supernatant was added with acid ninhydrin and glacial acetic acid in a test tube. The mixture was heated for 30 min at 98 °C in a water
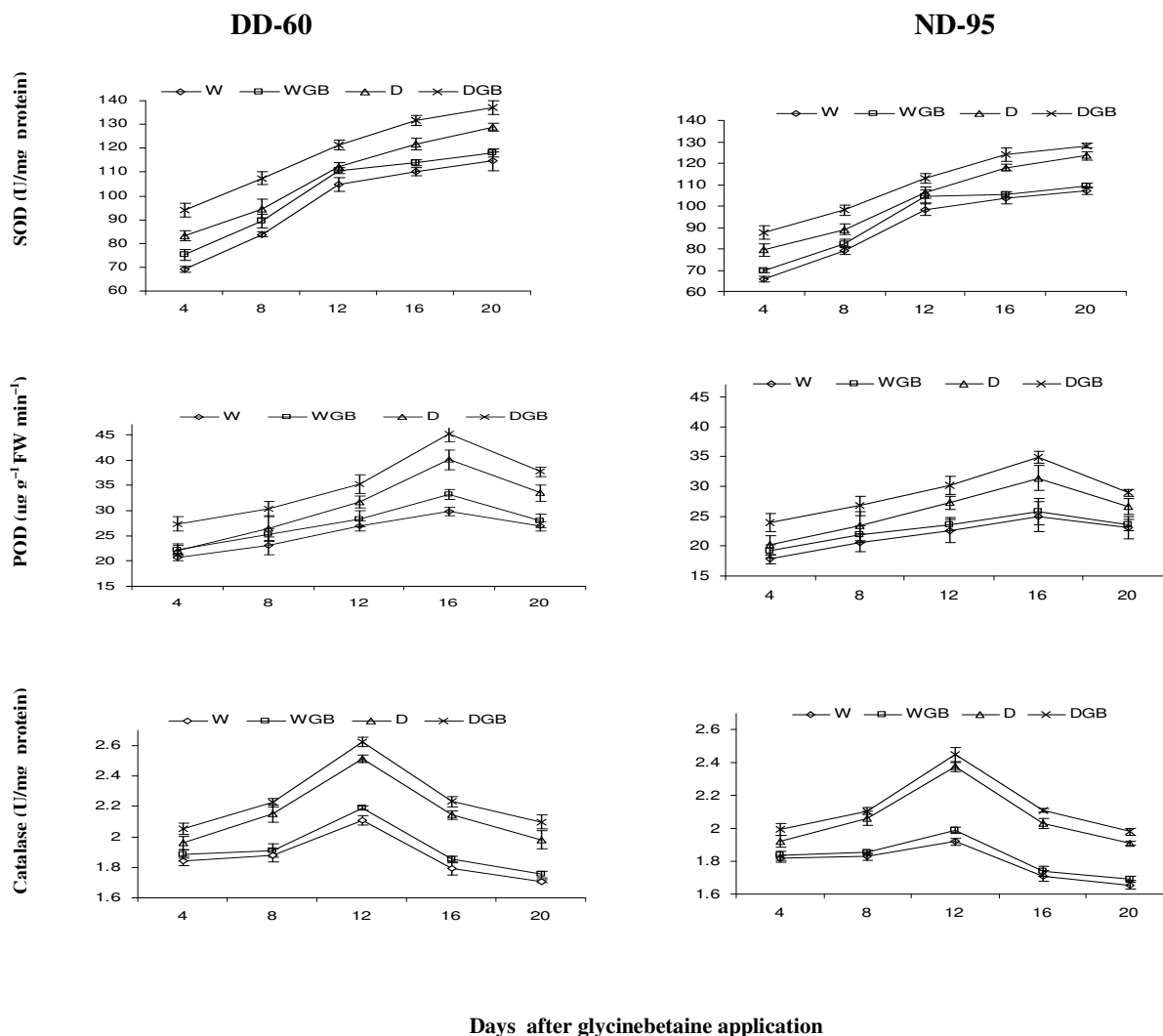
580

DD-60   **ND-95**

Days after glycinebetaine application

Fig 3. Influence of glycinebetaine (GB) application on superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) activity of two maize cultivars (DD-60 and ND-95) under drought ± SE. W: well-water, WGB: glycinebetaine (GB) in well water conditions, D: drought stress, DGB: glycinebetaine (GB) in drought conditions

bath and then allowed to cool at room temperature. The mixture was extracted with toluene and absorbance was determined using a UV-visible spectrophotometer at 520 nm.

