

<sup>1</sup>Department of Botany, University of Agriculture, Faisalabad, Pakistan

<sup>2</sup>Second affiliation: King Saud University, Riyadh, Saudi Arabia

<sup>3</sup>Department of Botany and Microbiology, King Saud University, Riyadh, Saudi Arabia

## Modulation of growth parameters, proline content and mineral nutrients in maize (*Zea mays* L.) by exogenously applied glycinebetaine at different growth stages under salt stress

Khalid Nawaz<sup>1</sup>, Muhammad Ashraf<sup>1,2</sup>, \*Nudrat Aisha Akram<sup>1</sup>, F. Al-Qurainy<sup>3</sup>

(Received March 20, 2010)

### Summary

Effect of glycinebetaine (0, 50 and 100 mM) applied as a foliar spray at different growth stages i.e. vegetative, reproductive and both at the vegetative and reproductive stages on growth, proline accumulation and ion accumulation was investigated in plants of two maize cultivars under saline conditions. Salt stress caused a significant reduction in growth, leaves and roots  $K^+$  and  $Ca^{2+}$  while,  $Na^+$  and  $Cl^-$  concentrations of both maize cultivars was significantly increased due to salt stress. Foliar applied glycinebetaine at the vegetative stage was more effective in ameliorating the adverse effects of salt stress than when applied at the reproductive or at both vegetative and reproductive stages. Foliar applied glycinebetaine did not change the leaf proline concentration in the salt stressed plants of both cultivars. However, exogenously applied glycinebetaine significantly reduced  $Na^+$  accumulation in the roots and leaves, while that of  $K^+$  increased, thereby resulting in higher  $K^+/Na^+$  and  $Ca^{2+}/Na^+$  ratios under saline conditions.

### Introduction

Many plants accumulate low molecular weight compatible solutes when exposed to different environmental stresses including salt stress (GRIEVE and MASS, 1984; PARK et al., 1995). Glycinebetaine (GB) is one of widely known compatible solutes that accumulate in a number of plants grown under stress conditions. GB is actively involved in stress tolerance of plants, because it acts as a potential osmoprotectant in addition to a number of roles it plays in plant metabolism (ASHRAF and FOOLAD, 2007). GB is known to regulate a number of physiological processes in plants subjected to salt stress such as stomatal conductance, transpiration (AGBOMA et al., 1997a; RAZA et al., 2006), ion homeostasis (CUIN and SHABALA, 2005; RAZA et al., 2007), changes in antioxidant capacity (TÜRKAN and DEMIRAL, 2004; RAZA et al., 2007).

However, it has been observed that in many crop plants the inherent synthesis/accumulation of GB is lower than the desired level to ameliorate the adverse effects of osmotic stress caused by various environmental stresses (SUBBARAO et al., 2001; ASHRAF and FOOLAD, 2007). Although biosynthetic pathway of GB was promoted in some non-accumulating species by genetic engineering, transgenic plants still were not capable of producing GB levels equivalent to those in natural GB accumulators (SAKAMOTO and MURATA, 2000; 2002). With these results in mind, a number of scientists proposed a shotgun approach where enhanced internal GB level in plants could be achieved through exogenous application of GB (HARINASUT et al., 1996; MÄKELA et al., 1999; RAZA et al., 2006; 2007; ASHRAF and FOOLAD, 2007). There is accumulating evidence that exogenous application of GB to low-accumulating or non-accumulating plants may help to reduce the adverse effects of environmental stresses (MÄKELA et al., 1998; YANG and LU, 2005; 2006; CHEN and MURATA, 2008; BANU et al., 2009). Uptake and accumulation of different ions in plants under salt stress are also affected due to exogenous application

of GB. For example, exogenous application of GB enhanced endogenous level of GB and  $K^+$  in wheat. Higher endogenous GB caused an increase in  $K^+$  and a decrease in  $Na^+$  accumulation in the leaves and roots of wheat plants. This phenomenon led to a better maintenance of  $K^+/Na^+$  and  $Ca^{2+}/Na^+$  ratios in the shoots (RAZA et al., 2007). In tomato, exogenous application of GB resulted in reduced accumulation of  $Na^+$  and  $Cl^-$  (HEUER, 2003).

Proline accumulation is one of the most important modifications induced by salt stress in plants, and often considered to be involved in stress resistance (MISRA and SAXENA, 2009). It plays a substantial role in osmotic adjustment under saline conditions (HYUN et al., 2003; KHEDR et al., 2003; AKRAM et al., 2006). Enhanced salt tolerance (KHEDR et al., 2003; NOREEN and ASHRAF, 2009) by stabilization of proteins, prevention of denaturation of enzymes, acting as a hydroxyl radical scavenger (HSU et al., 2003), protection of protein turnover machinery, and up-regulation of stress protective proteins (MISRA and SAXENA, 2009) have been attributed to high accumulation of proline.

Certain maize cultivars, including many inbred lines are unable to synthesize GB because they are defective in the first step of betaine biosynthesis (RHODES et al., 1989). Although some maize cultivars can accumulate GB, the synthetic capacity is relatively low. The accumulation of GB in some stressed maize plants is only in the range of 2-5 mmol  $g^{-1}$  fresh weight which is about 10-fold lower than those in stress-tolerant plants (GRIEVE and MAAS, 1984; KISHITANI et al., 1994; ALLARD et al., 1998). Keeping in view the above mentioned reports about 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 improving stress tolerance in different plant species. One of the most common modes of exogenous application of GB i.e., foliar application may increase the levels of glycinebetaine in plants that are unable to synthesize this compound. Therefore, foliar application of GB seems to be an effective and efficient alternative to genetic engineering to improve crop productivity under stress conditions.

Thus, the primary objective of the present study was to assess up to what extent GB applied exogenously at different growth stages could ameliorate the adverse effects of salt stress on maize. It is not known if exogenous GB application has beneficial effects on ion uptake in maize. A further aim was to determine whether exogenous GB applied as a foliar spray could regulate ion homeostasis in maize plants subjected to salt stress.

