Improving Salt Tolerance of *Zea Mays* L. Plants by Presoaking Their Grains in Glycine Betaine.

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Absatract: The present investigation was carried out to study the effect of post-emergence soil treatment with different salinity levels (50, 100, and 150 mM NaCl) on growth characteristics, endogenous phytohormones (IAA, GA_3, zeatin, zeatin riboside, ABA and ethylene), photosynthetic pigments, carbohydrate contents, mineral ions composition, total amino nitrogen and proline, total soluble protein and protein profile of 30-day old *Zea mays* L. plants cv. Giza 2 resulted from grains soaked in water or the osmoprotectant glycine betaine for 12 hours at 28°C ± 2. Glycine betaine was shown to improve salt tolerance in *Zea mays* L. cv. Giza by protecting endogenous phytohormones from damage induced by salinity stress. It is able also to improve the vital properties of stressed *Zea mays* plants through protecting chloroplast membrane, preserving photosynthetic pigments, enhancing osmotic adjustment and turgor maintenance by accumulating organic osmolytes (total amino nitrogen, proline, total soluble protein and total soluble sugars) and inorganic osmolytes (K, Mg and Ca ions). Glycine betaine was shown also to enable *Zea mays* plants to cope with salinity via alteration of gene expression which led to the appearance of new proteins and / or increased the intensities of the other that may be involved in increasing the tolerance of *Zea mays* plants.

Key words: Zea mays - tolerance - salinity - stress - growth - phytohormones - metabolic activities - SDS - PAGE - protein electrophoresis - glycine betaine.

INTRODUCTION

Sodium chloride salinity inhibits growth and reduces yield of many plant species (Bernstein *et al.*, 2001). Progressive gradual decreases in fresh and dry weight were observed with progressive increase in salinity stress maintained for barley (AL-Kafaf *et al.,* 1990). Salt stress also reduced the final number of leaves on the main stem (Maas and Grieve, 1990) and decreased leaf appearance rate and blade area of wheat (Grieve and Francois, 1992). The depression of relative growth rate by salt stress varied in various types. In this respect, Erdei *et al.* (1996) reported that maize was more severely affected than sorghum.

Abiotic stresses impact on the hormone systems of plants. Therefore, stresses inhibit growth through their effects on the hormonal balance of the plant (Lerner and Amzallag, 1994).

Morgan (1990) concluded that ABA and cytokinins have opposite effects and roles in drought and osmotic stress. ABA levels appear to rise and cytokinin level to fall; both changes favor stomatal closure and possibly reduced resistance to water flow. Elevated ABA levels in the leaves are typical response to water deficit induced by drought or salinity (Chapin, 1991).

Li and Lin (1994) working on maize seedlings found that exogenous application of benzyladenine increased photosynthetic rate and photosynthetic electron transport in water-stress seedling compared with untreated stressed seedlings. They suggested that benzyladenine protected photosynthetic membranes under water stress of roots. Hare and Cress (1997) concluded that a reduction of cytokinin supply from root alters gene expression in the shoot. They suggested that cytokinins may impact on methylation reactions as methyl group carriers which are known to be important in the biochemical acclimation to environmental stress. Ethylene affects a wide variety of stresses such as water stress (McKeon *et al.,* 1982), osmotic stress (Chrominski *et al.*, 1988), and low-temperature stress (Wang, 1989; Field, 1990).

Castrillo and Fernandez (1990) found that there is a significant positive correlation between chlorophyll content and osmotic potential. Ebukanson (1989) recorded that increasing water stress increased carotene, phytol and protochlorophyll levels in maize seedling. Moreover, salt stress induced important alterations of the

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chloroplast structure such as swelling of thylakoids which might be related to the severe drop in chlorophyll contents (Dubacq *et al.*, 1993).

Soluble sugars are considered as the principle organic osmotica in a number of glycophytes subjected to saline conditions (Greenway and Munns, 1980). In this respect, Munns *et al.* (1982) reported that NaCl treatment increased the concentration of soluble carbohydrates in the growing tissues of barley while starch remained unchanged. Some authors suggested that the carbohydrate accumulation in various plants under salinity stress was attributed to the reduction in utilization of carbohydrates, either as a source of carbon or as a source of energy for the formation of new cells and tissues (Pèrez-Alfocea *et al.*, 1993 and Mohamed and Alain, 1995). On the other side EL-Shahaby (1981) and Abdel-Backey (1996), observed that, sugars and consequently the total carbohydrates contents at low and moderate salinity levels were lowered due to their utilization in growth and other plant activities.