Lipid peroxidation

The lipid peroxidation was estimated in terms of malondialdehyde (MDA), which is an end product of lipid peroxidation, as described by De Vos et al. (1991). The leaf samples (0.5 g) were homogenized in 10% trichloroacetic acid. The homogenate was centrifuged at 15000 g for 5 min at 25 °C and 4 mL of 0.5% thiobarbituric acid in 20 % trichloroacetic acid was added to 2 mL aliquot of the supernatant. MDA content was calculated by absorbance at 532nm and measurements were corrected for non-specific turbidity by subtracting the absorbance at 600 nm. The concentration of MDA was calculated using an extinction coefficient of 155 µM-1/cm.

Electrolyte leakage

Membrane permeability was estimated by electrolyte leakage (EL) according to Valentovic et al., (2006) with a few modifications. Leaves samples (0.5 g) were excised, washed with deionized water, and placed in test tubes containing 20 mL distilled ionized water and incubated at 25°C. The electrical conductivity of bathing solution (L_1) was determined. The samples were then autoclaved at 120°C for 20 min to release all electrolytes, cooled to 25°C and the final electrical conductivity (L_2) was determined. The EL was expressed following the formula EL= (L_1/L_2) × 100.

Soluble protein and antioxidant enzymes assay

Frozen leaf samples (0.5 g) were ground in liquid nitrogen with mortar and pestle and then homogenized in 10 ml extraction buffer of 50 mM sodium phosphate buffer (pH 7.0),

1 mM EDTA-Na$_2$, 2% polyvinylpyrrolidine-40 (PVP-40). The homogenate was centrifuged at 11000 g for 15 min at 4 °C. One millilitre of Bradford solution was added to 100 μL crude extract and absorbance was recorded at 595 nm for estimation of soluble protein content, according to the method of Bradford (1976). The supernatant was collected and further used for antioxidant enzymes activity analysis. The superoxide dismutase (SOD) activity was determined by using assay kits obtained from Nanjing Jiancheng Bioengineering Institute, China. Assay for SOD was performed according to the protocol described with the assay kits. SOD activity was defined as the amount of enzyme required for 1 mg tissue proteins in 1 mL of a reaction mixture SOD inhibition rates to 50 % as measured at 550 nm. The peroxidase (POD) activity was estimated with the help of method described by Upadhyaya et al. (1985). The reaction mixture containing 3.9 mL of 50 mM phosphate buffer (pH 7.0) with 28 μL guaiacol, 100 μL enzyme extract and 19 μL H$_2$O$_2$. At 420 nm at least 2 min per 30 seconds noted the absorbance and used the absorbance change 0.01 as a POD activity. The catalase (CAT) activity was assayed by following the operational process of ready kits provided by Nanjing Jiancheng Bioengineering Institute, China. The kit based upon principle that the reaction of CAT decomposing H$_2$O$_2$ through adding ammonium molybdate quickly suspended and the rest of the H$_2$O$_2$ with ammonium molybdate produced a kind of flaxen complex that can read in 405 nm.

Growth and yield components

Leaf area of maize plants was measured with Li-3100 leaf area meter (Li-Cor, Lincoln, NE) CI-203 (CID, Inc., USA) after 17 days of GB application. At harvest 33 plants (11 plants from each replicate) representing each treatment were sampled randomly and quantified to measure the growth, yield and yield components. Harvest index (HI) was calculated as follow HI (%): (grain yield/biological yield) x 100.

Statistical analysis

The experiment was laid out in RCBD design with three replications of each experimental unit. Data set was statistically analyzed by analysis of variance (ANOVA) technique using computer software SPSS 16.0 and Newman–Keuls test was applied for mean separation. Graphical presentation of the data was made by Microsoft Excel.

Acknowledgements

References

Ali Q, Ashraf M (2011) Exogenously applied glycinebetaine enhances seed and seed oil quality of maize (*Zea mays* L.) under water deficit conditions. Environ Exp Bot 71:249-259.

