Gas exchange and chlorophyll synthesis of maize cultivars are enhanced by exogenously-applied glycinebetaine under drought conditions

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ABSTRACT

Glycinebetaine acts as an osmoprotectant and is closely related with drought resistance. In the present study, glycinebetaine (GB) was exogenously-applied to two contrasting maize cultivars, Dongdan-60 and ND-95, to see whether GB improves drought resistance. Maize cultivars were grown with normal water supply till the heading stage and then exposed to two levels of soil moisture, well-watered control and drought-stressed, and then GB solution of 100 mmol was foliar applied five days after moisture treatments were imposed. The gas exchange and chlorophyll concentration were substantially declined in both maize cultivars under water stressed conditions. However, this reduction was less in Dongdan-60 than ND-95. Nonetheless, GB-treated plants considerably maintained higher gas exchange rate and chlorophyll concentration during drought stress than non-GB treated plants. The GB-induced improvement in gas exchange and chlorophyll synthesis under water stress ultimately resulted in improved growth and yield in both maize cultivars. Furthermore, the positive responses to exogenous GB application were more pronounced in Dongdan-60 as compared to ND-95 in all traits examined under water-deficit conditions. In conclusion, exogenously applied GB to maize crops could improve gas exchange, chlorophyll synthesis, growth and yield of maize.

Keywords: water-stressed conditions; photosynthesis; chlorophyll; yield; maize

The abiotic stresses adversely affect plant growth, development and yield performance of crop plants. Drought stress is one of the prime abiotic stresses in the world (Ashraf 2010). Understanding the plant responses to drought is of great importance for enhancing stress tolerance of crop plants. Changes in plant productivity due to changes in gas exchange, especially photosynthetic rate, have received much attention worldwide. The ability of crop plants to acclimate to different environments is directly or indirectly linked with their ability to acclimate at the level of photosynthesis (Chandra 2003). Drought stress damages the thylakoid membrane, disturbs its functions, and ultimately decreases photosynthesis and crop yield (Huseynova et al. 2007). The reduction of the photosynthetic activity under drought stress can be ascribed to both stomatal and non-stomatal factors. From a physiological perspective, leaf chlorophyll concentration is a parameter of significant interest in its own right. Studies by Randall et al. (1977) revealed that the majority of chlorophyll lost in response to water deficit occurs in the mesophyll cells with a less amount being lost from the bundle sheath cells.

The strategy of exogenous application of various organic osmolytes, osmoprotectants, and growth regulators such as abscisic acid, benzyladenine (Pospíšilová and Baťková 2004), proline (Ali et al. 2007), ascorbic acid (Dolatabadian et al. 2009), chitosan (Yang et al. 2009), glycinebetaine, salicylic acid, nitrous oxide, brassinosteroids (Farooq et al. 2010), methyl jasmonate (Wang 1999) to plants was regarded vital technique to alleviate the deleterious effects of drought stress in plants. Out of the various stress ameliorating materials, glycinebetaine (GB) is considered an effective one. Furthermore, some plant species cannot synthesize GB in sufficient amounts, and its exogenous ap-

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plication becomes indispensable to induce stress tolerance. GB is involved in higher plants as a defensive response to extreme conditions of salt, drought, temperature or light stress (Farooq et al. 2008). It stabilizes the structures and activities of enzymes and protein complexes and maintains the integrity of membranes against the damaging effects of abiotic stresses (Sakamoto and Murata 2002). It physiologically acts as an osmolyte to protect cells or as a catabolic source of methyl groups to facilitate various biochemical processes, protects the plant cells against osmotic inactivation and increases the water retention of cells (Ashraf and Foolad 2007).

