Pak. J. Bot., 39(5): 1647-1653, 2007.

IMPROVEMENT IN SALT TOLERANCE OF MAIZE BY EXOGENOUS APPLICATION OF GLYCINEBETAINE: GROWTH AND WATER RELATIONS

KHALID NAWAZ AND *MUHAMMAD ASHRAF

Department of Botany,
University of Agriculture, Faisalabad, Pakistan

Abstract

Effect of exogenous glycinebetaine (0, 50, and 100 m*M*) applied at different growth stages i.e., vegetative, reproductive and both at the vegetative and reproductive stages on growth and water relations was assessed in the plants of two maize cultivars, Golden and C-20 under salt stress. Salt stress impaired growth by reducing plant fresh and dry biomass of both maize cultivars. Imposition of salt stress also had adverse effects on leaf water potential, leaf osmotic potential and leaf turgor potential. However, salt induced reduction in growth of both maize cultivars was ameliorated by exogenously applied GB. Furthermore, GB applied at the vegetative growth stage was more effective in ameliorating the adverse effects of salt stress on both maize cultivars than when applied at the reproductive stage or at both the vegetative and reproductive growth stages. Leaf water potential and leaf turgor potential of the salt stressed plants of both cultivars were increased due to foliar application of GB. The ameliorative effect of GB on growth of both maize cultivars under saline conditions was due to GB-induced improvement in plant water status. The adverse effects of salt stress on maize can be alleviated by the exogenous application of GB at different growth stages by modulating water relations.

Introduction

Salt stress is one of the major abiotic stresses that suppress crop growth (Ashraf, 2004). This reduction in growth observed in many plants subjected to salinity stress is often correlated with salt-induced osmotic effect, nutrient deficiency or specific ion toxicity (Munns, 2002). To acclimatize adverse environmental conditions such as salt stress, plants have evolved a number of strategies including the accumulation of low molecular weight compounds known as compatible solutes (Hasegawa *et al.*, 2000). Glycinebetaine is one of several such compatible solutes that act as osmoprotectants. Glycinebetaine is known to increase stress tolerance in most crop plants. However, in many crop plants the natural synthesis/accumulation of GB is lower than the requisite level to ameliorate the adverse effects of osmotic stress caused by various environmental stresses (Yancey, 1994; Subbarao *et al.*, 2001). Although the introduction of the GB biosynthesis pathway into non-accumulating species by gene engineering has been successful (Sakamoto & Murata, 2002), the transgenic plants can accumulate low levels of GB as compared to GB accumulators (Sakamoto & Murata, 2000). With these reasons in minds a number of scientists have proposed a shot-gun approach by which enhanced internal GB level in plants could be achieved through exogenous application of GB (Harinasut *et al.*, 1996; Hayashi *et al.*, 1998a; Mäkela *et al.*, 1999; Ashraf & Foolad, 2007).

*Corresponding author e-mail: ashrafbot@yahoo.com

Exogenous application of GB to low-accumulating or non-accumulating plants may help reduce the adverse effects of environmental stresses (Mäkela *et al.*, 1998; Yang & Lu, 2005). Positive effects of exogenous application of GB on plant growth and final crop yield under salt stress have been reported in a number of crops such as tobacco, wheat, maize, barley, sorghum, soybean and common beans (Ashraf & Foolad, 2007). Exogenous application of GB increased the salt-tolerance ability of rice seedlings in terms of water relations, pigment stabilization, water oxidation and CO_2 assimilation, leading to an improvement in photosynthetic rate, chlorophyll concentration and growth characteristics (Gadallah, 2000; Cha-um *et al.*, 2006). Recently, Ashraf & Foolad (2007) suggested that effectiveness of foliar applied glycinebetaine depends on a number of factors including type of species, plant developmental stage at which it is applied, concentration of GB and number of applications.

