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EFFICACY OF EXOGENOUS GLYCINE BETAINE APPLICATION ON SORGHUM PLANTS GROWN UNDER SALINITY STRESS

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A pot experiment was conducted to evaluate the beneficial effect of glycine betaine application on salinity tolerance of sorghum plants subjected to two salinity levels (125 and 250 mM) for 18 days. Salinity greatly reduced sorghum growth and this effect was more pronounced at 250 mM than at 125 mM NaCl. Foliar application of glycine betaine at 75 mM mitigated to some extent the adverse effects of salinity on sorghum growth.

The measured osmotic pressure and solutes concentration (Na, K, Ca, Mg, QACs, total soluble sugar and betaine) increased while K/Na ratio decreased in sorghum sap with increasing salinity level. The major contributors to the measured osmotic pressure were found to be soluble sugars and K, however, this effect decreased with increasing salinity level while that of Na, proline and betaine increased. The obtained results clearly showed that betaine application reduced proline accumulation in sorghum sap under salinity treatments.

It was appeared that the expanding leaf had more osmolality, K, sugars, proline and betaine concentrations than the fully expanded leaf. Conversely, Na , Ca and Mg concentrations were higher in the fully expanded leaf than the expanding leaf.

Total N concentration increased, while that of C decreased with increasing salinity level. Hydrogen concentration slightly affected by salinity treatments. Stomatal conductance was markedly reduced with increasing salinity level; the main effect of foliar application of glycine betaine was not significant for this criterion.

Key words: glycine betaine, leaf expansion, nitrogen, osmolality, salinity, solutes, *Sorghum*, stomatal conductance

INTRODUCTION

Salinity has a considerable effect on world agriculture, with as much as half of the irrigated areas of land being affected by high salinity. The effects of salinity on plant growth are well documented and there is no doubt that they have been and continued to be the subject of great deal of research (Flowers and Yeo 1995). The interaction of salts with plant physiology process is obviously complex. There are many salt species, many mechanisms and many organs, tissues and cells involved (Shalhevet 1993). The adverse effects of salts

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on plants are generally divided into the osmotic effect, specific ion effect and excess exchangeable sodium effect on soil swelling and / or dispersion (Bresler *et al*. 1982).

The accumulation of non-toxic (thus compatible) osmoticaly active solutes like betaines, proline, amino acids, sugars and polyols occurs in response to salinity and other stresses; many but not all compatible solutes have been proposed to confer protection against oxidative damage by scavenging free radicals in addition to their roles in maintenance of osmotic equilibrium without perturbing macromolecule-solvent interactions (Laurie and Stewart 1990, Hare *et a*l. 1998, Sakamoto and Murata 2002).

Glycine betaine (N,N,N-tri-methylglycine) is a quaternary ammonium compound, accumulated by many species of angiosperms, and is thought to contribute to salt and drought tolerance (Gorham 1996, Wood *et al*. 1996). There is good evidence to suggest that it acts as a non-toxic cytoplasmic osmolyte and plays a central role in adaptation to stress (Sakamoto and Murata 2002). The osmoprotective effects of glycine betaine are now generally interpreted in terms of compatibility with macromolecular structure and function (Rhodes and Hanson 1993). Glycine betaine is metabolically inert and readily translocated from its site of synthesis in leaves to the other parts of plants; however it is readily degraded by soil microbes (Agboma *et al*. 1997).

There have been studies on the use of glycine betaine to alleviate the effects of salinity (Harinasut *et al*. 1996, Lutts 2000) and drought (Agboma *et al*. 1997) on plants and interest is increasing with better understanding of the physiological effects of salt and drought stresses. The objective of this research was to investigate the effect of foliar application of glycine betaine on salinity tolerance of a sorghum variety common in Egypt. A second objective was to monitor osmotic pressure, elements, some organic solutes, and stomatal conductance of grown plants to throw some lights on the effects of glycine betaine and salinity stress on physiological processes.