Maize (*Zea mays* L.) is the world's third most important cereal crop, and moisture is a crucial factor for successful production of maize (Konopka et al. 2009). Drought can occur at any stage of crop but the occurrence of water stress at the heading stage has the most adverse effect on yield. At present, with the aim of improving agricultural yield within the limited water resources, it is imperative to develop crops able to produce a high yield when growing in stressed environments. It is known that endogenous GB acts and an osmoprotectant in cells under water stress conditions (Hussain et al. 2009). It is also important to know the role that the exogenously-applied GB plays in drought resistance of crops. Therefore, in the present study, GB was exogenously applied to maize crops of two cultivars and the possible physiological mechanisms of protective functions under water-deficit conditions were discussed.

MATERIALS AND METHODS

Plant materials, growth conditions and soil moisture treatments. A pot study was carried out from February to July 2010 at the College of Agronomy and Biotechnology, Southwest University, Chongqing, China. The seeds of two contrasting maize (*Zea mays* L.) cultivars, Dongdan-60 and ND-95, were germinated in PVC nursery trays, a little in amount but frequent irrigation was applied to seedlings with the help of hand sprayer. The nursery trays were placed in a greenhouse where temperature ranged from 22 to 31°C and relative humidity from 64 to 72%. Two-week old seedlings were transplanted into plastic pots (34 cm in diameter, 24 cm in depth) filled with sandy loam soil containing organic matter 17.51 g/kg, total nitrogen 2.13 g/kg, total phosphorus 3.67 g/kg, total potassium 9.21 g/kg, alkali-hydro nitrogen content 88.22 mg/kg, available phosphorus 34.67 mg/kg, readily-available potassium 57.41 mg/kg and pH 6.32. Two maize seedlings were transplanted in each plastic pot, treatment comprised of 15 pots and each treatment factor of the experiment was replicated 3 times. The plastic pots were then shifted to wire-house after transplanting the seedlings. The seedlings were grown with normal water supply till the heading stage and then were divided into following four groups: (1) WW = well-watered and non-GB treated, (2) WW + GB = well-watered and GB treated, (3) DS = drought-stressed and non-GB treated, and (4) DS + GB = drought-stressed and GB treated. Well-watered and drought-stressed treatments were maintained at 80% and 35% soil field capacity, respectively. The glycinebetaine (GB) of 100 mmol was sprayed onto the leaves until liquid runoff. The pots were weighed daily to maintain the desired soil water levels by adding appropriate volumes of water. Moisture treatments were regularly monitored until harvesting by TRIME-EZ/-IT (IMKO Micromodultechnik GmbH, Ettlingen, Germany). Each pot was supplied with fertilizer at the rate of 12 g/pot (4 g at sowing, 4 g after 24 days after transplanting (DAP), 4 g after 45 (DAP), using NPK compound fertilizer with 15, 5 and 5% N, P_2O_5 and K_2O, respectively.

Measurement. Gas exchange and chlorophyll concentration were measured 10 days after foliar application of GB. Growth related attributes were measured during the life cycle and the remaining plants were harvested at maturity for assessing yield and yield components.

Leaf gas exchange. Measurements of gas exchange were made with a portable infrared gas exchange analyzer based photosynthesis system (Li-6400, Li-Cor, Lincoln, Nebraska, USA) on the 3rd leaf from top of each plant. These measurements were made from 8:45 a.m. to 10:00 a.m. with the following specifications: water vapor pressure into chamber was 3.6 mbar, ambient CO_2 concentration was 373 μmol/mol; temperature of leaf chamber varied from 34.4 to 37.7°C; ambient temperature was 33.23 to 37.41°C; molar flow of air per unit leaf area was 401.06 mmol/m^2/s; relative humidity ranged from 49.55 to 53.71%; PAR at leaf surface was maximum up to 1195 μmol/m^2/s. Water use efficiency (WUE) was calculated as ratio between net photosynthesis (A) and transpiration rate (E).