### Materials and methods

The seeds of two maize cultivars, Golden and C-20 were obtained from the Department of Plant Breeding and Genetics, University of Agriculture, Faisalabad, Pakistan. A sand culture pot experiment was conducted in the Botanic Garden of the University of Agriculture, Faisalabad, Pakistan (latitude = 31°- 30' N, longitude = 73°- 10' E and altitude = 184.4m) in a nethouse under natural sunlight during August-November, 2004-05 and 2005-06 where the average rainfall was  $49 \pm 1.22$  mm, maximum and minimum relative humidity 62%

\*Corresponding author

and 55%, and average temperatures were  $35 \pm 1.60$  and  $21 \pm 2.26$  °C, respectively. River sand was used as a growth medium. Ten seeds were sown per plastic pot (24.5 cm diameter and 28 cm deep), each containing 10 kg river sand. The sand was washed well with sufficient amount of tap water. Two liters of full strength Hoagland's nutrient solution were applied on alternate days to each pot so as to flush through all the salts previously present in the sand.

Salinity (NaCl) treatment (100 mM NaCl) was applied in full strength Hoagland's nutrient solution after 21 days of plants germination. Each time, two liters of each treatment solution were applied in the evening. Glycinebetaine (GB) treatments (0, 50 and 100 mM in 0.1% Tween-20 solution) were exogenously applied as a foliar spray to plants at different growth stages, i.e., vegetative (28-day old plants), reproductive (77-day old plants), and both vegetative and reproductive stages (28- and 77-day old plants). The experiment was laid out in a completely randomized design (CRD) with four factors. There were four replicates for each treatment. Two plants (91-day old) from each pot were uprooted carefully and washed with distilled water. After drying with blotting paper, roots were carefully removed, then the plant samples were oven-dried at 65 °C for one week and dry weights of both shoots and roots recorded.

#### Determination of mineral elements

The dried ground leaf or root material (0.1 g) was digested with sulphuric acid and hydrogen peroxide following WOLF (1982). After digestion, the volume of each sample was made up to 100 ml with distilled de-ionized water. Ions, i.e.,  $\text{Na}^+$ ,  $\text{K}^+$  and  $\text{Ca}^{2+}$  were determined with a flame photometer (Jenway PFP7). For  $\text{Cl}^-$  analysis, the ground leaf or root material (0.1 g) was extracted in 10 ml distilled water at 80 °C for 4 h. The  $\text{Cl}^-$  content was determined with a chloride analyzer (Model 926, Sherwood Scientific Ltd., Cambridge, UK).

#### Proline determination

Proline in the leaves was determined according to the method of BATES et al. (1973) after extraction at room temperature with 3% 5-sulfosalicylic acid solution. The proline concentration was determined from a standard curve and calculated on fresh weight basis.

#### Statistical Analysis

The data for all variables were computed using the MSTAT Computer Program (MSTAT Development Team, 1989) for working out analysis of variance. Four factors were cultivars, salt treatments, growth stages and different levels of glycinebetaine. Bar graphs using mean  $\pm$  S.E values were drawn using Microsoft Excel Software.

## Results

Salinity had a detrimental effect on growth (shoot and root dry biomass, leaf area, and number of leaves per plants (Fig. 1A-D) of two maize cultivars. However, cv. Golden had higher shoot dry biomass than that of cv. C-20 (Fig. 1A). Exogenously applied glycinebetaine (GB) improved almost all growth components, particularly leaf area, and shoot dry biomass production under saline conditions. Although both 50 and 100 mM GB levels applied foliarly had almost similar increasing effect on the growth of both cultivars under saline conditions, GB applied at the vegetative stage proved to be very effective in enhancing shoot dry weight of salt-stressed plants of both cultivars. Likewise, the effect GB applied at the vegetative stage was more prominent in improving number of leaves per plant (Fig. 1D) and leaf area of both maize cultivars than that applied at the other growth stages.

Addition of salt to the growth medium caused a significant ( $P \leq$

0.001) increase in the accumulation of  $\text{Na}^+$  and  $\text{Cl}^-$  in the roots and leaves of both cultivars (Fig. 2 A-D). Cultivar Golden had lower  $\text{Na}^+$  and  $\text{Cl}^-$  concentrations in the leaves and roots than those in cv. C-20 under saline conditions. Foliar application of GB significantly ( $P \leq 0.05$ ) reduced the accumulation of  $\text{Na}^+$  in the leaves and roots of both cultivars. Overall, 50 mM GB was the most effective GB concentration in reducing  $\text{Na}^+$  accumulation in the leaves and roots of both cultivars under saline conditions. Exogenous application of GB at different growth stages also had a significant effect on leaf  $\text{Na}^+$ .

Exogenous application of GB did not significantly ( $P \leq 0.05$ ) alter the accumulation of  $\text{Cl}^-$  in the leaves of both cultivars under normal or saline conditions. Excessive amount of NaCl in the rooting medium significantly reduced  $\text{K}^+$  and  $\text{Ca}^{2+}$  concentrations in the leaves and roots of both maize cultivars (Fig. 3 A-D). Application of 50 mM GB and 100 mM GB increased the accumulation of  $\text{K}^+$  in the leaves and roots of salinized plants of both cultivars, whereas they did not significantly alter leaf  $\text{K}^+$  of non-stressed plants. Accumulation of  $\text{K}^+$  in the leaves was generally higher when GB was applied at the vegetative stage or at the vegetative and reproductive stages than applied at the reproductive stage under saline conditions. Of different levels of GB, 50 mM GB was found to be more effective in promoting the accumulation of  $\text{K}^+$  in the roots, particularly when applied to cv. Golden at the vegetative stage and 100 mM GB to cv. C-20 under saline conditions.