MATERIALS AND METHODS

Plant materials and growth conditions: Grains of sorghum (*Sorghum bicolor* L. var. Hybrid 113) which were obtained from the Egyptian Ministry of Agriculture were used in these investigations. This cultivar is commonly grown in Egypt and used as a source of grains for animal and sometimes human utilisation (Ibrahim 1999), and can tolerate up to 300 mM NaCl in germination stage (unpublished observations).

The grains were surface sterilised with bleach solution (10%) for ten minutes and washed thoroughly with distilled water; then soaked for 6 hours in distilled water and germinated in plastic boxes for 48 hours on moist tissue. Ten germinated grains were sown in plastic pots (15 ×10 cm) filled with 1 kg soil of John Innes Compost. The pots were kept in a greenhouse, and the plants were subjected to natural day/night conditions (minimum / maximum air temperature and relative humidity were: 29.2/35.2 °C and 63/68%, respectively at mid-day during the experimental period). After 12 days the plants in each pot were thinned to 4 per pot. Then the pots were allocated as following: control, irrigation with 125 mM NaCl (+ 6.25 mM $CaCl_2$), irrigation with 250 mM NaCl (+12.5 mM $CaCl_2$), glycine betaine (GB) control, GB + 125 mM NaCl (+6.25 mM $CaCl_2$) and GB + 250 mM NaCl (+12.5 mM $CaCl_2$). The plants were sprayed with glycine betaine (75 mM) weekly at night. After thinning, NPK (2:1:1) fertiliser was added with the rate 150 kg/ha. The plants were harvested 18 days from starting salt treatments for growth and biochemical analysis.

Extraction of sap: The second fully expanded and the expanding leaves (6 replicates) were separated and used for sap extraction. Samples were frozen in 1.5 cm^3 polypropylene microcentrifuge tubes, thawed and crushed using a custom made metal rod with a tapered end and centrifuged at 9000 g (Gorham *et al*. 1985).

Measurements of osmotic pressure: Osmotic pressure of leaf sap was measured in a Wescor 5100B Vapor Pressure Osmometer.

Metallic ions analysis: Na, K, Ca and Mg concentrations were measured in the leaf sap by ion chromatography in a Dionex 2010i ion chromatograph as described by Gorham and Hardey (1990).

Determination of quaternary ammonium compounds: Quaternary ammonium compounds were determined in leaf sap by the periodide method of Grieve and Grattan (1983).

Determination of betaine, proline and soluble sugars: A mixed sample (400 µl) from each treatment was used to evaluate the concentration of betaine, proline and soluble sugars (sucrose + glucose + fructose) in sorghum sap by high performance liquid chromatography.

Determination of C, N and H: C, N and H percentages in a mixed sample of shoot dry weight for each treatment was determined using LECO (CHN 2000 Analyzer Corporation).

Measurements of stomatal conductivity: Stomatal conductivity (6 replicates) on the upper and lower side of the second fully expanded leaf was measured by AF4 Porometer (Delta-T Devices).

Statistical analysis: The main effect of factors (salinity and glycine betaine and, on some occasions leaf expansion (or leaf surface) and their interactions were evaluated by GLM using SPSS program. Tukey's test were used to asses the significant ($P < 0.05$) differences between means. Correlation coefficients between salinity tolerance index and the evaluated criteria of salinity treated plants was also estimated by SPSS program.

RESULTS

Changes of growth criteria

Salinity treatments (125 mM and 250 mM) markedly reduced sorghum shoot fresh and dry weights and length. The main effect of glycine betaine was significant for shoot fresh and dry weights and not significant for shoot length. Foliar application of glycine betaine mitigated the adverse effect of salinity on sorghum fresh and dry weights. Salinity tolerance index was significantly raised in sorghum plants sprayed with glycine betaine (Table 1).