Anjum SA, Wang LC, Farooq M, Hussain M, Xue LL, Zou CM (2011a) Brassinolide application improves the drought tolerance in maize through modulation of enzymatic antioxidants and leaf gas exchange. J Agron Crop Sci 197:177-185.

Anjum SA, Wang LC, Farooq M, Khan I, Xue LL (2011b) Methyl jasmonate-induced alteration in lipid peroxidation, antioxidative defense system and yield in soybean under drought. J Agron Crop Sci 197 (4):296-301.

Ali B, Hasan SA, Hayat S, Hayat Q, Yadav S, Fariduddin Q, Ahmad A (2008) A role for brassinosteroids in the amelioration of aluminium stress through antioxidant system in mung bean (*Vigna radiata* L. Wilczek). Environ Exp Bot 62:153–159.

Ashraf M, Foolad MR (2007) Roles of glycinebetaine and proline in improving plant abiotic stress resistance. Environ Exp Bot 59:206–216.

Bates LS, Waldren RP, Teare ID (1973) Rapid determination of free proline for water-stress studies. Plant Soil 39:205–207.

Blokhina O, Virolainen E, Fagerstedt KV (2003) Antioxidants, oxidative damage and oxygen deprivation stress. Ann Bot 91:179–194.

Bradford MN (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Chem 72:248-254.

De Vos C, Schat HM, De Waal MA, Vooijs R, Ernst W (1991) Increased to copper-induced damage of the root plasma membrane in copper tolerant *Silene cucubalus*. Plant Physiol 82:523–528.

Duan B, Yang Y, Lu Y, Korpelainen H, Berninger F, Li C (2007) Interactions between drought stress, ABA and genotypes in *Picea asperata*. J Exp Bot 58:3025–3036.

Farooq M, Aziz T, Hussain M, Rehman H, Jabran K, Khan MB (2008) Glycinebetaine improves chilling tolerance in hybrid maize. J Agron Crop Sci 194:152–160.

Farooq M, Wahid A, Lee DJ, Cheema SA, Aziz T (2010) Comparative time course action of the foliar applied glycinebetaine, salicylic acid, nitrous oxide, brassinosteroids and spermine in improving drought resistance of rice. J Agron Crop Sci 196:336-345.

Gadallah MAA (1999) Effects of proline and glycinebetaine on *Vicia faba* responses to salt stress. Biol Plant 42:249–257.

Gill SS, Tuteja N (2010) Polyamines and abiotic stress tolerance in plants. Plant Signal Behav 5:26-33.

Gindaba J, Rozanov A, Negash L (2004) Response of seedlings of two Eucalyptus and three deciduous tree species from Ethiopia to severe water stress. For Ecol Manage 201:119-129.

Gong JR, Zhao AF, Huang YM, Zhang XS, Zhang CL (2006) Water relations, gas exchange, photochemical efficiency, and peroxidative stress of four plant species in the Heihe drainage basin of Northern China. Photosynthetica 44:355–364.

Hussain M, Malik MA, Farooq M, Khan MB, Akram M, Saleem MF (2009) Exogenous glycinebetaine and salicylic acid application improves water relations, allometry and quality of hybrid sunflower under water deficit conditions. J Agron Crop Sci 195:98-109.

Jaleel CA, Gopi R, Manivannan P, Kishorekumar A, Sankar B, Panneerselvam R (2006) Paclobutrazol influences vegetative growth and floral characteristics of *Catharanthus roseus* (L.) G. Don. Indian J Appl Pure Biol 21:369–372.

Jiang Y, Huang B (2002) Protein alterations in tall fescue in response to drought stress and abscisic acid. Crop Sci 42:202-207.

Khamssi NN, Golezani KG, Najaphy A, Zehtab S (2011) Evaluation of grain filling rate, effective grain filling period and resistance indices under acclimation to gradual water

deficit stress in chickpea cultivars. Aus J Crop Sci 5:1044-1049.

Kumar SG, Mattareddy A, Sudhakar C (2003) NaCl effects on proline metabolism in two high yielding genotypes of mulberry (*Morus alba* L.) with contrasting salt tolerance. Plant Sci 165:1245–1251.

Lv S, Yang A, Zhang K, Wang L, Zhang J (2007) Increase of glycinebetaine synthesis improves drought tolerance in cotton. Mol Breed 20:233–248.