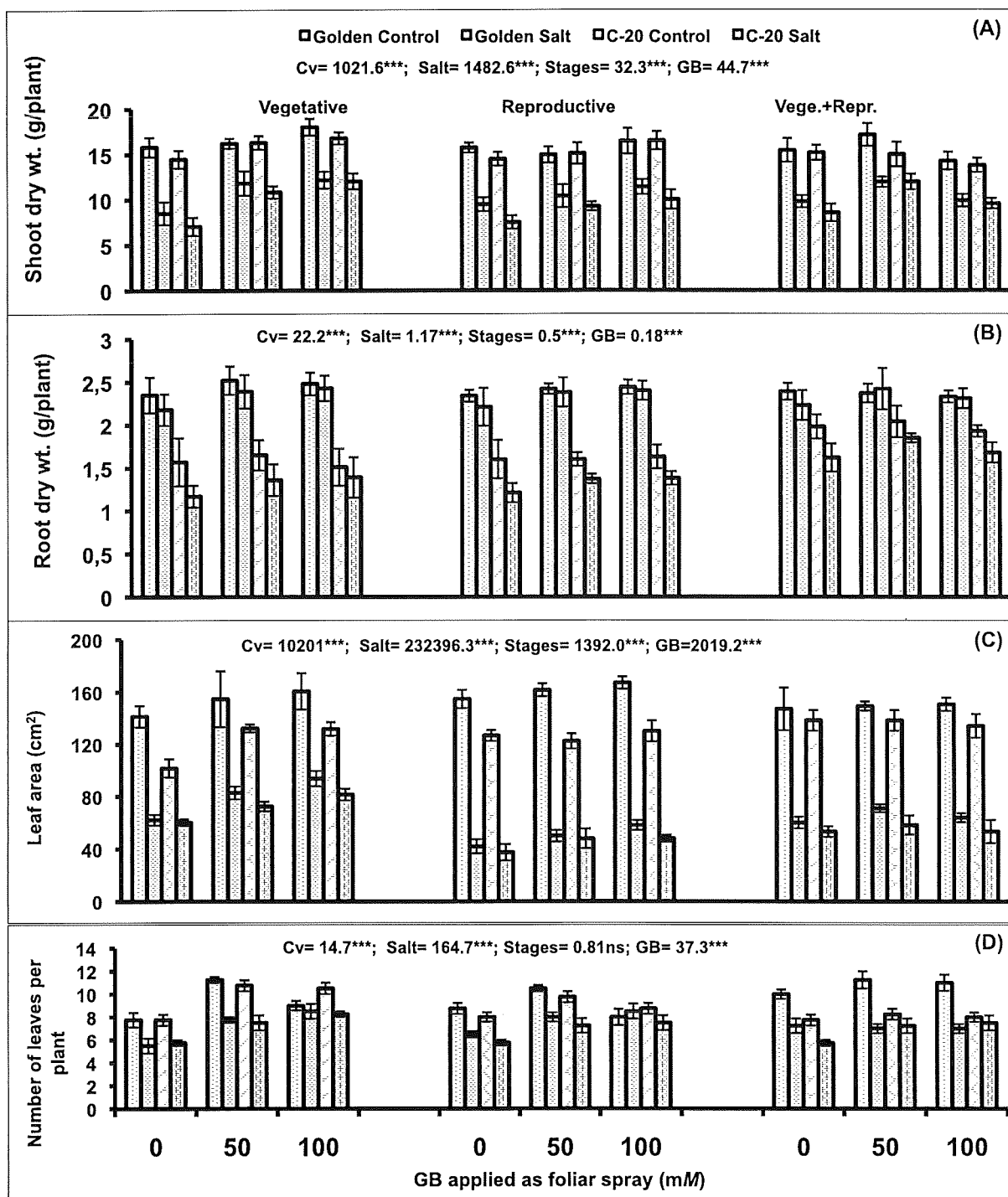
Exogenous application of GB, particularly, at the vegetative stage had a significant increase in leaf  $\text{Ca}^{2+}$  of both cultivars under saline conditions. However, GB applied exogenously at different growth stages did not significantly affect the root  $\text{Ca}^{2+}$  in both cultivars (Fig. 3 C and D).

Exogenous application of 50 mM GB was more effective than 100 mM GB in increasing the leaf  $\text{K}^+/\text{Na}^+$  and the reverse was true in case of root  $\text{K}^+/\text{Na}^+$  ratio in both maize cultivars under both normal and saline conditions (Fig. 4 C and D). Foliar application of GB at the vegetative stage was more effective in increasing leaf  $\text{K}^+/\text{Na}^+$  ratio as compared with GB applied at other growth stages. Under saline conditions, leaf  $\text{Ca}^{2+}/\text{Na}^+$  ratio increased particularly at 50 mM. However, foliar applied GB did not cause a significant effect on root  $\text{Ca}^{2+}/\text{Na}^+$  ratio of both cultivars. Application of GB at the vegetative stage was more effective in improving leaf  $\text{Ca}^{2+}/\text{Na}^+$  and root  $\text{Ca}^{2+}/\text{Na}^+$  ratios under saline conditions than that applied at the other growth stages (Fig. 4 A and B).

Exogenously applied GB (50 and 100 mM) significantly ( $P \leq 0.05$ ) affected the leaf proline concentration in both cultivars, but the cultivars did not differ significantly. Proline accumulation in the leaves of salt stressed plants of both cultivars was increased when GB was applied at the reproductive stage or at both the vegetative and reproductive stages, whereas proline accumulation remained almost unchanged when GB was applied at the vegetative stage (Fig. 4 E).

## Discussion

Salt stress caused a significant reduction in growth of both maize cultivars, but the response of the two cultivars was different with cv. C-20 being more sensitive to salt stress than cv. Golden. The reduction in growth and dry matter production of plants is attributed to several reasons. The most important reason is the reduced water and nutrient transport under stress conditions, which occurs due to the accumulation of high amounts of soluble salts in the rooting medium, thereby resulting in impaired plant growth. However, the reduction in growth of the maize cultivars was compensated by foliar application of GB, because foliar application of GB with increased growth of both cultivars. These results are similar to some earlier findings in which foliar application of GB resulted in a significant improvement in salt tolerance of maize plants (YANG and LU, 2005; 2006). Similarly, in rice, a marked improvement in salt tolerance was



**Fig. 1:** Shoot and root dry weights, leaf area and number of leaves/plant 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 vegetative and reproductive stages. (\*\*\*) = significant at 0.001 level; ns = non-significant).

observed due to exogenous application of GB (HARINASUT et al., 1996; LUTTS, 2000).

Changes in growth of both maize cultivars due to imposition of salt stress or foliar applied GB might have been due to changes in leaf growth (leaf area). It is well documented that biomass production is closely related to light interception or leaf area index (LAI) as observed in different agricultural crops (MONTEITH, 1977) and other vegetation types (HIROSE et al., 1997). From the results of the present study, it is also clear that foliar application of GB increased leaf area

of both cultivars under both normal and saline conditions. Thus, it is plausible to propose that reduction in growth of both maize cultivars was due to their reduced leaf growth, and GB-induced growth improvement was also due to GB-induced increase in leaf area (photosynthesizing tissue).