Changes of measured osmotic pressure and solutes concentration

The main effect of salinity, glycine betaine and leaf expansion was significant for sorghum sap osmotic pressure (Fig. 1). The osmotic pressure was raised with increasing salinity level. Glycine betaine added more increase to sorghum osmotic pressure. Correcting the measured osmotic pressure to shoot water content indicated that this increase resulted from solutes accumulation rather than water loss. Osmotic pressure of the expanding leaves was markedly higher than that of the fully expanded leaves. The interactions (salinity × glycine betaine) and (glycine betaine × leaf expansion) were also significant. Glycine betaine appeared to change the measured osmotic pressure at

Table 1

Effect of exogenously added glycine betaine on growth criteria of sorghum plants grown under salinity conditions

Treatments	Shoot fresh weight (g)	Shoot dry weight (g)	Shoot length (cm)	Salinity tolerance index (STI)
Control	33.29^{a}	4.21^{a}	146.14^{a}	–
125 m M NaCl	17.13^{b}	3.08^{b}	106.0	73.15^{a}
250 mM NaCl	13.17^{c}	2.32^{c}	97.57^{c}	55.11^{b}
Glycine betaine (control)	35.16^{a}	4.58^{a}	141.85^{a}	–
125 mM NaCl + glycine betaine	19.51^{b}	3.37^{b}	112.14^{b}	80.01^{c}
250 mM NaCl + glycine betaine	16.41$^{b\,d}$	3.07$^{b\,d}$	100.00^{bd}	72.92^{a}

STI = (Shoot d.wt of stressed plants / Shoot d.wt of unstressed plants) × 100
Values in each column, and labeled with the same letter are not significantly different at $P < 0.05$

125 mM more higher than at 250 mM NaCl concentration. Furthermore, spraying with glycine betaine was effective in case of the fully expanded leaf only.

Sorghum plants appeared to have a low Na concentration. The concentration of metallic ions in sorghum sap (Na, K, Mg and Ca) increased, while K/Na ratio decreased with increasing salinity level as compared with that of control plants. The fully expanded leaf had a higher concentration of Na, Mg and Ca than the expanding leaf. On the other hand, K and K/Na ratio was higher in the expanding leaf than in the fully expanded leaf. In this investigation, glycine betaine significantly decreased Ca concentration and non-significantly affected the other evaluated ions (Fig. 2).

Regarding quaternary ammonium compounds (QACs), all main effects (salinity, betaine and leaf expansion) were significant. QACs concentrations in sorghum sap increased with increasing salinity levels. Plants sprayed with glycine betaine had a higher concentration than non-sprayed plants. Fully expanded leaf had a lower concentration than the expanding leaf.

Soluble sugars, proline and glycine betaine were accumulated in sorghum sap in response to the applied salt stress and this effect was more pronounced at 250 mM than at 125 mM (Fig. 3). Expanding leaf accumulated more of these solutes than the fully expanded leaf. Spraying with betaine increased the