Keeping in view the above reports on the role of exogenous GB on various plant species there is a need for better understanding of GB mechanism of action and the magnitude of its effects in different plant species to improve crop stress tolerance. Thus, the primary objective of carrying out the present study was to assess that up to what extent GB applied exogenously at different growth stages could ameliorate the adverse effects of salt stress on maize.

Materials and Methods

Seed material of two maize cultivars, Golden and C-20 was obtained from the Department of Plant Breeding and Genetics, University of Agriculture, Faisalabad, Pakistan. A greenhouse (sand culture) experiment was conducted in the Botanical Garden of the University of Agriculture, Faisalabad, Pakistan in a net house under natural sunlight during the appropriate growth season with the following environmental conditions: average rainfall 49 ± 1.22 mm and the maximum and minimum relative humidity 52 and 45%, respectively and the average maximum and minimum temperatures 35 ± 1.60 and 21 ± 2.26 °C, respectively. The sand was washed well with sufficient amount of tap water. Ten seeds of each cultivar were sown in each pot. Two liters of full strength Hoagland's nutrient solution were applied on alternate days to each pot. This volume was sufficient to flush through all the salts previously present in the sand. After 14 days of germination, plants were thinned to five plants per pot. The salt (NaCl) treatment (10 dS m^{-1}) in full strength Hoagland's nutrient solution was applied after 21 days of sowing. The salt concentration was increased step-wise by applying 5 dS m^{-1} solution on alternate days until the appropriate concentration was attained. Glycinebetaine treatments (0, 50 and 100 m*M* in 0.1% Tween-20 solution) were applied as a foliar spray to the maize plants at different growth stages i.e., vegetative, reproductive and both vegetative and reproductive stages. The experiment was laid out in a completely randomized design (CRD) with four factors. There were four replicates for each treatment. After two weeks of foliar application of GB at the reproductive stage two plants from each pot were uprooted carefully and washed with distilled water. Roots were carefully removed and fresh biomass of shoots and roots of the blotted dry plants were recorded. The plant samples were then oven-dried at 65ºC for one week and dry weights recorded. Before harvest, leaf water potential, leaf osmotic potential and leaf turgor potential were measured in the second leaf from top of each plant

Water relations: The second leaf from top (fully expanded youngest leaf) was excised at 6:30 to 8:30 a.m. to determine the leaf water potential with a Scholander type pressure chamber (Arimad-2-Japan). A proportion of the same leaf used for water potential measurements, was frozen into 2 cm^3 polypropylene tubes at −40 °C in an ultra-low freezer for 2 weeks, after which time plant material was thawed and the frozen sap was extracted by crushing the material with a glass rod. After centrifugation (8000 × *g*) for 4 min, the sap was directly used for osmotic potential determination using a vapor pressure osmometer (Wescor 5500). Leaf turgor pressure was calculated as the difference between leaf water potential and leaf osmotic potential values. Leaf osmotic potential values were corrected for the dilution of the symplastic sap by apoplastic water, which occurs when sap is expressed. Apoplastic water was considered 10% following Wilson *et al.*, (1980).

Statistical Analysis of the data: Four factor completely randomized design (Analysis of variance technique) of the data was computed for all attributes by using the MSTAT Computer Program (MSTAT Development Team, 1989). Four factors were varieties, salt treatments, growth stages and different levels of glycinebetaine. Bar graph using mean ± S.E values was drawn by using Microsoft Excel software. The Duncan's New Multiple Range test at 5% level of probability was used to test the differences among mean values following Snedecor & Cochran (1980).

Results and Discussion

Salt stress had an inhibitory effect on growth (total plant fresh and dry biomass) of two maize cultivars. But cultivars differed significantly i.e., cv. Golden higher in plant fresh and dry biomass than cv. C-20 (Fig. 1a) being glycinebetine applied at different growth stages improved plant fresh and dry biomass production under saline conditions, but GB applied at the vegetative stage was very effective in improving plant dry weight of salt-stressed plants of both cultivars.