Comparison of some earlier reports in the literature with the present study shows that the effective and efficient doses of GB depends on the species (MÄKELA et al., 1996; AGBOMA et al., 1997a; 1997b; 1997c), mode of application (HEUER, 2003), plant growth stage at

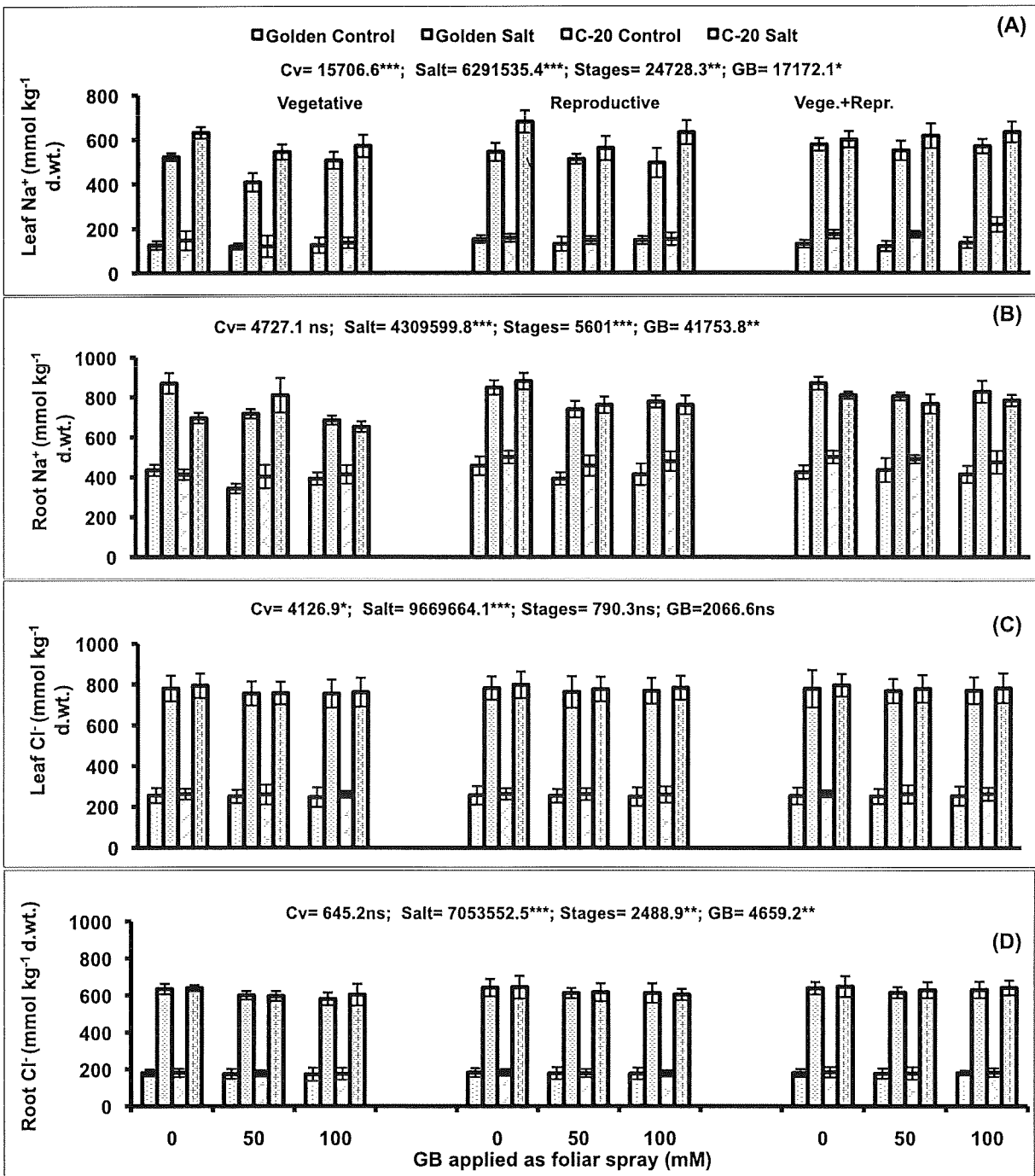


Fig. 2: Leaf and root Na<sup>+</sup> and Cl<sup>-</sup> concentrations of two maize (*Zea mays* L.) cultivars when different levels of GB were applied as foliar spray to salt stressed or non-stressed plants at the vegetative, reproductive, or the vegetative and reproductive stages. (\*, \*\*, \*\*\* = significant at 0.05, 0.01 and 0.001 levels, respectively; ns = non-significant).

which it is applied (AGBOMA et al., 1997a), concentration of GB applied (RAZA et al., 2006; 2007), and number of foliar applications (IBRAHIM et al., 2006). In the present study, effect of exogenous application of GB varied when applied at different growth stages. For example, 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. The differential response of maize plants to exogenous application of GB at different growth stages may have been due to its role in biosynthesis and transport of hormones like cytokinins (BADENOCH-JONES et al.,

1996) which may have a role in the transport of photoassimilates (TAIZ and ZEIGER, 2006). Secondly, the plants which received GB at the reproductive stage had been subjected to salt stress for a longer period without GB application than those which were sprayed with GB at the vegetative stage. This factor may have played a vital role in the differential response of maize plants to GB application at different growth stages.

Proline accumulation has been correlated with tolerance to salinity stress in most plant species (KAVI KISHOR et al., 1995). Thus, GB may play a protective role rather osmoregulatory role in preventing cell injury from salt stress as has earlier been reported in some studies