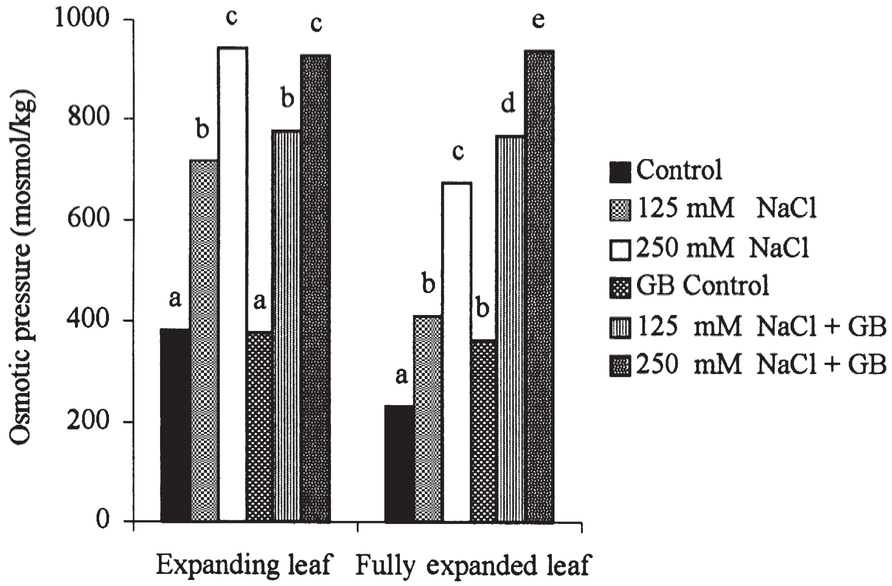

Fig. 1. Effect of exogenously added glycine betaine on measured osmotic pressure of sorghum plants grown under salinity conditions (Bars in grouping labeled with the same letter are not significantly different at $P = 0.05$. Abbreviation: GB = glycine betaine)

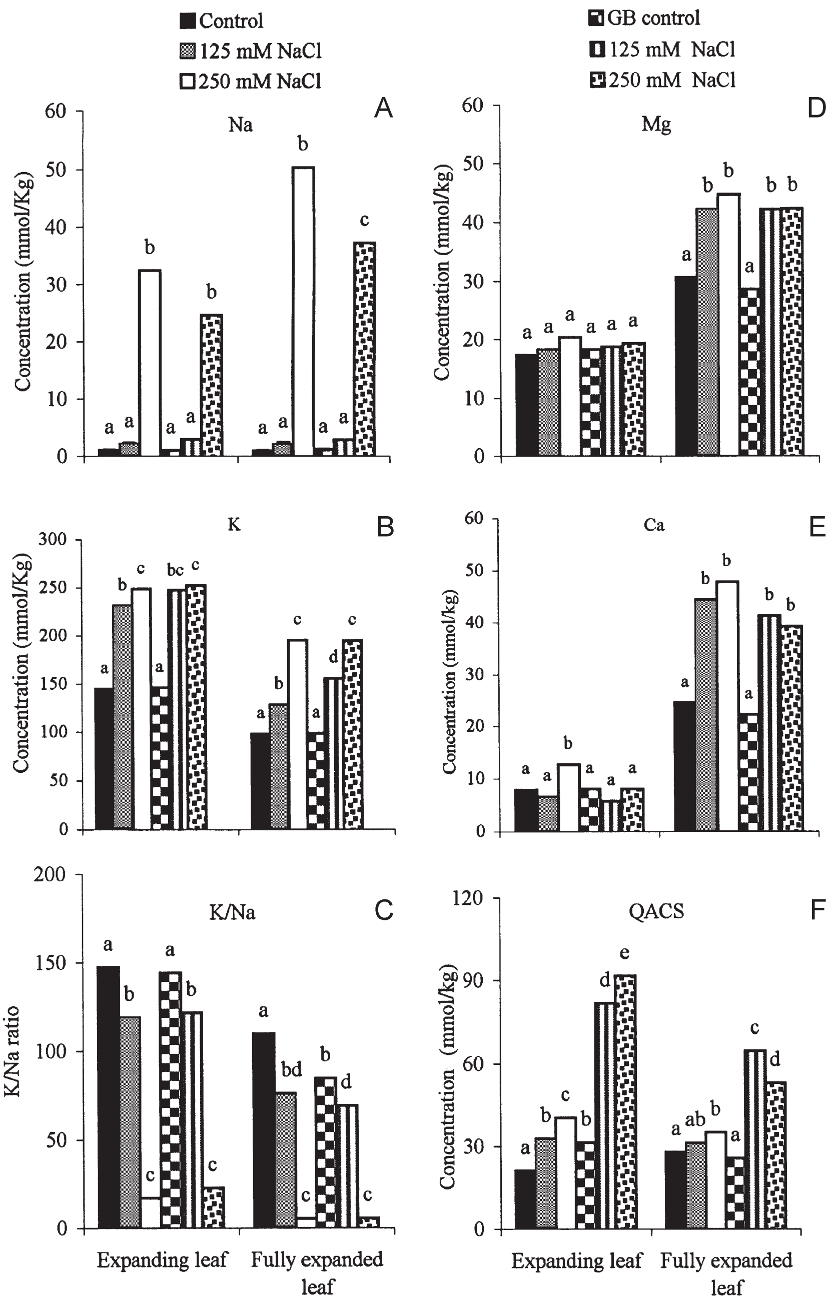

Fig. 2. Effect of exogenously added glycine betaine on metallic ions and quaternary ammonium compounds (QACS) concentration of sorghum plants grown under salinity conditions. Bars in grouping labeled with the same letter are not significantly different at $P < 0.05$

concentration of soluble sugars and betaine in leaf sap. The concentration of proline was reduced in the expanding leaf, and increased in fully expanded leaf, as compared to non-sprayed plants.

