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INFLUENCE OF EXOGENOUSLY APPLIED GLYCINEBETAINE ON GROWTH AND GAS EXCHANGE CHARACTERISTICS OF MAIZE (*ZEA MAYS* L.)

Asma Maqsood, [1]Muhammad Shahbaz and Nudrat Aisha Akram
Department of Botany, University of Agriculture, Faisalabad.
[1]Corresponding author's e-mail: shahbazmuaf@yahoo.com

Many inbred lines of maize are unable to synthesize glycinebetaine (GB). The present study was conducted to assess the influence of exogenously applied GB on growth and photosynthetic attributes of maize (*Zea mays* L.). Two maize varieties were C-20 and C-77 (C stands for Comb). Exogenously applied different levels i.e. control (without spray), water spray, 50 or 100 mmol L^{-1} of glycinebetaine had no increasing or decreasing effect on shoot length, shoot fresh and dry weights. Among different gas exchange attributes only stomatal conductance (g_s) and transpiration rate (E) increased while photosynthetic rate (A), sub-stomatal CO_2 conc. (C_i) and water use efficiency (WUE) were unaffected by foliar spray of GB. Chlorophyll *a* was high at 100 m*M* of GB for C-20, whereas in case of C-77 water spray gave maximum value. There was no effect of GB on chlorophyll *b*.
Key words: Glycinebetaine, maize, photosynthetic attributes

INTRODUCTION

Environmental factors restricted the productivity and spatial distribution of plants, particularly many agronomical and horticultural crop plants of commercial importance. Among these factors, drought and salt play very significant roles in reducing agricultural production worldwide (Boyer, 1982). When plants experience the unfavorable environmental conditions associated with high levels of salt, drought or low temperature, plant cells protect themselves from the stress of high concentrations of intracellular salts by accumulating a variety of small organic metabolites that are referred to collectively as compatible solutes e.g. glycinebetaine and proline (Bohnert *et al.*, 1995).
Glycinebetaine is a zwitterionic, fully *N*-methyl-substituted derivative of Gly that is found in a large variety of microorganisms, higher plants, and animals (Rhodes and Hanson, 1993) under stress conditions. Some crops including sugar beet (*Beta vulgaris* L. var. altissima), accumulate betaine in high quantities (Beib, 1994), while others such as barley and wheat are moderate accumulators (Wyn Jones and Storey, 1981). It has been reported that certain maize (*Zea mays*) genotypes, including many inbred lines, are unable to synthesize GB because they are defective in the first step of betaine biosynthesis (Rhodes *et al.,* 1989). Whereas those can synthesize GB, the synthetic capacity is very low. For example, stressed maize plants accumulated GB only in a range of 2-5 µmol $(g\ F.W)^{-1}$ (Rhodes *et al.*, 1987, 1989), which was about 5 to 10 fold lower than that by stress tolerant plants, such as Spartina sorghum (*Sorghum bicolor*). Salt sensitive grass species such as *Zea mays* exhibit a fairly low capacity for betaine accumulation (Hitz and Hanson, 1980; Rhodes *et al.*, 1987; Storey *et al.*, 1977) and some maize genotypes lack betaine almost completely (Rhodes and Rich, 1988 and Rhodes *et al.*, 1987).
Under non-stress conditions, Makela *et al.* (1996b) investigated that plants are able to utilize foliarly applied glycinebetaine and to translocate it to almost all plant parts, especially developing organs. Thus, foliar applications may increase the levels of glycinebetaine in plants that are unable to synthesize this compound. However, at water stress levels, exogenously applied glycinebetaine to crops showed positive responses. The foliar application of a fertilizer containing glycinebetaine during the vegetative stages of wheat development enhances grain yield due to an increase in seed number per spike (Diaz-Zorita *et al.*, 2001). As some lines of maize are unable to synthesize GB so the primary objective of this study was to observe the effect of foliar application of GB on growth and photosynthetic attributes of maize. As in many studies it is accumulated under stress conditions, our hypothesis was to observe the effect under normal conditions.