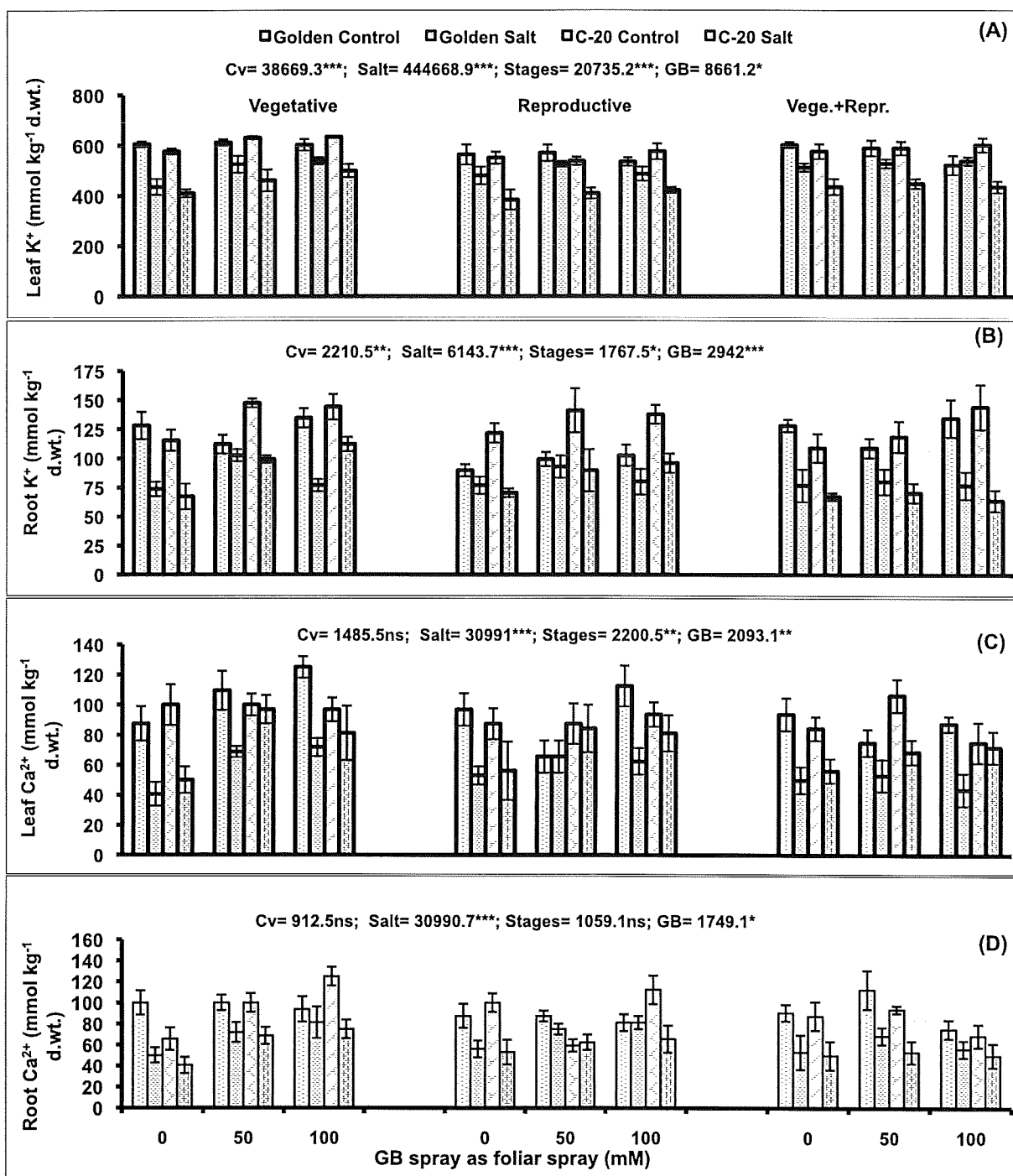


Fig. 3: Leaf and root  $K^+$  and  $Ca^{2+}$  concentration of two maize (*Zea mays* L.) cultivars when different levels of GB were applied as foliar spray to salt stressed or non-stressed plants at the vegetative, reproductive, or the vegetative and reproductive stages. (\*, \*\*, \*\*\* = significant at 0.05, 0.01 and 0.001 levels, respectively; ns = non-significant).

on different crops, e.g., in *Trifolium alexandrinum* (VARSHNEY et al., 1988), species of *Triticum*, *Agropyron* and *Elymus* (WYN JONES et al., 1984), and in pea (*Pisum sativum* L.) (NOREEN and ASHRAF, 2009). Although in the present study, salt stress increased proline accumulation in both maize cultivars, it remained unchanged in salinized plants due to GB application. From these findings, it is plausible to propose that GB may have not modulated the biosynthesis of proline (HASSINE et al., 2008; MISRA and SAXENA, 2009). Exogenous application of GB significantly reduced  $Na^+$  accumulation in the roots and leaves of both cultivars, while increased that of  $K^+$ .

Similarly,  $K^+/Na^+$  and  $Ca^{2+}/Na^+$  ratios of both cultivars were also increased with GB application under saline conditions. These results can be explained in view of some earlier reports in which it has been demonstrated that exogenous GB application reduces the accumulation of  $Na^+$  and maintains or promotes the accumulation of  $K^+$  in the shoots of most plant species (LUTTS et al., 1999; MÄKELA et al., 1999; RAHMAN et al., 2002; RAZA et al., 2007). For example, GB-treated rice plants had significantly lower  $Na^+$  and higher  $K^+$  concentrations in the shoots, compared with those of the untreated plants. The ameliorative effect of exogenously applied GB on rice