From these solutes, K and soluble sugars (sucrose + glucose + fructose) were the major contributors to the measured osmotic pressure (38.0% and 38.7%, respectively for control plants), however this effect decreased with increasing salinity level. It is interesting to notice that the contribution of glycine betaine, proline and sodium to the measured osmotic pressure increased with salinity treatments.

Salinity tolerance index (STI), expressed on shoot dry weight basis, was negatively correlated (r = –0.712, $P = 0.02$) with Na concentration of sorghum sap and non-significantly correlated with the other solutes concentration.

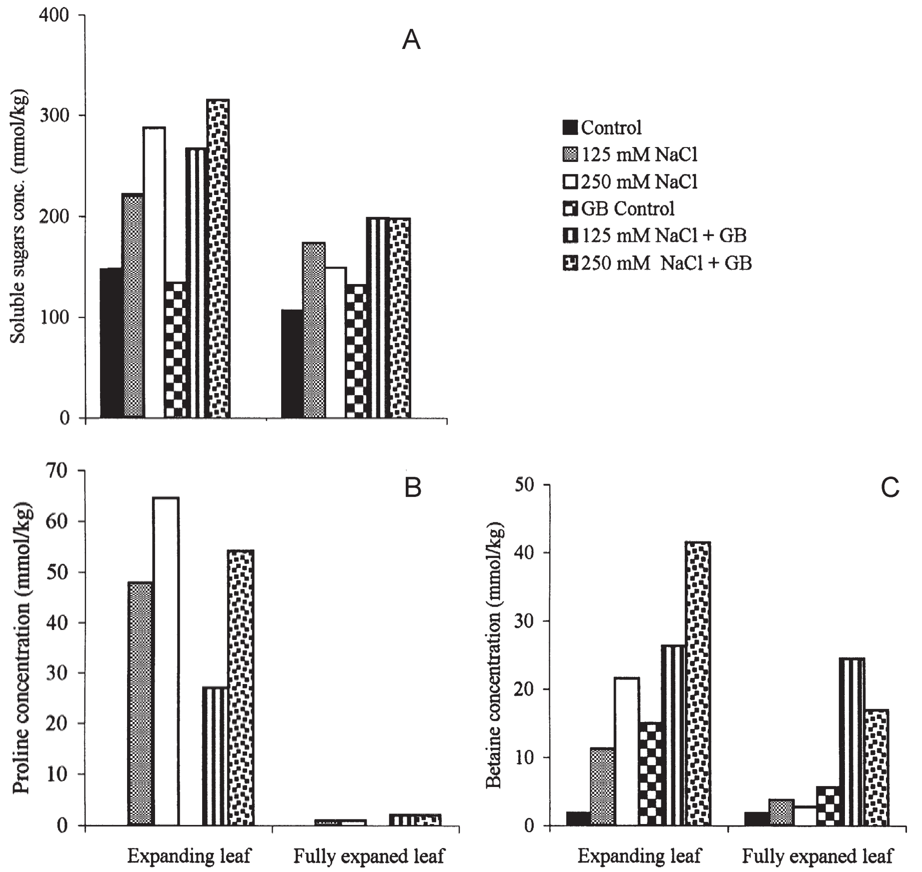

Fig. 3. Effect of exogenously added glycine betaine on sugars (A), proline (B) and betaine (C) concentration of sorghum plants grown under salinity conditions

Changes of N, C and H concentration

Examination of results of elemental analysis (% d.wt., Fig. 4) showed that N concentration was increased with salinity, while C was decreased. Little reduction effect was observed for H element. Glycine betaine treatments appeared to reduce N concentration and improved to some extent C and H concentration of sorghum as compared by non-treated plants. Relating N concentration to total shoot dry weight, indicated that N content decreased in sorghum at 250 mM NaCl stress as compared with control plants. Furthermore, glycine betaine application appeared to improve N content of sorghum under salinity conditions.