MATERIALS AND METHODS

The experiment was conducted in Botanic Garden of the University of Agriculture, Faisalabad, supplied with natural conditions during August-November 2004. There were two maize varieties i.e. C-20 and C-70 (C stands for comb), four GB treatments and four replicates. Seeds were obtained from Ayub Agriculture Research Institute, Faisalabad. Four levels of GB were control (without spray), water spray, 50 or 100 mmol L^{-1} of GB. Tween 20 @ 0.1 % was used as surfactant with GB application. After eight weeks of sowing foliar application of GB was given to each plant. The data were collected after three weeks of spray and two plants were uprooted from each replicate for shoot fresh weight, shoot length and total leaf area per plant. The plants were oven dried at 65 °C until the constant weight and then computed dry weight.

Gas exchange characteristics

Measurements of net CO_2 assimilation rate (*A*), transpiration rate (*E*), stomatal conductance (g_s) and sub-stomatal CO_2 concentration (C_i) were made on fully expanded youngest leaf of eleven weeks old plants using an open system LCA-4 ADC portable infrared gas analyzer (Analytical Development Company, Hoddesdon, England). These measurements were made from 10:15 to 12:45 with the following specifications/adjustments: leaf surface area 11.35 cm^2, ambient CO_2 concentration (Cref) 352 µmol mol^{-1}, temperature of leaf chamber varied from 31.5 to 37.8 °C, leaf chamber gas flow rate (v) 251 µmol s^{-1}, Molar flow of air per unit leaf area (Us) 221.06 mol m^{-2} s^{-1}, ambient pressure 99.2 kPa, water vapor pressure into chamber ranged from 0.0006 to 0.00089 MPa, PAR (Q_{leaf}) at leaf surface was maximum upto 1048 µmol m^{-2} s^{-1}.

Chlorophyll contents

The chlorophyll *a*, *b* and total chlorophyll were determined according to the method of Arnon (1949). The fresh material i.e. 0.15 g was extracted in 10 ml of 80 % acetone and then absorbance was read at 663 and 645 nm using a spectrophotometer (Hitachi-220, Japan).

The chl. *a* and *b* were calculated by the following formulas:

Chl. *a* (mg/g) = [12.7(OD663)-2.69(OD645)]xV/1000xW

Chl. *b* (mg/g) = [22.9(OD645)-4.68(OD663)]xV/1000xW

Where

V = Volume of the extract (ml)

W = Weight of fresh leaf tissue (g)

Statistical analysis of data

Analysis of variance technique was employed for carrying out statistical analysis of data collected (Steel and Torrie, 1980). The mean values were compared with least significance difference test (LSD) following Snedecor and Cochran (1980).

RESULTS

Exogenous application of glycinebetaine had neither increasing nor decreasing effect on shoot fresh and dry weights of maize. Shoot length and varietal difference were non significant under different foliar spray levels of GB. Data for total leaf area/plant showed that effect of glycinebetaine was significant ($p \leq 0.05$) when applied exogenously. Total leaf area per plant was maximum when plants were sprayed by water in C-20 whereas in case of C-77 it was maximum under control conditions. In all other levels total leaf area/plant remained almost same (Fig. 1).

Chlorophyll *a* contents of both varieties affected significantly by exogenous foliar levels of GB. But these levels had no effect on chlorophyll *b* of both varieties as interaction of GB vs varieties for chlorophyll *b* was non significant. Varietal difference and GB alone had not increasing or decreasing effect on both chlorophyll *a* and *b* contents. Highest level of GB i. e. 100 mmol/L was prominent as maximum chlorophyll *a* concentration was observed at this level in C-20. Whereas in case of C-77 water spray gave highest chlorophyll *a* contents i.e. 0.932 mg g^{-1}. GB foliar application had non-significant effect on total chlorophyll (Fig. 1).

Foliarly applied GB had non-significant effect on photosynthetic rate. Varieties also did not differ significantly in this attribute. Various exogenous levels of GB had significantly increased transpiration rate. But varietal difference was non significant. Maximum transpiration rate was observed at the highest levels of GB i.e. 50 and 100 mmol/L in both varieties. Stomatal conductance increased significantly by GB application, but varieties did not differ significantly. GB level of 100 mmol/L had maximum increased value for stomatal conductance as compared to all other levels.