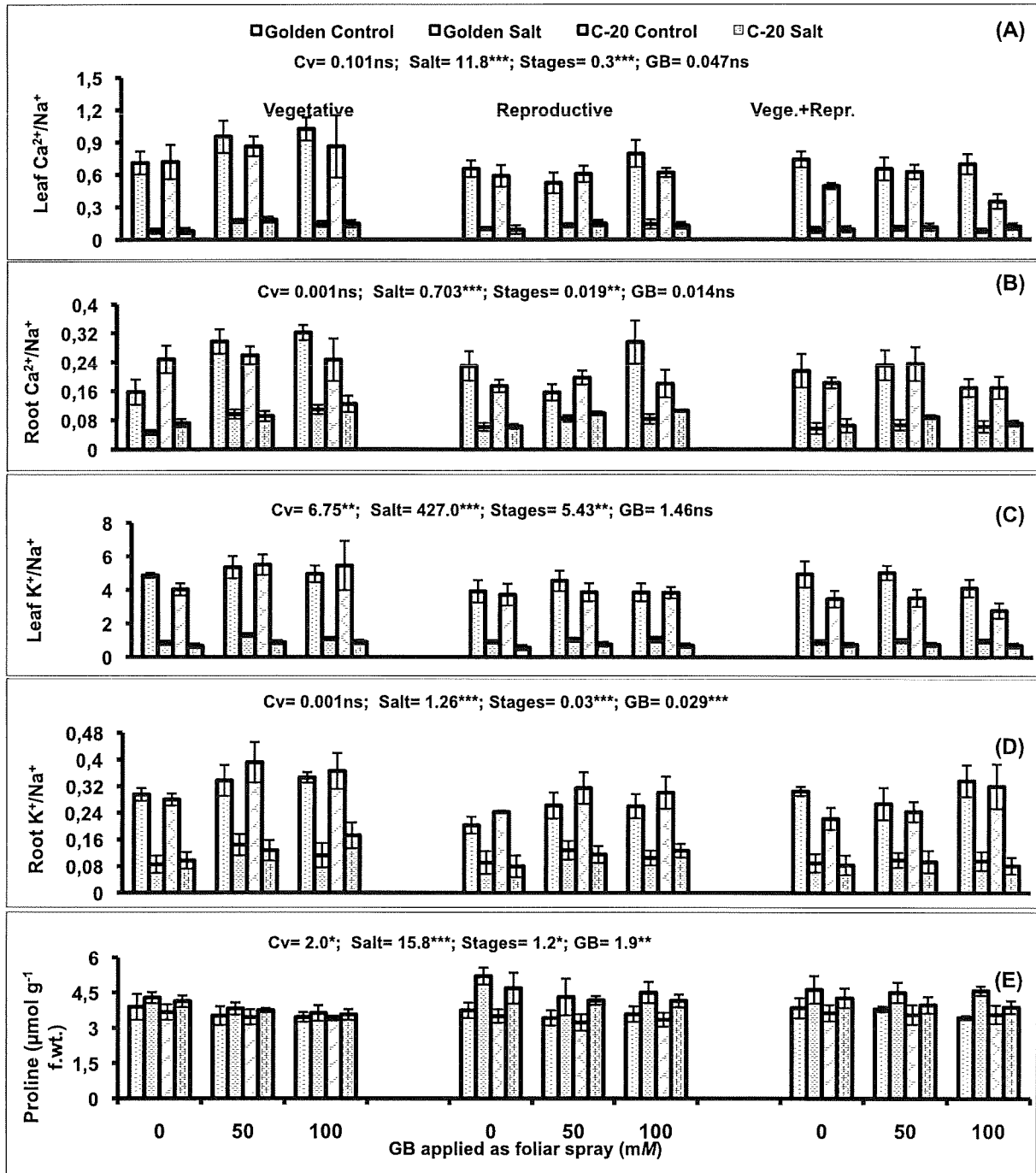


Fig. 4: Leaf and root  $Ca^{2+}/Na^+$  and  $K^+/Na^+$  ratios and leaf proline of two maize (*Zea mays* L.) cultivars when different levels of GB were applied as foliar spray to salt stressed or non-stressed plants at the vegetative, reproductive, or the vegetative and reproductive stages. (\*, \*\*, \*\*\* = significant at 0.05, 0.01 and 0.001 levels, respectively; ns = non-significant).

seedling growth was due to GB-induced greater accumulation of  $Na^+$  in the root and its decreased transportation to the shoot (RAHMAN et al., 2002). Exogenous application of GB to rice plants exposed to salt stress resulted in reduced  $Na^+$  accumulation and maintenance of  $K^+$  concentration in the shoot (LUTTS et al., 1999). Recently, RAZA et al. (2007) have found that exogenous GB applied as a foliar spray to wheat plants particularly at the vegetative stage reduced the accumulation of  $Na^+$ , but it did not alter that of  $K^+$ , thereby, resulting into improved  $K^+/Na^+$  ratio. There are a number of reports and reviews available in the literature that show that plants use different transporters for the uptake of  $Na^+$ ,  $K^+$  and  $Ca^{2+}$ , which play

important roles in maintaining cellular  $K^+/Na^+$  and  $Ca^{2+}/Na^+$  ratios (QUINTERO and BLATT, 1997; SANTA-MARIA et al., 1997; AMTMANN and SANDERS, 1998; TESTER and DAVENPORT, 2003; ASHRAF, 2004; MUNNS, 2005). Furthermore, it is widely accepted that GB maintains integrity of biological membranes and protects various transporters for proper functioning under various environmental stresses (MURATA et al., 1992; MANSOUR, 1998; YEO, 1998; HASEGAWA et al., 2000; SAKAMOTO and MURATA, 2002; ASHRAF and FOOLAD, 2007). For example, MANSOUR (1998) proposed that GB protects various transporters for normal functioning under salt stress. Root applied 0.5-5 mM GB maintained cytosolic  $K^+$  homeostasis in barley

preventing NaCl-induced K<sup>+</sup> leakage from the cell, possibly through enhanced H<sup>+</sup>-ATPase activity (CUIN and SHABALA, 2005). Recently, exogenous application of a range of compatible solutes including GB also reduced oxidative stress-induced K<sup>+</sup> leakage from the roots of *Arabidopsis thaliana*. Furthermore, they have suggested that the beneficial effect of GB in salt stressed plants may have been due to the protective effect of GB on the integrity of plasma membrane and its associated transporter proteins, and scavenging free radicals (CUIN and SHABALA, 2007). From the results of the present study and all these reports, it can be concluded that leaf applied GB might have direct beneficial effect on salt stressed plants of maize cultivars by providing stabilization of biological membranes and macromolecules (proteins, PS-II, and transporters) which resulted in Na<sup>+</sup> discrimination against K<sup>+</sup> and Ca<sup>2+</sup> (improved K<sup>+</sup>/Na<sup>+</sup> and Ca<sup>2+</sup>/Na<sup>+</sup> ratio) rather than simply providing protection against osmotic stress.

By summarizing all the results, it is clear that foliar application of GB was effective in ameliorating the adverse effects of salinity on growth of both maize cultivars. Moreover, foliar application of GB was more effective when applied at the vegetative stage than applied at the reproductive or at both the vegetative and reproductive stages. Beneficial effect of GB applied as a foliar spray caused enhanced Na<sup>+</sup> discrimination against K<sup>+</sup> and Ca<sup>2+</sup> (improved K<sup>+</sup>/Na<sup>+</sup> and Ca<sup>2+</sup>/Na<sup>+</sup> ratio) in salt stressed maize plants.