STI was positively correlated with C and H (r = 0.92–0.98, $P = 0.001$) concentrations and negatively correlated (r = –0.74, $P = 0.03$) with N concentration of sorghum plants.

Changes of stomatal conductance

The main effect of salinity was to reduce stomatal conductance of sorghum leaf (Fig. 5). The upper leaf surface had a lower conductance than the lower surface. The main effect of glycine betaine was not significant. The interactions (salinity × leaf surface) was significant and this reflected the fluctuations of the effect of salinity on the upper leaf surface.

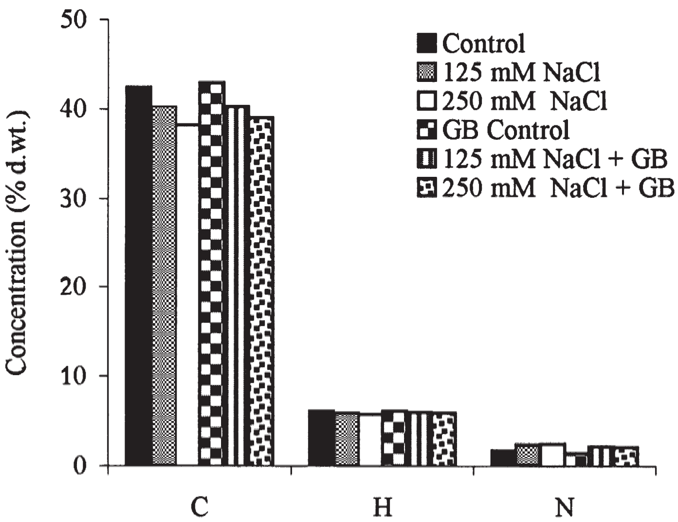

Fig. 4. Effects of exogenously added glycine betaine on total C, H and N concentration of sorghum plants grown under salinity conditions

DISCUSSION

In the present study the growth of used sorghum was severely reduced by salinity stress. Suppressed growth and damage to tissue under salinity could result from physiological drought, injury to cell membranes, Ca^{2+}–Na^{+} interaction, salt accumulation in the apoplast resulting in cell dehydration, decrease in the photosynthetic surface, the cost of osmotic adjustment and nutrient deficiencies (Yeo 1983, Shalhevet 1993). Glycine betaine application mitigated the adverse effects of salinity on growth criteria of sorghum. The beneficial effects of glycine betaine may be related to its role in osmotic balance and at the same time compatiblity with cells metabolism (Rhodes and Hanson 1993, Hare *et al*. 1998, Sakamoto and Murata 2002).

The measured osmotic pressure of sorghum plants increased with increasing salinity level. Application of glycine betaine, on some occasions, added more increase to sorghum osmotic pressure. This increase in the osmotically active compounds represents an important cellular response to salinity and allows cell to re-establish turgor and extract additional water from the soil (Mullet and Whittsitt 1996).

The data presented here indicated that K and soluble sugars are the major contributors to the measured osmotic pressure of sorghum sap. The contribu-