In present study, analyses of variance of data show that sub-stomatal CO_2 concentration, water use efficiency and C_i/C_a ratio remained unaffected by foliar application of GB. Varieties also showed uniform behavior under different GB levels (Fig. 2).

DISCUSSION

In present study shoot fresh and dry weights were not affected by foliar application of different levels of glycinebetaine. However, Hewer (2003) reported that fresh weights for both roots and shoots decreased in rice with the application of GB. In contrast, it was also investigated that shoot fresh weight of rice variety Pokkali and shoot and root dry weights of IR-28 increased by GB application (Damaral and Turkan, 2005). Our results for leaf area supported to the study of Lopez *et al.* (2002). They found that leaf area was increased significantly in unstressed plants when 10 m*M* glycinebetaine was applied but at 30 m*M* glycinebetaine produced no increase in leaf area. There are some reports about plants ability to utilize foliar-applied glycinebetaine and translocate it to

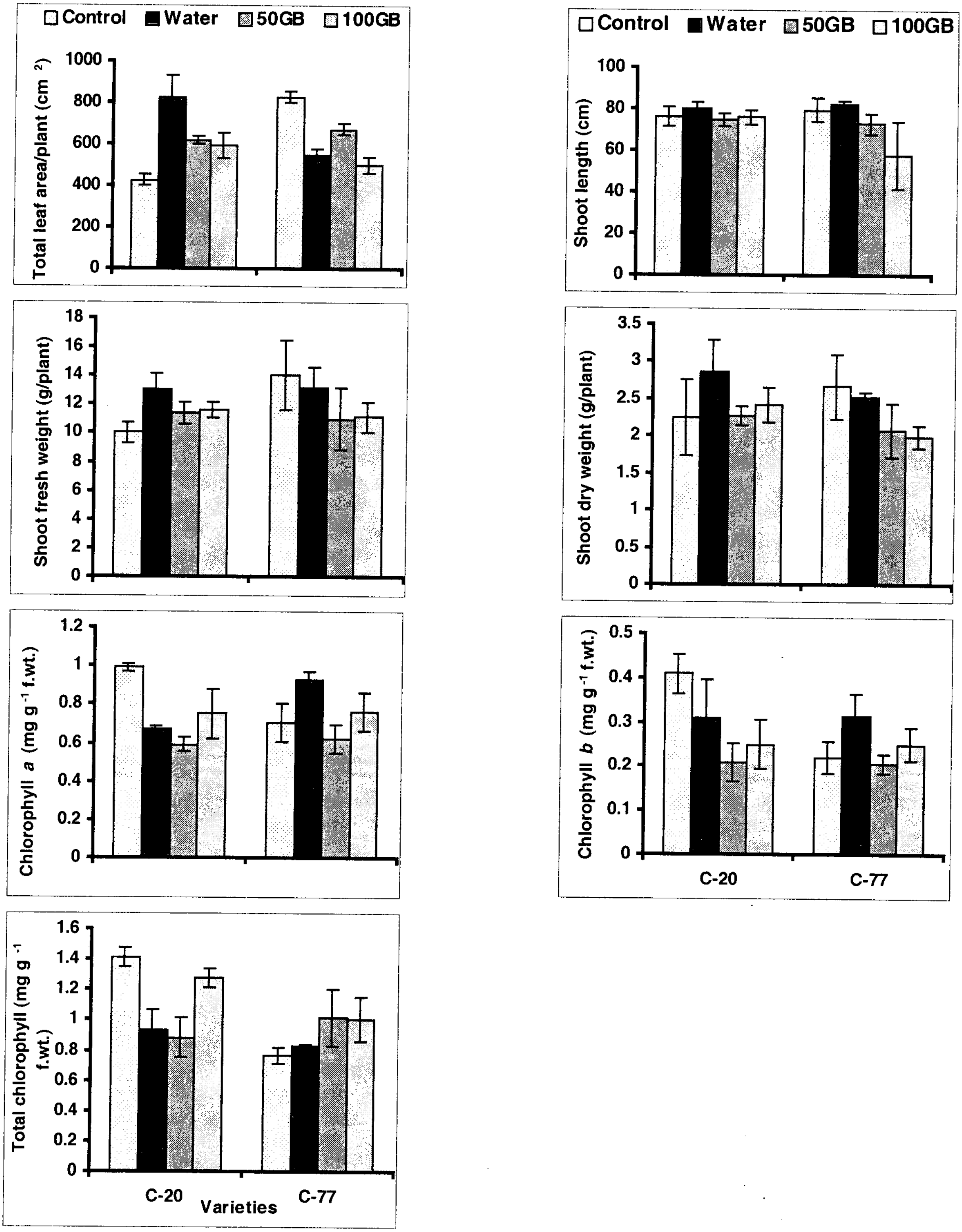

Fig. 1. Growth parameters and chlorophyll contents of maize (*Zea mays* L.) when 56-day old plants were subjected to various levels of foliar application of glycinebetaine (GB)
0 m*M* GB ☐ Water spray ■ 50 m*M* GB ▨ 100 m*M* GB ☐