## References

- AGBOMA, M., JONES, M.G.K., PELTONEN-SAINIO, P., RITA, H., PEHU, E., 1997a: Exogenous glycinebetaine enhances grain yield of maize, sorghum and wheat grown under two supplementary watering regimes. *J. Agron. Crop Sci.* 178, 29-37.
- AGBOMA, P.C., PELTONEN-SAINIO, P., HINKKANEN, R., PEHU, E., 1997b: Effect of foliar application of glycinebetaine on yield components of drought-stressed tobacco plants. *Exp. Agric.* 33, 345-352.
- AGBOMA, P.C., SINCLAIR, T.R., JOKINEN, K., PELTONEN-SAINIO, P., PEHU, E., 1997c: An evaluation of the effect of exogenous glycinebetaine on the growth and yield of soybean: timing of application, watering regimes and cultivars. *Field Crop Res.* 54, 51-64.
- AKRAM, N.A., SHAHBAZ, M., ATHAR, H.R., ASHRAF, M., 2006: Morpho-physiological responses of two differently adapted populations of *Cynodon dactylon* (L.) Pers. and *Cenchrus ciliaris* L. to salt stress. *Pak. J. Bot.* 38, 1581-1588.
- ALLARD, F., HOUDE, M., KROL, M., IVANOV, A., HUNER, N.P.A., 1998: Betaines improve freezing tolerance in wheat. *Plant Cell Physiol.* 39, 1194-1202.
- AMTMANN, A., SANDERS, D., 1998: Mechanisms of Na<sup>+</sup> uptake by plant cells. *Adv. Bot. Res.* 29, 75-112.
- ASHRAF, M., 2004: Some important physiological selection criteria for salt tolerance in plants. *Flora* 199, 361-376.
- ASHRAF, M., FOOLAD, M.R., 2007: Roles of glycine betaine and proline in improving plant abiotic stress resistance. *Environ. Exp. Bot.* 59, 206-216.
- BADENOCH-JONES, J., PARKER, C.W., LETHAM, D.S., SINGH, S., 1996: Effect of cytokinins supplied via xylem at multiplies of endogenous concentrations on transpiration and senescence in de-rooted seedlings of oat and wheat. *Plant Cell Environ.* 19, 504-516.
- BANU, M.N., HOQUE, M.A., WATANABE-SUGIMOTO, K., MATSUOKA, K., NAKAMURA, Y., SHIMOISHI, Y., MURATA, Y., 2009: Proline and glycinebetaine induce antioxidant defense gene expression and suppress cell death in cultured tobacco cells under salt stress. *J. Plant Physiol.* 166, 146-156.
- BATES, L.S., WALDREN, R.P., TEARE, I.D., 1973: Rapid determination of free proline for water stress studies. *Plant Sci.* 39, 205-207.
- CHEN, T.H., MURATA, N., 2008: Glycinebetaine: an effective protectant against abiotic stress in plants. *Trends Plant Sci.* Aug 12, (Epub ahead of print).
- CUIN, T.A., SHABALA, S., 2005: Exogenously supplied compatible solutes rapidly ameliorate NaCl-induced potassium efflux from barley roots. *Plant Cell Physiol.* 46, 1924-1933.
- CUIN, T.A., SHABALA, S., 2007: Compatible solutes reduce ROS-induced potassium efflux in *Arabidopsis* roots. *Plant Cell Environ.* 30, 875-885.
- GRIEVE, C.M., MAAS, E.V., 1984: Betaine accumulation in salt stressed sorghum. *Physiol. Plant.* 61, 167-171.
- HARINASUT, P., TSUTSUI, K., TAKABE, T., NOMURA, M., KISHITANI, S., 1996: Exogenous glycine betaine accumulation and increased salt tolerance in rice seedlings. *Biosci. Biotech. Biochem.* 60, 366-368.
- HASEGAWA, P.M., BRESSAN, R.A., ZHU, J.K., BOHNERT, H.J., 2000: Plant cellular and molecular response to high salinity. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 51, 463-499.
- HASSINE, B.A., GHANEM, M.E., BOUZID, S., LUTTS, S., 2008: An inland and a coastal population of the Mediterranean xero-halophyte species *Atriplex halimus* L. differ in their ability to accumulate proline and glycinebetaine in response to salinity and water stress. *J. Exp. Bot.* 59, 1315-1326.
- HEUER, B., 2003: Influence of exogenous application of proline and glycinebetaine on growth of salt-stressed tomato plants. *Plant Sci.* 165, 693-699.
- HIROSE, T., ACKERLY, D.D., TRAW, M.B., RAMSEIER, D., BAZZAZ, F.A., 1997: CO<sub>2</sub> elevation, canopy photosynthesis, and optimal leaf area index. *Ecology* 78, 2339-2350.
- HSU, S.Y., HSU, Y.T., KAO, C.H., 2003: The effect of polyethylene glycol on proline accumulation in rice leaves. *Biol. Plant.* 46, 73-78.
- HYUN, D.Y., LEE, I.S., KIM, D.S., LEE, S.J., SEO, Y.W., LEE, Y.I., 2003: Selection of azetidine-2-carboxylic acid resistant cell lines by in vitro mutagenesis in rice (*Oryza sativa* L.). *J. Plant Biotechnol.* 5, 43-49.
- IBRAHIM, M., ANJUM, A., KHALIQ, N., IQBAL, M., ATHAR, H.R., 2006: Four foliar applications of glycinebetaine did not alleviate adverse effects of salt stress on growth of sunflower. *Pak. J. Bot.* 39, 1561-1569.
- KAVI KISHOR, P.B., HONG, Z., MIAO, G.H., HU, C.A.A., VERMA, D.P.S., 1995: Overexpression of  $\Delta^1$ -pyrroline-5-carboxylate synthetase increases proline production and confers osmotolerance in transgenic plants. *Plant Physiol.* 108, 1387-1394.
- KISHITANI, S., WATANABE, K., YASUDA, S., ARAKAWA, K., TAKEBE, T., 1994: Accumulation of glycinebetaine during cold acclimation and freezing tolerance in leaves of winter and spring barley plants. *Plant Cell Environ.* 17, 89-95.
- KHEDR, A.H.A., ABBAS, M.A., WAHID, A.A.A., QUICK, W.P., ABOGADALLAH, G.M., 2003: Proline induces the expression of salt-stress responsive proteins and may improve the adaptation of *Pancreaticum maritimum* L. to salt-stress. *J. Exp. Bot.* 54, 2553-2562.
- LUTTS, S., 2000: Exogenous glycinebetaine reduces sodium accumulation in salt-stressed rice plants. *Intl. Rice Res. Notes* 25, 39-40.
- LUTTS, S., MAJERUS, V., KINET, J.M., 1999: NaCl effects on proline metabolism in rice (*Oryza sativa*) seedlings. *Physiol. Plant.* 105, 450-458.
- MÄKELA, P., MANTILA, J., HINKKANEN, R., PEHU, E., PELTONEN-SAINIO, P., 1996: Effect of foliar applications of glycinebetaine on stress tolerance, growth and yield of spring cereals and summer turnip rape in Finland. *J. Agron. Crop Sci.* 176, 223-234.
- MÄKELA, P., JOKINEN, K., KONTTURI, M., PELTONEN-SAINIO, P., PEHU, E., SOMERSALO, S., 1998: Foliar application of glycinebetaine – a novel product from sugar beet as an approach to increase tomato yield. *Ind. Crops Prod.* 7, 139-148.
- MÄKELA, P., KONTTURI, M., PEHU, E., SOMERSALO, S., 1999: Photosynthetic response of drought and salt stressed tomato and turnip rape plants to foliarly applied glycinebetaine. *Physiol. Plant.* 105, 45-50.
- MANSOUR, M.M.F., 1998: Protection of plasma membrane of onion epidermal cells by glycine betaine and proline against NaCl stress. *Plant Physiol. Biochem.* 36, 767-772.
- MISRA, N., SAXENA, P., 2009: Effect of salicylic acid on proline metabolism in lentil grown under salinity stress. *Plant Sci.* 177, 181-189.
- MONTEITH, J.L., 1977: Climate and the efficiency of crop production. In: *Britain Philosophical Trans-actions of the Royal Society, London* 281,