The effect of salt stress on Ca^{++} nutrition is particularly interesting since Ca^{++} is an important factor in the resistance of plants to salt stress (Kent and Läuchli, 1985). Kurth *et al.* (1986) suggested that under saline conditions cells must develop a sufficiently low Ψs to reverse the flow of water, either by uptake of ions from the medium or by synthesis and transport of organic osmotica otherwise, cell enlargement will stop. Rodriguez *et al.* (1997) concluded that inorganic ions contributed relatively more to Ψs than did organic solutes. In this respect, Koyro (1997) and Rodriguez *et al.* (1997) found that salinity increased the concentration of Na^+ and Cl^- and reduced the concentration of K^+ and Ca^{++}.

Salinity induces distinct protein changes in root and shoot tissues of barley. (Ramagopal *et al.,* 1987; Hurkman and Tanaka, 1987). In this respect, Ebad *et al.* (1987) found a progressive and consistent decrease in concentration of total protein of maize plants with increasing NaCl. Moreover, Dell' Aquila and Spada (1992) working on germinating wheat embryos detected a novel expression of the 26 kDa protein (osmotin) under salinity conditions. In addition, Hamada (1994) proved that low concentration of NaCl stimulated soluble protein production, but higher concentrations decreased the content of soluble proteins. However, plants subjected to salt stress showed increased levels of total free amino acids (Dubey and Pessarakli, 1995).

In many plants, free proline accumulates in response to the imposition of a wide range of biotic and abiotic stresses (Hare and Cress, 1997). Carceller *et al.* (1999) found that the accumulating proline in maize cultivar showed a higher osmotic adjustment capacity than the non accumulating one. Dalta-pyrroline-5-carboxylate (P5C) synthase, an enzyme involved in the biosynthesis of proline, the gene of such enzyme was induced by dehydration and high salt (Yoshiba *et al.,* 1995, 1997). Moreover, Ginzberg *et al.* (1998) found a rapid increase in a steady-state transcription of genes that encode P5C's in alfalfa roots upon exposure to 90 mM NaCl suggesting that there are salt inducible genes.

Glycine betaine is a particulary widespread and effective osmoprotectant (Yancey, 1994; Gorham, 1995; Papageorgiou and Murata, 1995). Betaine acts as an osmoregulatory substance, maintaining the osmotic balance with the environment thus prevents damage from cellular dehydration (LeRudulier *et al.*, 1984; Robinson and Jones, 1986). Harinasut *et al.* (1996) found that exposure of 28-day-old rice seedlings to 150 mM NaCl for 6 days induced drastic decreases in relative water contents, chlorophyll, and protein in leaves. This effect was largely prevented when the seedlings were treated for 4 days with 15 mM glycine betaine before the exposure to NaCl. Mäkelä *et al.* (1996) found that labeled glycine betaine was translocated to all plant parts of turnip rape plants after one day of foliar application. Such results indicate that plants are able to translocate foliar applied glycine betaine from their leaf to other organs. They also suggested that glycine betaine is quite inert end product in plant cells being mainly phloem-mobile.

The present work was carried out to investigate the effect of post-emergence soil treatment with different salinity levels (50, 100, and 150 mM NaCl) on growth characteristics, endogenous phytohormones (IAA, GA_3, zeatin, zeatin riboside, ABA and ethylene), photosynthetic pigments, carbohydrate contents, mineral ions content, total amino nitrogen and proline, total soluble protein and protein profile of *Zea mays* L. plants cv. Giza 2 resulted from grains soaked in water or the osmoprotectant glycine betaine.

MATERIALS AND METHODS

The tested plant in the present investigation was *Zea mays* L.cv. Giza 2 (Gz 2). The grains of this line were obtained from Agriculture Research Centre, Ministry of Agriculture.