The results for different growth attributes of two maize cultivars show that salt stress caused a marked reduction in growth of both maize cultivars. However cultivars differed significantly in their response to salt stress i.e., cv. Golden being more tolerant to salt than cv. C-20. However, adverse effects of salt on the growth of maize plants were mitigated by the foliar application of GB. These results are in agreement with some earlier findings in which a significant improvement in salt tolerance of maize plants was reported due to foliar application of GB (Yang & Lu, 2005).

In the present study, the alleviating effect of foliar application of GB varied when applied at different growth stages. GB applied at the vegetative growth stage was more effective in improving the growth of both maize cultivars under salt stress than when applied at other growth stages.

Salt stress caused a significant reduction in all leaf water relation parameters such as water potential, osmotic potential and turgor potential of both cultivars (Fig. 2). Different levels of GB i.e., 50 m*M* and 100 m*M* increased (less negative values) leaf water potential in both cultivars, particularly under saline conditions. GB applied at the vegetative stage particularly 50 m*M* was effective in reducing leaf osmotic potential of both stressed and non-stressed plants, but no difference in this variable was discerned when GB applied at other growth stages. Exogenous application of different levels of GB enhanced leaf turgor potential of salt stresses plants of both maize cultivars. However, the GB level 50 m*M* applied at the vegetative stage was more effective than 100 m*M* in increasing leaf turgor potential of both maize cultivars under normal and saline conditions.