- 277-294.
- MSTAT DEVELOPMENT TEAM, 1989: MSTAT user' guide: A microcomputer programme for the design management and analysis of agronomic research experiments. Michigan State University East Lansing, USA.
- MUNNS, R., 2005: Genes and salt tolerance: bringing them together. *New Phytol.* 167, 645-663.
- MURATA, N., MOHANTY, P.S., HAYASHI, H., APAGEORGIU, G.C., 1992: Glycinebetaine stabilizes the association of extrinsic proteins with the photosynthetic oxygen-evolving complex. *FEBS Lett.* 296, 187-189.
- NOREEN, Z., ASHRAF, M., 2009: Changes in antioxidant enzymes and some key metabolites in some genetically diverse cultivars of radish (*Raphanus sativus* L.). *Environ. Exp. Bot.* 67, 395-402.
- PARK, S., SMITH, L.T., SMITH, G.M., 1995: Role of glycinebetaine and related osmolytes in osmotic stress adaptation in *Yersinia enterocolitica* ATCC 9610. *Appl. Environ. Microbiol.* 61, 4378-4381.
- QUINTERO, F.J., BLATT, M.R., 1997: A new family of K<sup>+</sup> transporters from *Arabidopsis* that are conserved across phyla. *FEBS Lett.* 415, 206-211.
- RAHMAN, M.S., MIYAKE, H., TAKEOKA, Y., 2002: Effects of exogenous glycinebetaine on growth and ultra structure of salt-stressed rice seedlings (*Oryza sativa* L.). *Plant Prod. Sci.* 5, 33-44.
- RAZA, S.H., ATHAR, H.R., ASHRAF, M., 2006: Influence of exogenously applied glycinebetaine on the photosynthetic capacity of two differently adapted wheat cultivars under salt stress. *Pak. J. Bot.* 38, 341-351.
- RAZA, S.H., ATHAR, H.R., ASHRAF, M., HAMEED, A., 2007: Glycinebetaine-induced modulation of antioxidant enzymes activities and ion accumulation in two wheat cultivars differing in salt tolerance. *Environ. Exp. Bot.* 60, 368-376.
- RHODES, D., RICH, P.J., BRUNK, D.G., JU, G.C., RHODES, J.C., PAULY, M.H., HANSEN, L.A., 1989: Development of two isogenic sweet corn hybrids differing for glycinebetaine content. *Plant Physiol.* 91, 1112-1121.
- SAKAMOTO, A., MURATA, N., 2000: Genetic engineering of glycinebetaine synthesis in plants: current status and implications for enhancement of some stress tolerance. *J. Exp. Bot.* 51, 81-88.
- SAKAMOTO, A., MURATA, N., 2002: The role of glycinebetaine in the protection of plants from stress: clue from transgenic plants. *Plant Cell Environ.* 25, 163-171.
- SANTA-MARIA, G.E., RUBIO, F., DUBCOVSKY, J., NAVARRO, A.R., 1997: The *HAK1* gene of barley is a member of a large gene family and encodes a high-affinity potassium transporter. *Plant Cell* 9, 2281-2289.
- SUBBARAO, G.V., WHEELER, R.M., LEVINE, L.H., STUTTE, G.W., 2001: Glycinebetaine accumulation, ionic and water relations of red-beet at contrasting levels of sodium supply. *J. Plant Physiol.* 158, 767-776.
- TAIZ, L., ZEIGER, E., 2006: *Plant Physiology*, 4<sup>th</sup> edition, Sinauer Associates, Sunderland.
- TESTER, M., DAVENPORT, R., 2003: Na<sup>+</sup> tolerance and Na<sup>+</sup> transport in higher plants. *Ann. Bot.* 91, 503-507.
- TÜRKAN, I., DEMIRAL, T., 2004: Does exogenous glycinebetaine affect antioxidant system of rice seedlings under NaCl treatment? *Plant Physiol.* 161, 1089-1100.
- VARSHNEY, K.A., GANGWAR, L.P., GOEL, N., 1988: Choline and betaine accumulation in *Trifolium alexandrinum* L. during salt stress. *Egypt. J. Bot.* 31, 81-86.
- WOLF, B., 1982: A comprehensive system of leaf analysis and its use for diagnosing crop nutrient status. *Commun. Soil Sci. Plant Anal.* 13, 1035-1059.
- WYN JONES, R.G., GORHAM, J., MCDONNELL, E., 1984: Organic and inorganic solute contents as selection criteria for salt tolerance in the Triticeae. In: Staples, R., Toennissen, G.H. (eds.), *Salinity tolerance in plants: Strategies for crop improvement*, 189-203. Wiley and Sons, New York.
- YANG, X., LU, C., 2005: Photosynthesis is improved by exogenous glycinebetaine in salt stressed maize plants. *Physiol. Plant.* 124, 343-352.
- YANG, X., LU, C., 2006: Effects of exogenous glycinebetaine on growth, CO<sub>2</sub> assimilation, and photosystem II photochemistry of maize plants. *Physiol. Plant.* 127, 593-602.
- YEO, A., 1998: Molecular biology of salt tolerance in the context of whole-plant physiology. *J. Exp. Bot.* 49, 915-929.

Address of the author:

Nudrat Aisha Akram, E-mail: nudrataaauaf@yahoo.com