Growth conditions:

The experiment was carried out in the green house, Faculty of Science, Ain Shams University. Uniform sterilized grains (Gz 2) were divided into two groups; the grains of the first group were soaked in tap water, while the grains of the second group were soaked in 100 mM glycine betaine over night (about 12 hours) at 28°C ± 2. Plastic pots were used, each pot was filled with 800 g of washed dried sand and a plastic tube was inserted in the middle of each pot to prevent leaching of the salt in the bottom and to ensure equal distribution of water and nutrient. Eight uniform *Zea* grains of each previously soaked groups were sown in each pot and irrigated with water to 80% of their saturation capacity and supplied with half strength Hoagland nutrient solution once a week.

After one week the growing seedlings were divided into 2 groups. The seedlings of the 1st or the 2nd group which were previously soaked in pure water or 100 mM of glycine betaine, were divided into 4 subgroups receiving NaCl concentrations (0.0, 50, 100 and 150 mM). The different saline solutions were added once, and the soil water saturation capacity of each pot, was adjusted daily at 80%. After 30 days from planting and 3 weeks of treatments plants were harvested at random from the different treatments and divided into shoots and roots to measure the different growth parameters, phytohormones (auxins, gibberellins, cytokinins and ABA) were determined in the extracts of fresh tissues, ethylene in fresh plants, photosynthetic pigments in the extract of fresh leaves and protein profile in the extract of plants (at 24 hours and 3 weeks after treatment). The carbohydrate contents and ion contents were estimated in the oven dried tissues at 80°C. Statistical analysis of growth parameters (5 samples), metabolic activities (3 samples) were analyzed using L.S.D. at 5% level of probability according to SAS program (1982).

Extraction, Separation and Estimation of Endogenous Phytohormones:

The method of extraction was essentially similar to that adopted by Shindy and Smith (1975). To estimate the amounts of acidic hormones [indole acetic acid (IAA), gibberellic acid(GA_3) and abscisic acid(ABA)], the plant hormone fractions and standard ones were methylated according to Vogel (1975) to be ready for GC analysis. The retention time (RT) of peaks of authentic samples was used in identification and characterization of peaks of samples under investigation. However, cytokinin fractions (zeatin and zeatin riboside) were extracted as previously mentioned for the acidic hormones and were detected using High Performance Liquid Chromatography (HPLC), standards of zeatin and zeatin riboside were used and the peak area of the standard were also used in identification and detection of cytokinin in each sample (Müller and Hilgenberg, 1986). The method of ethylene determination was essentially similar to that adopted by Kao and Yang (1983). The plant material used for ethylene measurement was 30-days old maize plants subjected to different treatments and those of control ones. The peak area and ethylene concentration were measured automatically by the software attached to the GC.

Estimation of Photosynthetic Pigments:

Chlorophyll a, Chlorophyll b and Carotenoids were determined in the leaves of the investigated plant. The spectrophotometric method recommended by Metzner *et al.* (1965) was used. The pigment contents were calculated as mg g^{-1} fresh weight of leaves.

Estimation of Carbohydrates:

Soluble sugars was extracted from dry powdered tissue by ethanol according to the method described by Homme *et al.*(1992). The anthrone sulphuric acid method described by Whistler *et al.* (1962) was used for the determination of soluble sugars. According to Naguib (1963) a known weight of dried plant residue which remained after extraction of soluble sugars was heated under reflux in 1.5 N H_2SO_4 for 6 hours at 100°C. The extract was filtered and made up to volume. Polysaccharide content was estimated by anthrone-sulphuric acid method (Whistler *et al.,* 1962). Total carbohydrates were calculated as the sum of the amounts of soluble sugars and polysaccharides of the same sample. All data were calculated as mg glucose equivalent 100 g^{-1} dry weight.

Estimation of Certain Minerals:

The dried matter digested according to the method of Chapman and Pratt (1978). One g of ground plant material (oven dried at 80°C) was weighed into a 250 ml digestion flask which has been previously washed with acid and distilled water. Ten ml mixture of concentrated nitric acid and perchloric acid (70%) at the ratio 4: 1 (v/v) were added. The samples were digested on electric heater until dense white fumes appeared and finally the solution became clear and was about 5 ml. The samples were then left to cool and diluted with

distilled water. Filtration was carried out using filter paper Whatman No. 42. then the filtrates were quantitatively transferred into a 50 ml volumetric flask. Each sample was made up to volume with distilled water. The solutions were stored for potassium, sodium, calcium and magnesium determinations. Sodium and potassium were estimated by the flame emission technique as adopted by Ranganna (1977). Magnesium and calcium were determined simultaneously by ICP Spectroscopy according to the method of Soltanapour (1985). Data were calculated as mg $100g^{-1}$ dry weight.