Estimation of chlorophyll concentration. Chlorophyll concentration in the 3rd leaf from top was determined using the method by Arnon (1949). A fresh leaf sample of 0.1 g was cut into small pieces and placed in 15 mL centrifuge tube, along with 10 mL of miscible liquids by 95.5% acetone and absolute ethyl alcohol in 1:1 ratio, then covered with black plastic bag and kept at dark place until the sample changed into white. In order to determine carotenoids, chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*), and chlorophyll *a* + *b* (Chl *a* + *b*), concentrations, 1 mL of the filtered extract was diluted with 6 mL of absolute ethanol and the absorbance was measured at 470 nm, 645 nm, 652 nm and 663 nm.

Growth and yield components. At harvest, 27 plants (9 plants from each replicate) from each treatment were sampled randomly and quantified for yield and yield components.

Statistical analysis. All the data were subjected to statistical analysis and the means were tested by the Newman–Keuls test at 5% level of significance. All the statistical analyses were conducted using the statistical package SPSS 16.0.

RESULTS

Growth. Both cultivars showed parallel decreases in plant height, shoot fresh weight per plant, shoot dry weight per plant, number of leaves per plant, and cob length under water deficit conditions. However, Dongdan-60 demonstrated drought-tolerance due to less reduction in the growth-related traits than ND-95 under drought conditions. GB spray noticeably improved the maize growth which clearly indicated the positive role of GB in drought mitigation. GB-treatment to Dongdan-60 improved the plant height (4.48%), shoot fresh weight per plant (11.39%), shoot dry weight per plant (20.81%), number of leaves per plant (8.94%), and cob length (6.40%) under drought conditions. However, the values for ND-95 for the above mentioned traits were 3.51, 9.04, 16.32, 7.37, and 5.78%, respectively (Table 1).

Yield. Under water-deficit conditions, the yield of both maize cultivars deceased in various degrees in comparison to well-watered control. The decrease in grain yield per plant in Dongdan-60 under drought stress was 34.85% compared to well-watered control, while yield in ND-95 reduced by 38.28%. The drought-induced reduction in yield and yield components was substantially alleviated by GB application in both cultivars under drought stress. Exogenously applied GB increased number of kernels per cob in Dondan-60 by 13.79% and that in ND-95 by 6.98% when exposed to drought. Grain yield per plant was increased by 12.94% and 10.68% for Dongdan-60 and ND-95, respectively (Table 2). Irrespective of cultivar, the greater effectiveness of exogenous GB was found in drought-stressed treatments compared to the well-watered control.

Gas exchange. Net photosynthetic rate (A), transpiration rate (E), and stomatal conductance (g_s) in both maize cultivars decreased noticeably under water stress. The decreases in g_s caused a reduction in intercellular CO_2 concentration. The reduction in gas exchange attributes in ND-95 was greater than in Dongdan-60, which suggested that a great excess of photons existed in Dongdan-60. GB application was able to restore the gas exchange traits up to some extent under drought stress condition. GB improved A by 16.28%, E 8.33%, g_s 21.60%, and C_i 2.32% in Dongdan-60 under drought, whereas, elevated these traits by 10.30%,

Table 1. Growth-related traits of two maize cultivars as affected by exogenous application of glycinebetaine (GB) under drought

Cultivars	Treatments	Plant height (cm)	Shoot FW/ plant (g)	Shoot DW/ plant (g)	No. of leaves/plant	Cob length (cm)
Dongdan-60	WW	201.00 ± 2.00^{a}	254.52 ± 4.30^{b}	66.66 ± 1.33^{a}	13.05 ± 0.07^{a}	20.08 ± 0.59^{ab}
	WW + GB	207.01 ± 1.15^{a}	268.87 ± 1.10^{a}	71.00 ± 1.15^{a}	13.69 ± 0.02^{a}	21.09 ± 0.18^{a}
	DS	186.33 ± 2.91^{c}	197.88 ± 2.89^{d}	48.00 ± 1.53^{c}	10.51 ± 0.04^{b}	17.97 ± 0.20^{c}
	DS + GB	194.67 ± 0.88^{b}	220.42 ± 1.69^{c}	58.00 ± 1.73^{b}	11.45 ± 0.05^{b}	19.12 ± 0.20^{b}
ND-95	WW	199.00 ± 2.89^{a}	252.70 ± 3.89^{a}	64.00 ± 2.00^{a}	12.44 ± 0.07^{b}	19.69 ± 0.38^{a}
	WW + GB	204.33 ± 1.20^{a}	261.77 ± 3.61^{a}	68.97 ± 0.99^{a}	12.71 ± 0.02^{a}	20.65 ± 0.23^{a}
	DS	181.67 ± 1.76^{b}	190.76 ± 5.49^{c}	47.00 ± 1.73^{c}	10.04 ± 0.04^{d}	17.64 ± 0.27^{c}
	DS + GB	188.04 ± 1.20^{b}	208.00 ± 3.61^{b}	54.67 ± 2.03^{b}	10.78 ± 0.05^{c}	18.66 ± 0.35^{b}