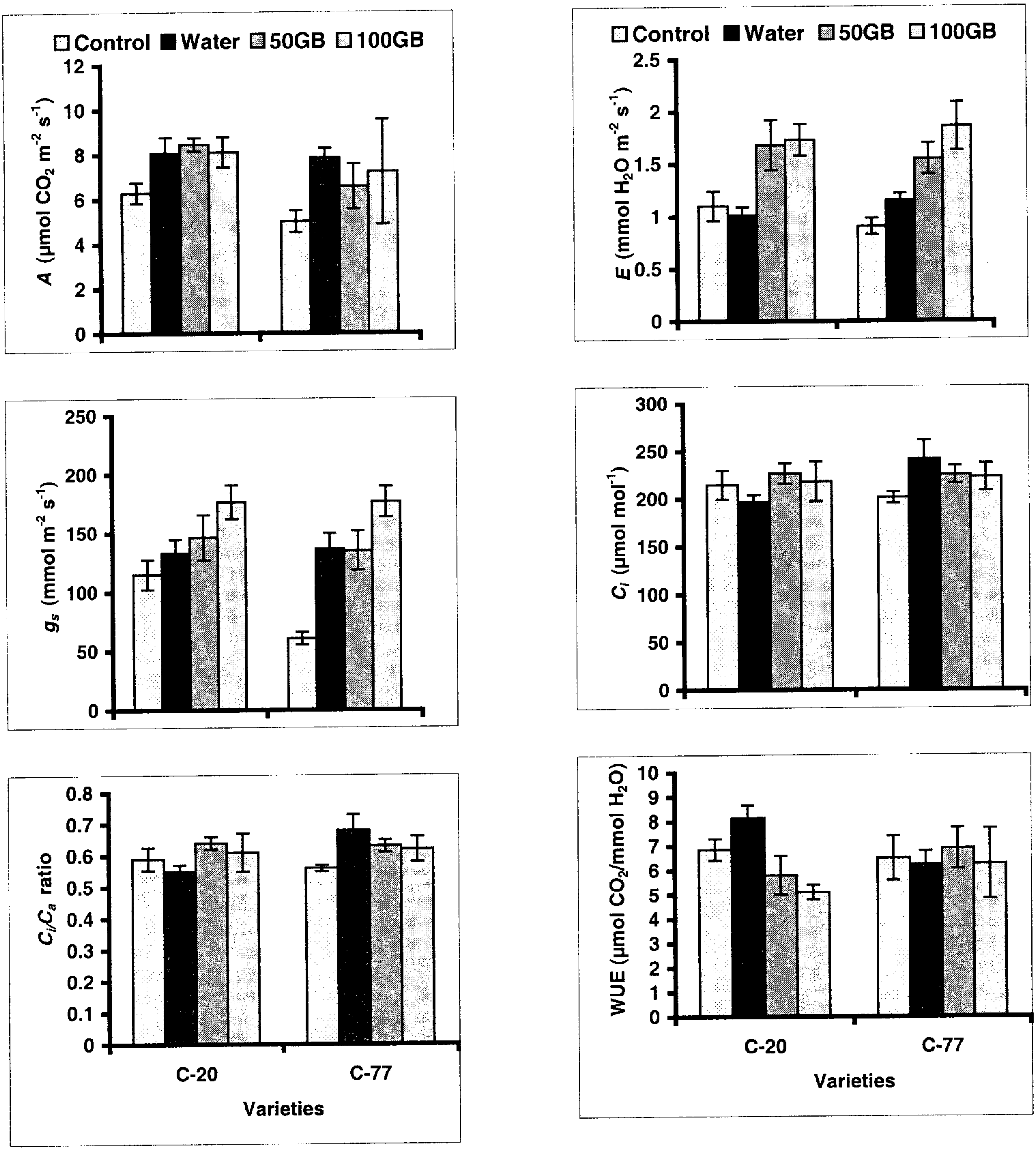

Fig. 2. Photosynthetic attributes of maize (*Zea mays* L.) when 56-day old plants were subjected to various levels of foliar application of glycinebetaine (GB).
0 m*M* GB ☐ Water spray ■ 50 m*M* GB ▨ 100 m*M* GB ☐

almost all plant parts, especially developing organs (Makela *et al.*, 1996b). Thus, foliar application may increase the levels of glycinebetaine in plants that are unable to synthesize this compound. The application of exogenous glycinebetaine to crops at a range of water stress levels showed positive responses examined in glasshouse experiments with tobacco (*Nicotiana tobaccum*), increases in the leaf area and in the fresh and dry weights of leaves were reported by Agboma *et al.* (1997b). Makela *et al.* (1997) found an increased in the growth rates of turnip rape (*Brassica rapa* sp. oleifera) and pea (*Pisum sativum*). Similar results were reported for soybean (*Glycine max* L. Merr.), where exogenous glycinebetaine tended to increase photosynthetic activity, nitrogen (N) fixation, leaf area and seed yield (Agboma *et al.*, 1997c).

There are certain reports that showed the profound effect of GB on chlorophyll contents. In maize lines, GB enhances membrane stability and chlorophyll fluorescence (Yang *et al.*, 1996). In tomato GB increased protein and chlorophyll contents (Makela *et al.*, 2000). Our results for chl. *a* and *b* were not agreed to earlier study but total chlorophyll was affected by foliar application of glycinebetaine. The chlorophyll content of tomato leaves increased upto 18% due to glycinebetaine application especially in well-watered plants with a 100 m*M* conc. of glycinebetaine. Whapman *et al.* (1993) have reported an increase in the chlorophyll content of tomato leaves when treated with seaweed extracts containing glycinebetaine and other betaines.