Estimation of Amino Nitrogen:

The tissue extract of soluble – N was deproteinized using ethanol acetone mixture and the free amino acids were then determined Spectro-colourimetrically with ninhydrin according to the method described by Muting and Kaiser (1963).

Estimation of Proline:

Free proline was extracted and determined according to the method described by Bates *et al.* (1973).

Estimation of Total Soluble Proteins:

The total soluble protein concentration was determined in fresh tissues according to the method described by Bradford (1976) using the Bio-Rad protein assay which is a dye-binding assay based on the differential colour change of a dye in response to various concentrations of protein (Bio Rad Technical Bulletin 1051, 1977).

Protein Electrophoresis:

The extracted protein samples were fractionated by sodium dodecyle sulphate-polyacrylamide gel electrophoresis (SDS-PAGE). A solution of 12% SDS-polyacrylamide slab gel was prepared according to the method of Laemmli (1970) as modified by Studier (1973) and equal volumes of proteins extracted were loaded. Scanning of the separated protein bands was analysed by the Gel Documentation System (GDS) which comparing polypeptide maps; molecular protein markers, band intensity and molecular weight of each polypeptide in relation to standard markers using gel proanalyzer version 3 MEDIA CYBERNE TICE Imaging Experts Software.

RESULTS AND DISCUSSION

Growth Characteristics:

It has been found in the present work that the growth responses of *Zea mays* L. cv. Giza 2 plants, due to post-emergence treatments with different levels NaCl (50, 100, 150 mM), varied considerably, ranging from stimulation to severe reduction (Table 1). Low salinity level (50 mM) is promotive on inducing significant increases in shoot length, circumference of stem, mean leaf area per plant, root length, number of adventitious roots, fresh and dry weights of shoots, roots and whole plant as compared with control values. The values of growth parameters were generally lowered by increasing salinity, and were more pronounced using the highest concentration of NaCl (150 mM) compared to untreated control plants (Table 1).

The inhibitory effects of water stress induced by salinity on the growth parameters of the experimental plants are in accordance with the results obtained by Lutts *et al*. (1995); Moons *et al*. (1995) and Lutts *et al*. (1996) working on different cultivars of rice, and Ali (2000) working on sunflower and cotton plants. These inhibitory effects may be attributed to the fact that salt stress is known to retard plant growth through several facets of plant activities such as osmotic adjustment (Lutts *et al*., 1996), protein and nucleic acid synthesis (Bejaoui, 1985), ion uptake (Rodriguez *et al.,* 1997), hormonal balance (Foda *et al.*, 1991), photosynthesis (Singh and Dubey, 1995), ion toxicity (Gorham *et al.,* 1994, Kohl, 1997), disturbances of plasma membrane structure and function (Morales *et al.,* 1998), or more likely, a combination of these with other facets of metabolism may be involved.

It has been found, in the present investigation that presoaking *Zea mays* grains in 100 mM glycine betaine reduced the inhibition of growth induced by increasing soil salinity. Therefore, glycine betaine increased the leaf area of *Zea mays* cv. Giza 2 exposed to 150 mM NaCl by 22.9% as compared with that of stressed plants. Glycine betaine also significantly increased the fresh weights of differently stressed plants and their dry weights over those of the corresponding stressed controls (Table 1).

The increase in leaf area following glycine betaine treatment which maximizes the photosynthetic activity (increase in total carbohydrate) and biomass production could be attributed to the physiological ability of glycine betaine to prevent cellular dehydration, induced by soil salinity and to maintain turgor pressure and photosynthetic activity under conditions of low water potentials (Borowitzka, 1981; Agboma *et al.,* 1997b; Mäkelä *et al.,* 1999).