Manivannan P, Jaleel CA, Kishorekumar A, Sankar B, Somasundaram R, Sridharan R, Panneerselvam R (2007) Drought stress induced changes in the biochemical parameters and photosynthetic pigments of cotton (*Gossypium hirsutum* L.). Ind J Appl Pure Biol 22:369–372.

Munne-Bosch S, Penuelas J (2003) Photo and antioxidative protection, and a role for salicylic acid during drought and recovery in field-grown *Phillyrea angustifolia* plants. Planta 217:758-766.

Ozkur O, Ozdemir F, Bor M, Turkan I (2009) Physiochemical and antioxidant responses of the perennial xerophyte *Capparis ovata* Desf. to drought. Environ Exp Bot 66:487–492

Parida AK, Dagaonkar VS, Phalak MS, Umalkar GV, Aurangabadkar LP (2007) Alterations in photosynthetic pigments, protein and osmotic components in cotton genotypes subjected to short-term drought stress followed by recovery. Plant Biotechnol Rep 1:37–48.

Passardi F, Cosio C, Penel C, Dunand C (2005) Peroxidases have more functions than a Swiss army knife. Plant Cell Rep 24:255–265.

Pirzad A, Shakiba MR, Salmasi SZ, Mohammadi SA, Hadi H, Darvishzadeh R (2011) Effect of irrigation regimes and plant density on harvest index of German chamomile (*Matricaria chamomilla* L.). Aus J Agric Eng 2: 120-126.

Ruiz-Sánchez MC, Domingo R, Pérez-Pastor A (2007) Daily variations in water relations of apricot trees under different irrigation regimes. Biol Plant 51:735-740.

Shahenshah, Isoda A (2010) Effects of water stress on leaf temperature and chlorophyll fluorescence parameters in cotton and peanut. Plant Prod Sci 13:269-278.

Shao HB, Chen XY, Chu LY, Zhao XN, Wu GY, Yong B (2006) Investigation on the relationship of proline with wheat antidrought under soil water deficits. Colloid Surf B Biointerf 53:113–119.

Sharma P, Dubey RS (2005) Lead toxicity in plants. Braz J Plant Physiol 17:35-52.

Smirnoff N (1998) Plant resistance to environmental stress. Curr Opin Biotechnol 9:214–219.

Tahi H, Wahbi S, Modafar CE, Aganchich A, Serraj R (2008) Changes in antioxidant activities and phenol content in tomato plants subjected to partial root drying and regulated deficit irrigation. Plant Biosyst 142:550–562.

Tai FJ, Yuan ZL, Wu XL, Zhao PF, Hu XL, Wang W (2011) Identification of membrane proteins in maize leaves, altered in expression under drought stress through polyethylene glycol treatment. Plant Omics J 4:250-256.

Upadhyaya A, Sankhla D, Davis TD, Sankhla N, Smith B.N (1985) Effect of paclobutrazol on the activities of some enzymes of activated oxygen metabolism and lipid peroxidation in senescing soybean leaves. J Plant Physiol 121:453-461.

Valentovic P, Luxova M, Kolarovic L, Gasparikova O (2006) Effect of osmotic stress on compatible solutes content, membrane stability and water relations in two maize cultivars. Plant Soil Environ 52:186-191.

Wang B, Li Z, Eneji AE, Tian X, Zhai Z, Li J, Duan L (2008) Effects of coronatine on growth, gas exchange traits, chlorophyll content, antioxidant enzymes, and lipid peroxidation in maize (*Zea mays* L.) seedlings under simulated drought stress. Plant Prod Sci 11:283-290.

Wang QF, Hou YH, Miao JL, Li GY (2009) Effect of UV-B radiation on the growth and antioxidant enzymes of Antarctic sea ice microalgae Chlamydomonas sp. ICE-L. Acta Physiol Plant 31:1097–1102

Weigel P, Weretilnyk EA, Hanson AD (1986) Betaine aldehyde oxidation by spinach chloroplasts. Plant Physiol 82:753–759.

Zhang J, Kirkham MB (1994) Drought-stress-induced changes in activities of superoxide dismutase, catalase and peroxidase in wheat species. Plant Cell Physiol 35:785-791.

Zwart FJ, Slow S, Payne RJ, Lever M, George PM, Gerrard JA, Chambers ST (2003) Glycinebetaine and glycinebetaine analogues in common foods. Food Chem 83:197–204.