WW – well-watered; WW + GB – Glycinebetaine application in well-watered conditions; DS – drought stress; DS + GB – Glycinebetaine application in drought stress conditions. Values in the table are mean ± SE. Values followed by the same letter within columns are not significantly different according to the Newman–Keuls test ($P < 0.05$)

Table 2. Yield and yield components of two maize cultivars as affected by exogenous application of glycinebetaine (GB) under drought

Cultivars	Treatments	Kernel rows/cob	Kernel number/row	Kernels/cob	Cobs/plant	Grain yield/plant (g)
Dongdan-60	WW	14.10 ± 0.06^{a}	37.06 ± 0.71^{a}	549.00 ± 3.62^{b}	1.64 ± 0.03^{a}	178.00 ± 1.73^{b}
	WW + GB	14.23 ± 0.09^{a}	37.93 ± 0.12^{a}	565.33 ± 0.33^{a}	1.69 ± 0.01^{a}	184.93 ± 2.58^{a}
	DS	13.60 ± 0.06^{b}	31.43 ± 0.27^{c}	384.13 ± 2.21^{d}	1.25 ± 0.01^{b}	132.52 ± 2.04^{d}
	DS + GB	13.80 ± 0.06^{b}	33.13 ± 0.46^{b}	437.10 ± 3.55^{c}	1.41 ± 0.01^{ab}	149.67 ± 1.86^{c}
ND-95	WW	14.03 ± 0.09^{ab}	37.14 ± 0.94^{a}	553.47 ± 7.52^{a}	1.61 ± 0.01^{a}	177.10 ± 3.66^{a}
	WW + GB	14.20 ± 0.12^{a}	37.63 ± 0.35^{a}	567.00 ± 3.06^{a}	1.67 ± 0.01^{a}	184.60 ± 3.12^{a}
	DS	13.47 ± 0.24^{b}	31.13 ± 1.26^{c}	377.33 ± 8.60^{c}	1.23 ± 0.02^{c}	128.00 ± 4.04^{c}
	DS + GB	13.67 ± 0.07^{ab}	32.51 ± 0.36^{b}	403.67 ± 4.48^{b}	1.36 ± 0.02^{b}	145.67 ± 2.91^{b}

Legend: Same as Table 1

7.03%, 18.12%, and 1.61% in ND-95, respectively (Table 3). GB-treated plants always maintained higher gas exchange rates than non-GB treated plants in both maize cultivars during the whole drought stress period.

Chlorophyll concentration. Although drought stress decreased chlorophyll concentration in both maize cultivars, the reduction was smaller in Dongdan-60 than in ND-95. Dongdan-60 was able to better withstand the water stressed conditions by maintaining higher concentration of chlorophylls than ND-95. Furthermore, GB-induced increases in Chl *a*, Chl *b*, Chl *a* + *b* and carotenoids concentration was 4.23, 6.49, 2.96, and 3.91% in Dongdan-60, whereas, 3.10, 4.40, 2.18, and 2.87% in ND-95 under water-deficit conditions (Table 4).