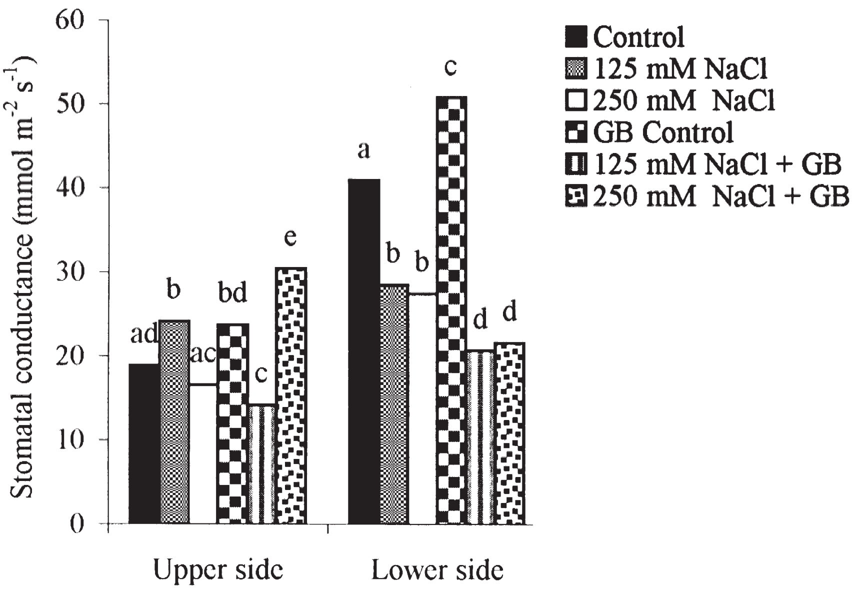

Fig. 5. Effects of exogenously added glycine betaine on stomatal conductance (upper and lower leaf side) of sorghum plants grown under salinity conditions

tion of these solutes decreased with increasing salinity level, while that of Na increased. Accordingly, K/Na ratio decreased with salinity. This confirms the previous findings of Premachandra *et al*. (1992). The interesting finding was that the contribution of betaine and proline to the measured osmotic pressure also increased with increasing salinity level. This may confirm the hypothesis that betaine and proline act as a compatible solutes in the cytosol to counteract the accumulation of Na in the vacuole.

The expanding leaf sap of used sorghum had a higher osmotic pressure, K, K/Na ratio, total QACs, sugars, glycine betaine and proline than the expanded leaf sap and the vice versa was for Mg, Ca and occasionally Na. The increased osmotic pressure and the accompanied increase in the beneficial solutes is necessary for water translocation to maintain turgor and growth of the growing apex (Kramer and Boyer 1995).

It is clear from the obtained results that the application of glycine betaine reduced the accumulation of proline in sorghum plants under salinity stress. This is compatible with the finding of Larther *et al.* (1996) in rape leaf discs. They observed that, whatever the osmoticum, proline accumulation was lowered when glycine betaine was added to the stressing media. They suggested that glycine betaine might exert some effect at the membrane level which inhibit the osmoinduced proline response.

The obtained results indicated also that salinity stress increased N concentration of sorghum shoot and this is compatible with the results of Gorham *et al*. (1986) and Prasad *et al*. (1997). Such accumulation may be related to the increase in total soluble nitrogen mainly resulting from a sharp increase in total free amino acids, total soluble proteins and glycine betaine (Gorham *et al*. 1986, Hamed *et al*. 1994). Others have shown that salinity reduces N accumulation in plants (Pessarakli and Tucker 1988, Khan *et al*. 1990). Application of glycine betaine reduced N concentration in sorghum. However, correcting N concentration to total shoot dry weight indicated that salinity had a negative effect on N content, and glycine betaine improved N content of salinity stressed sorghum plants. This may reveal that the observed increase of N concentration under salt stress resulted from reduction of plant growth.

The observed reduction of C% of used sorghum is in a good conformity with the usual observation of photosynthesis reduction under salinity stress (Shalhevet 1993, Singh *et al*. 1996). Reduction of stomatal conductance with salinity treatments was also clear from the presented data. Increasing resistance to transpiration, osmotic adjustment and other adaptive mechanisms leading to xerophytism enable the plants to tolerate tissue water deficit (Tanguilig *et al*. 1987, Singh *et al.* 1996). The main effect of glycine betaine was not significant for stomatal conductance.

From this study it could be clear that the foliar application of glycine betaine exerted a repairing effect on some physiological disorders of salinity upon sorghum plants.

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