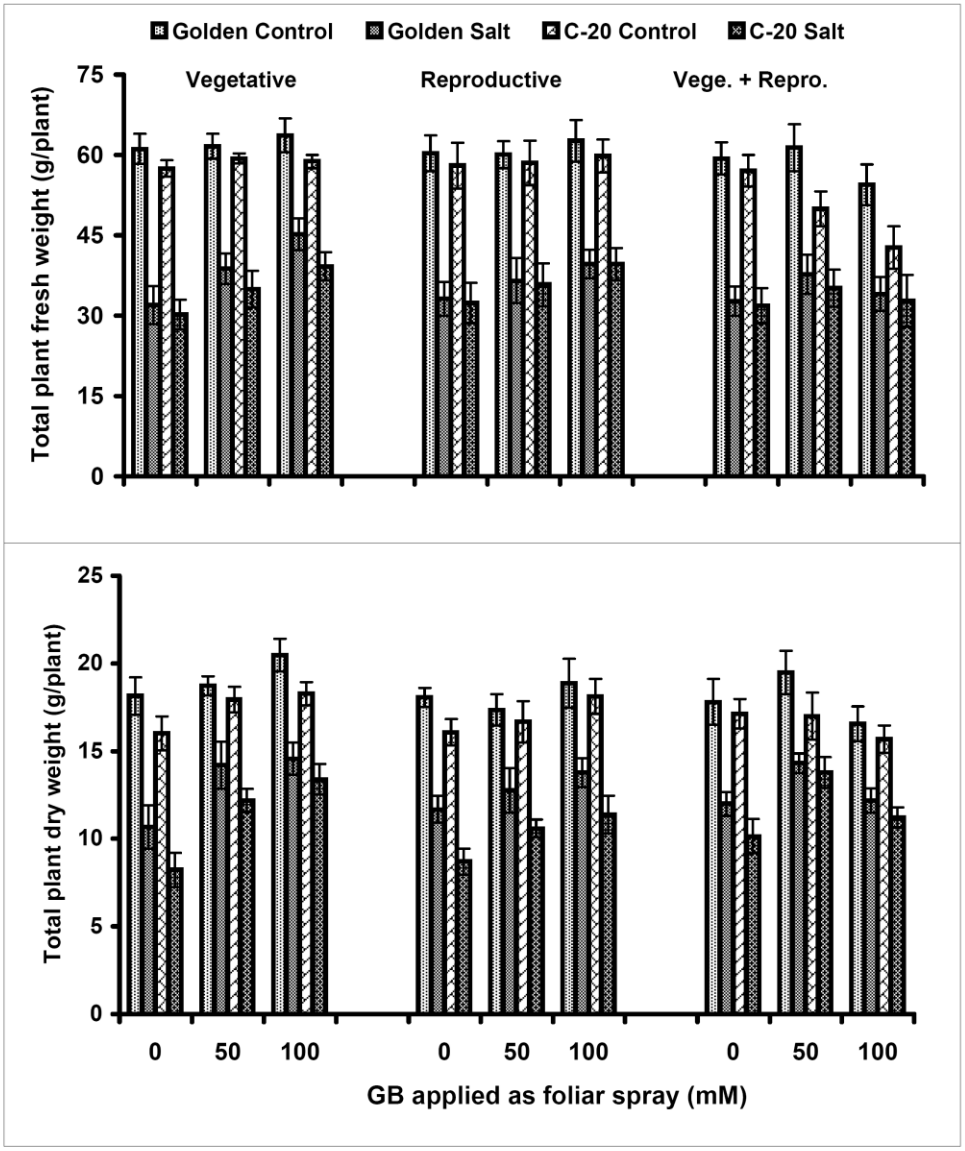

Fig. 1. Total plant fresh and dry weight of two maize (*Zea mays* L.) cultivars when different levels of GB were applied as foliar spray to salt-stressed or non-stressed plants at the vegetative, reproductive, or the vegetative and reproductive stages.