Endogenous Phytohormones:

The detectable amounts of IAA and GA_3 showed significant reduction in NaCl-treated maize plants (Table 2). The magnitude of reduction was much more pronounced by applying the highest concentration of NaCl (150 mM). The values of reduction in response to 50, 100 and 150 mM NaCl were 77.9%, 84.3% and 90.3%, respectively for IAA, 41.6%, 53.3% and 82.3%, respectively for GA_3, relative to those detected in untreated control plants (Table 2) The decrease in auxin and gibberellin contents in response to high levels of salinity stress were observed by Hassanein (1999a) working on rice plant and EL-Khawas (1999) working on *Trigonella foenum graecum* plant.

On the other hand, the amounts of zeatin and zeatin riboside in *Zea mays* plants increased mostly by increasing NaCl concentration. The gradual increase in the detectable amounts of cytokinins (zeatin and zeatin riboside) in response to NaCl treatments (50, 100 and 150 mM) (Table 3) indicated that salt stress may induce the biosynthesis of zeatin and zeatin riboside in maize plants and consequently increasing their ability to cope with salinity stress. Similar results were obtained by Reid and Wample (1985); Foda *et al.* (1991) and Hassanein (1999a). However, Thomas *et al.* (1992) found that, the concentration of free cytokinins (zeatin and zeatin riboside) decreased during salt stress.

In addition, the amount of ABA detected in *Zea mays* plants, was found to increase in NaCl treated maize plants (Table 2). The magnitude of increase was directly proportional to the increase in the concentration of NaCl. Therefore, 1.95, 3.85 and 6.9 fold increases in ABA were observed in response to 50, 100 and 150 mM NaCl treatments, respectively. Chandler and Robertson (1994) reported that ABA is implicated in the control of physiological and molecular processes involved in the desiccation tolerance in seeds as well as vegetative tissue. In addition, La Rosa *et al*. (1987) found that ABA stimulated osmotic adjustment and involved in adaptation of tobacco callus to NaCl.

Concerning the ethylene content, it has been found in the present work that 2.25, 21.0 and 53.5 fold increases in ethylene contents were recorded in response to 50, 100 and 150 mM NaCl treatment compared with the control value (Table 2). The stimulation of ethylene production in the present work is a common response of plants to stress (Chrominski *et al.,* 1988; Khan *et al.,* 1990). Ethylene production is probably part of the acclimation process that plants develop to cope with the condition of salinity stress. Therefore, the increase in ethylene and ABA contents along with decrease in auxin and gibberellin levels under salinity stress might be responsible for the observed stress symptoms such as growth inhibition.

Higher levels of auxins (IAA), gibberellins (GA_3) and cytokinins (zeatin and zeatin riboside) and lower contents of ABA and ethylene were observed in glycine betaine-treated maize plants exposed to soil salinity (Table 2). In this respect, Mäkelä *et al.* (1998) found that ABA transport and compartmentation may have been altered in leaves of salt-or drought-stressed tomato previously sprayed with glycine betaine. Moreover they suggested that cytokinins are also affected. Therefore glycine betaine may act as a second messenger in inducing growth through playing a role in alleviating the harmful effect of soil salinity on metabolic activities relevant to growth particularly the endogenous phytohormones.

It could be concluded that the capacity of *Zea mays* L. cv. Giza 2 to tolerate salinity appeared to be higher in glycine betaine treated plants then in untreated ones. This depends on the potentiality of glycine betaine-treated plants to synthesize adequate levels of endogenous phytohormones and to protect such levels from destruction and/or transformation.

Photosynthetic Pigments:

Increase the salt concentration from 50 to 150 mM NaCl caused significant decreases in chlorophyll a, chlorophyll b and carotenoid contents of *Zea mays* leaves compared with those of untreated plants (Table 3). The highest concentration of NaCl (150 mM) severely reduced all previously mentioned photosynthetic pigments. The magnitude of decrease in chlorophyll (b) was much higher than that of chlorophyll (a). Moreover, the chlorophyll a / chlorophyll b ratio increased by increasing the salinity level till 100 mM indicating that chlorophyll a in stressed maize plant is relatively more stable to salinity treatment than chlorophyll b (Table 4). These results are in a good agreement with those of Singh *et al.* (1994) in *Vigna radiata*, Muthukmarasamy and Panneerselvam (1997) in peanut and Hassanein (1999a) in rice plants.