DISCUSSION

Crop losses as a consequence of drought stress can be ameliorated by application of osmoprotectants to crop plants. Exogenously applied GB can rapidly penetrate through leaf surface and be easily transported to other plant organs, where it would contribute to improvement in stress tolerance (Mäkelä et al. 1998). Response to drought stress differs noticeably among different crop cultivars due to their inherent differences in drought-tolerance (Huang and Zhao 2001). The effect of drought stress on plant growth related attributes was greater in the drought sensitive ND-95 than the drought tolerant Dongdan-90 of wheat as demonstrated by Chandrasekar et al. (2000). The GB-induced drought tolerance could be attributed to lowering of the osmotic potential due to net solute accumulation in response to water stress, which might help to preserve the metabolic processes, contribute to growth of plants through maintaining turgor in cells (Chimenti et al. 2002), and ultimately increase drought tolerance. These results are in agreement with Hussain et al. (2009) who reported that exogenous application of GB improved the growth in sunflower under drought.

Table 3. Gas exchange traits of maize as affected by exogenous application of glycinebetaine (GB) under drought

Cultivars	Treatments	Net photo-synthesis (μmol CO$_2$/m^2/s)	Transpiration rate (mmol H$_2$O/m^2/s)	Stomatal conductance (mol/m^2/s)	Water use efficiency (mmol CO$_2$/mol H$_2$O)	Intercellular CO$_2$ (μmol/mol)
Dongdan-60	WW	20.91 ± 0.56^{b}	3.68 ± 0.09^{a}	0.227 ± 0.012^{a}	5.68 ± 0.07^{d}	259.33 ± 0.88^{a}
	WW + GB	22.40 ± 0.32^{a}	3.85 ± 0.03^{a}	0.248 ± 0.003^{a}	5.81 ± 0.11^{c}	262.66 ± 1.71^{a}
	DS	15.66 ± 0.34^{d}	2.64 ± 0.04^{c}	0.162 ± 0.009^{c}	5.92 ± 0.22^{b}	248.56 ± 2.16^{b}
	DS + GB	18.21 ± 0.41^{c}	2.86 ± 0.05^{b}	0.197 ± 0.004^{b}	6.38 ± 0.25^{a}	254.33 ± 1.45^{ab}
ND-95	WW	19.49 ± 0.39^{a}	3.63 ± 0.06^{a}	0.216 ± 0.007^{ab}	5.37 ± 0.08^{c}	262.00 ± 2.65^{a}
	WW + GB	20.37 ± 0.15^{a}	3.75 ± 0.02^{a}	0.226 ± 0.006^{a}	5.43 ± 0.06^{bc}	265.67 ± 1.20^{a}
	DS	13.79 ± 0.40^{c}	2.56 ± 0.04^{c}	0.149 ± 0.006^{c}	5.56 ± 0.11^{ab}	248.67 ± 2.33^{b}
	DS + GB	15.21 ± 0.27^{b}	2.74 ± 0.04^{b}	0.176 ± 0.004^{bc}	5.65 ± 0.02^{a}	252.68 ± 1.20^{b}

Legend: Same as Table 1

Table 4. Chlorophyll contents (mg/g FW) of two maize cultivars as affected by exogenous application of glycinebetaine (GB) under drought