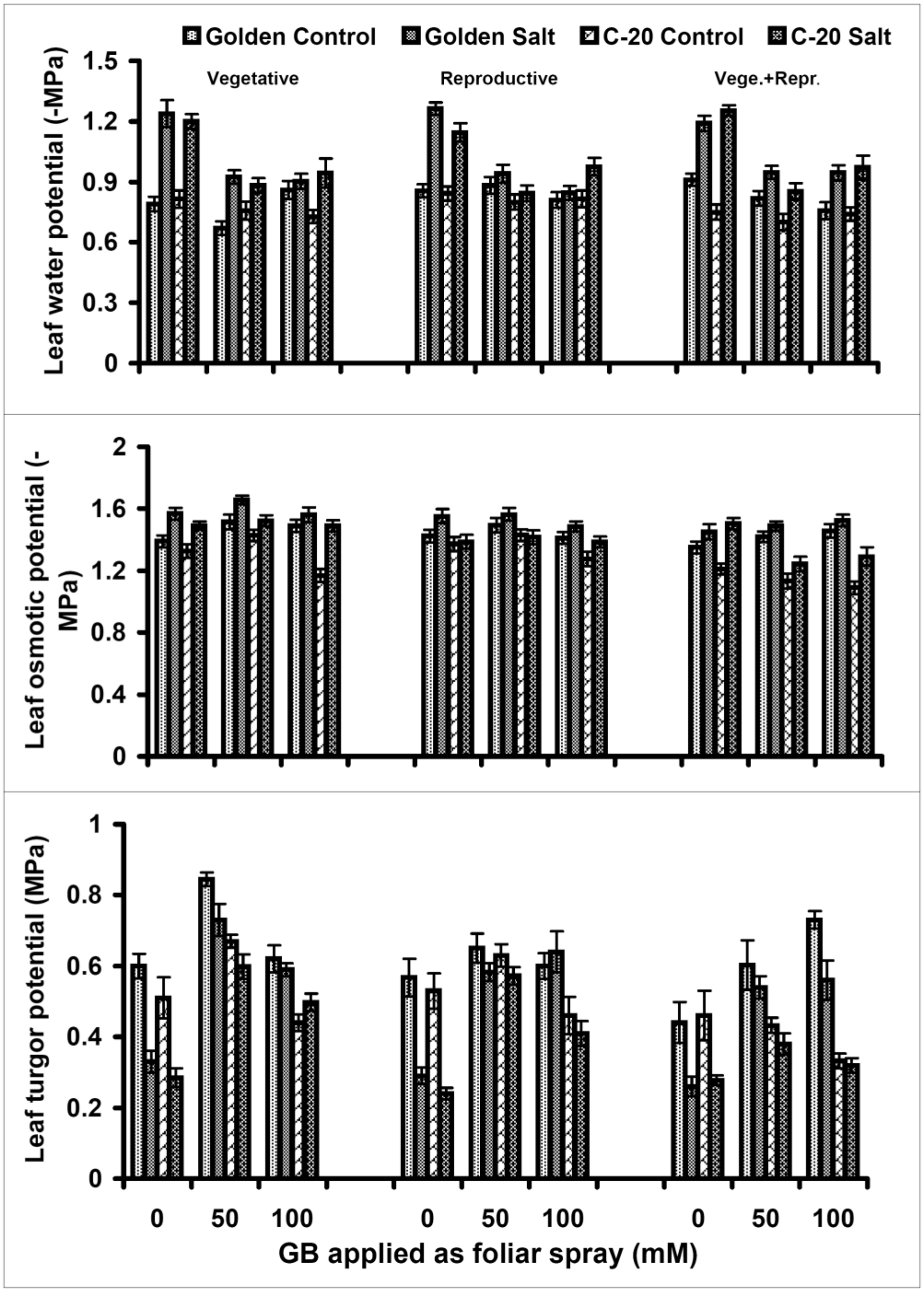

Fig. 2. Leaf water relation components of two maize (*Zea mays* L.) cultivars when different levels of GB were applied as foliar spray to salt-stressed or non-stressed plants at the vegetative, reproductive, or the vegetative and reproductive stages.

Water potential, solute potential and turgor potential are inter-related in plant cells and are markedly affected when plants are exposed to salt stress. In the present study, salt stress also had an adverse effect on water relation parameters of the two maize cultivars. Foliar application of GB improved leaf water potential and leaf turgor potential, whereas leaf osmotic potential slightly decreased in the salt stressed plants due to GB application. It is well known that plants under saline conditions can maintain their turgor by osmotic adjustment, which is one of the common mechanisms of salinity resistance in crop plants (Hernandez & Almansa, 2002; Chaparzadeh *et al.*, 2003). Although accumulation of GB increased in both non-stressed and salt stressed plants due to foliarly applied GB (data not shown), leaf osmotic potential was not greatly changed due to accumulation of GB. From these findings, it is plausible to propose that changes in GB accumulation caused slight changes in leaf osmotic potential which resulted in improved leaf turgor potential and thus contribute in osmoregulatory process. Furthermore, GB might also have a protective role in preventing cell injury from salt stress as has earlier been reported in some studies on different crops e.g., in *Trifolium alexandrinum* (Varshney *et al.*, 1988), *Triticum*, *Agropyron* and *Elymus* (Wyn Jones *et al.*, 1984).

Overall, foliar application of GB was found to be effective in mitigating the adverse effects of salinity on growth of both maize cultivars. In addition, foliar application of GB when applied at the vegetative stage was more effective than applied at the other growth stages. Although exogenous application of GB caused slight changes in leaf osmotic potential, it significantly improved plant water status which might have contributed to better growth of both maize cultivars under salt stress.

References

Anonymous. 1989. MSTAT user's guide: A microcomputer programme for the design management and analysis of agronomic research experiments. Michigan State Univ. East Lansing, USA.

Ashraf, M. 2004. Some important physiological selection criteria for salt tolerance in plants. *Flora*, 199: 361-376.

Ashraf, M. and M.R. Foolad. 2007. Improving plant abiotic-stress resistance by exogenous application of osmoprotectants glycinebetaine and proline. *Env. Exp. Bot*., 59: 206-216.

Chaparzadeh, N., R.A. Khavari-Nejad, F. Navari-Izzo and A. Izzo. 2003. Water relations and ionic balance *Calendula oficinalis* L. under salinity conditions. *Agrochimica*, 47: 69-79.

Cha-um, S., K. Supaibulwatana and C. Kirdmanee. 2006. Water relation, photosynthetic ability and growth of Thai Jasmine rice (*Oryza sativa* L. sp. indica cv. KDML 105) to salt stress by application of exogenous glycinebetaine and choline. *J. Agron. Crop Sci*., 192: 25-36.