Makela *et al.* (1996) examined the effect of foliarly applied GB in enhancing stomatal conductance and solutes concentration in stress plants. The water use efficiency of GB-accumulated plants is indicated by the low *E* with the high stomatal resistance (rs) or low G as stomata closure, causing on high LWC under drought/salt-stress conditions (Yordanov *et al.*, 2001 and Lopez *et al.*, 2002). Several studies had shown that engineering of enhanced GB synthesis could improve drought and chilling tolerance in maize plants (Quan *et al.*, 2004). Exogenously applied GB increases salt tolerance (Nomura *et al.*, 1995; Lilius *et al.*, 1996; Hayashi *et al.*, 1997; Makela *et al.*, 1998) in non accumulating plants and microbes. High concentrations of GB do not interfere with cytoplasmic functions and it efficiently stabilizes the structure and function of many macromolecules. Thus, it belongs to a group of compounds that are known collectively as compatible solutes. It is an extremely efficient compatible solute (Le Rudulier *et al.*, 1984) and its presence is strongly associated with the growth of plants in dry and/or saline environments (Rhodes and Hanson, 1993).

It has been well documented that, *in vitro,* GB stabilizes the structures and activities of enzymes and protein complexes and maintains the integrity of membranes against the damaging effects of excessive salt, cold, heat and freezing (Gorham, 1995). The issue was reviewed recently, from the respective, protective effect of GB on the photosynthetic machinery, by Papageorgiou and Murata (1995). Foliar-applied GB result in increased stomatal conductance (Makela *et al.*, 1998) and net photosynthesis and probably thus better in growth (Makela *et al.*, 1997). But in present study, overall exogenous applied GB has little useful effect on maize, particularly in stomatal conductance and transpiration rate. While in most of parameters it remains uneffective.

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