Cultivars	Treatments	Chl *a*	Chl *b*	Chl *a*+*b*	Chl *a/b*	Carotenoids
Dongdan-60	WW	2.82 ± 0.03^{a}	2.01 ± 0.03^{ab}	4.05 ± 0.03^{a}	1.40 ± 0.02^{ns}	1.96 ± 0.03^{ab}
	WW + GB	2.87 ± 0.01^{a}	2.08 ± 0.02^{a}	4.11 ± 0.01^{a}	1.37 ± 0.01	1.99 ± 0.02^{a}
	DS	2.60 ± 0.02^{c}	1.85 ± 0.04^{c}	3.71 ± 0.02^{c}	1.41 ± 0.03	1.79 ± 0.04^{c}
	DS + GB	2.71 ± 0.03^{b}	1.97 ± 0.01^{bc}	3.82 ± 0.02^{b}	1.38 ± 0.01	1.86 ± 0.01^{bc}
ND-95	WW	2.82 ± 0.02^{a}	1.99 ± 0.02^{a}	4.03 ± 0.02^{a}	1.41 ± 0.02^{ns}	1.92 ± 0.02^{a}
	WW + GB	2.84 ± 0.01^{a}	2.05 ± 0.01^{a}	4.07 ± 0.01^{a}	1.38 ± 0.01	1.93 ± 0.01^{a}
	DS	2.58 ± 0.02^{c}	1.82 ± 0.03^{c}	3.67 ± 0.03^{c}	1.42 ± 0.02	1.74 ± 0.03^{b}
	DS + GB	2.66 ± 0.03^{b}	1.90 ± 0.02^{b}	3.75 ± 0.01^{b}	1.40 ± 0.02	1.79 ± 0.02^{b}

Legend: Same as Table 1

Assimilation and partitioning of assimilates during grain development are of vital value. A declining trend in yield and yield-related parameters was observed during present experiment under drought stress. Compared with ND-95, higher drought tolerance of the cultivar Dongdan-60 was indicated by its maintenance of better yield and yield traits under water deficit environment as observed in the present study. GB-treatment alleviated the detrimental effects of drought stress with subsequent increases in yield and yield related parameters both under stress and under non-stress conditions. The improvement in yield by GB-treatment is in line with Diaz-Zarita et al. (2001) and Hussain et al. (2009), who reported that GB-treatment enhanced the yield of water stressed wheat and sunflower plants.

In our study, the reduction in gas exchange attributes under drought stress was greater in drought sensitive ND-95 than in the drought tolerant Dongdan-90. GB application enhanced photosynthesis in water-deficit experiencing plants, mostly due to a greater stomatal conductance which caused higher CO_2 diffusion inside the leaf thus favoring higher photosynthetic rate (Ma et al. 2007). GB may maintain the photosynthetic capacity not only through increasing stomatal conductance but also by maintaining chloroplast ultrastructure under drought. GB might contribute to restrict cytoplasmic dehydration and maintain leaf turgor in plants subjected to water deficit conditions (Iqbal et al. 2008), thereby maintaining high photosynthetic activities. Thus, increased photosynthetic capacity could then lead to improved capability of the plant to allocate more assimilates to developing seeds (Mäkelä et al. 1998).

The efficacy of light captured to drive photosynthesis is strongly related to the chlorophyll concentration in the leaf. The change in chlorophyll contents was used to evaluate the influence of environmental stress on plant growth and yield. Many studies indicated that high chlorophyll concentrations are associated with improved yield under water-limited conditions (Verma et al. 2004). In our study, the observed reduction of chlorophyll in water stressed plants might be due to a reduction in the lamellar content of the light harvesting chlorophyll protein (Randall et al. 1977). The decreased Chl *a*, Chl *b* and Chl *a* + *b* contents under drought stress are consistent with Anjum et al. (2011) who reported the reduced Chl *a*, Chl *b* and Chl *a* + *b* contents under progressive drought stress in maize. The decrease in chlorophyll *a* may be caused by the inhibition of biosynthesis of precursors of Chl *a* under moisture stress as reported by Makhmudov (1983). The lower Chl *a/b* values suggest that the light harvesting complexes of thylakoid membranes could be affected seriously by drought stress (Parida et al. 2003). Moreover, Cicek and Cakirlar (2008) revealed that the soybean cultivars under salt stress seemed to acclimate to the stress by decreasing their Chl *a/b* ratio.

In conclusion, Dongdan-60 was able to withstand water stress as compared to ND-95 by maintaining higher growth, yield, gas exchange, and chlorophyll concentration. Furthermore, GB-induced improvement in growth and yield under water stress was mediated through enhanced gas exchange as well as chlorophyll concentration under water stress conditions.

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