Gadallah, M.A.A. 2000. Effects of acid mist and ascorbic acid treatment on the growth, stability of leaf membranes, chlorophyll content and some mineral elements of *Carthamus tinctorius*, the safflower. *Water Air Soil Pollut*., 118: 311-327.

Harinasut, P., K. Tsutsui, T. Takabe, M. Nomura, T. Takabe and S. Kishitani. 1996. Exogenous glycinebetaine accumulation and increased salt-tolerance in rice seedlings. *Biosci. Biotechnol. Biochem*., 60: 366-368.

Hasegawa, P.M., R.A. Bressan, J.K. Zhu and H.J. Bohnert. 2000. Plant cellular and molecular response to high salinity. *Annu. Rev. Plant Physiol. Plant Mol. Biol*., 51: 463-499.

Hayashi, H.A., A. Sakamoto, H. Nonaka, T.H.H. Chen and N. Murata. 1998a. Enhanced germination under salt conditions of seeds of transgenic *Arabidopsis* with bacterial gene (*CodA)* for choline oxidase. *J. Plant Res*., 111: 357-362.

Hernandez, J.A. and M.S. Almansa. 2002. Short-term effects of salt stress on antioxidant systems and leaf water relations of pea leaves. *Physiol. Plant*., 115: 251-257.

Mäkela, P., M. Kontturi, E. Pehu and S. Somersalo. 1999. Photosynthetic response of drought and salt stressed tomato and turnip rape plants to foliarly applied glycinebetaine. *Physiol. Plant*., 105: 45-50.

Mäkela, P., R. Munns, T.D. Colmer, A.G. Condon and P. Peltonen-Sainio. 1998. Effect of foliar applications of glycinebetaine on stomatal conductance, abscisic acid and solute concentrations in leaves of salt-or drought-stressed tomato. *Aust. J. Plant Physiol*., 25: 655-663.

Munns, R. 2002. Comparative physiology of salt and water stress. *Plant Cell Environ*., 25: 239-250.

Sakamoto, A. and N. Murata. 2000. Genetic engineering of glycinebetaine synthesis in plants: current status and implications for enhancement of some stress tolerance. *J. Exp. Bot*., 51: 81-88.

Snedecor, G.W. and W.G. Cochran. 1980. *Statistical Methods*. 7th Ed. The Iowa State University Press, Ames, IOWA.

Subbarao, G.V., R.M. Wheeler, L.H. Levine and G.W. Stutte. 2001. Glycinebetaine accumulation, ionic and water relations of red-beet at contrasting levels of sodium supply. *J. Plant Physiol*., 158: 767-776.

Varshney, K.A., L.P. Gangwar and N. Goel. 1988. Choline and betaine accumulation in *Trifolium alexandrinum* L. during salt stress. *Egypt J. Bot*., 31: 81-86.

Wilson, J.R., M.M. Ludlow, M.J. Fisher and E.D. Schulze. 1980. Adaptation to water stress of leaf water relations characteristics of some tropical forage grasses and legumes in a semi-arid environment. *Aust. J. Plant Physiol.*, 7: 207-220.

Wyn Jones, R.G., J. Gorham and E. McDonnell. 1984. Organic and inorganic solute contents as selection criteria for salt tolerance in the Triticeae. In: Staples, R. and G.H. Toennissen (Eds.), Salinity Tolerance in Plants: Strategies for Crop Improvement. Wiley and Sons, New York, pp. 189-203.

Yancey, P.H. 1994. Compatible and counteracting solutes. In: *Cellular* and *Molecular Physiology of Cell Volume Regulation*. (Ed.): K. Strange. CRC Press, Boca Raton, FL. pp. 81-109.

Yang, X. and C. Lu. 2005. Photosynthesis is improved by exogenous glycinebetaine in salt stressed maize plants. *Physiol. Plant*., 124: 343-352.

(Received for publication 20 June 2007)