The severe reduction in photosynthetic pigments in maize leaves in response to salinity treatment might be ascribed to the toxic action of NaCl on the biosynthesis of pigments, increasing their degradation and / or due to damage of the chloroplast thylakoid (Hashem, 2000). The observed severe reduction in Mg ion in salt-treated *Zea mays* plants (Table 5) which are essential for chlorophyll biosynthesis reinforced the view that salinity decreased chlorophyll biosynthesis. Moreover, the decrease in chlorophyll content in salt-stressed *Zea mays* plants concurrently with the increase in the proline level (Table 6) led to the suggestion that nitrogen may shifted to the synthesis of proline instead of chlorophyll (De La Rosa and Maiti, 1995).

It has been found in the present work that presoaking maize grains cv. Giza 2 in glycine betaine and growing the seedlings in increasing levels of NaCl partially overcame the adverse effects of the salinity stress by increasing chlorophyll a, chlorophyll b and carotenoid contents concomitantly with higher levels of total carbohydrates (Table 4) and soluble proteins (Table 6) than those of stressed plants without glycine betaine. In this respect, Harinasut *et al.* (1996) found that exposure rice seedlings to 150 mM NaCl induced drastic decrease in relative water contents, chlorophyll and protein in leaves. This effect was largely prevented when the seedlings were treated for 4 days with 15 mM glycine betaine before exposure to NaCl. They also found that the quantum yield of PSII was decreased by 27% under salt stress. This decrease was also prevented by glycine betaine treatment. This indicates that glycine betaine may stimulate the biosynthesis of chlorophylls and carotenoids and / or may retard their degradation. Also, glycine betaine can protect the chloroplasts and their membranes against the deteriorative effects of salinity stress and thus maintaining their integrity (Hashem, 2000).

Carbohydrate Contents:

Soluble sugar, polysaccharide and consequently the total carbohydrate contents were progressively and significantly decreased in both roots and shoots with increasing NaCl concentration, except the lower concentration (50 mM NaCl) which significantly increased these carbohydrate fractions in roots above those of the control plants (Table 5).

The reduction in total carbohydrate contents of maize shoots and roots in response to treatment with the highest NaCl concentration (150 mM) concomitantly with arrested growth rate (Table 1) and reduction in leaf photosynthetic pigments (Table 3) led to the conclusion that sodium chloride inhibited the photosynthetic activity and / or increased partial utilization of carbohydrates into other metabolic products. In this respect, Singh and Dubey (1995) authenticated that the overall reduction of growth parameters is probably due to high sensitivity of photosystem II and Hill reaction activity to salinity stress which resulted in reduction in photosynthetic capacity in saline stressed plants.

The integrity of chloroplasts and stabilizing their membranes in the stressed *Zea mays* plants in presence of glycine betaine (Hashem 2000), concomitantly with increases in leaf area (Table 1) and photosynthetic pigment contents (Table 3) might be responsible for the higher contents of photosynthate (soluble sugars, polysaccharides and total carbohydrate contents) and soluble protein (Table 6) than those of the corresponding stressed plants in absence of glycine betaine. This indicates that glycine betaine can be involved in increasing the cell osmotic potential which improves the tolerance against salinity.

Accumulation of soluble sugars in stressed *Zea mays* plants previously treated with glycine betaine plays a prominent role in alleviating the water stress induced by salinity either via osmotic adjustment (Ackerson, 1985) or by conferring some desiccation resistance to plant cells (Srivastava *et al.,* 1995).

Ion Contents:

The data presented in Table 5 provide evidence that Na^+ content in shoots and roots of maize plants were increased significantly with increasing NaCl salinization level in the soil. The accumulation of this ion in plant tissues was more pronounced in shoots than in roots. On the other hand the contents of K^+, Mg^{++} and Ca^{++} decreased in most cases with increasing salinity level, except the lowest concentration of NaCl which induced a high significant increase in Ca^{++} contents in shoot of the tested plants as compared with those of the untreated control plants. Also K^+ ions accumulated in salt stressed maize root by all NaCl concentration used (Table 5). Patil *et al.* (1996) proved that leaf sodium (Na^+) content of maize plants increased markedly with concomitant decrease in potassium (K^+) content at high salinity levels. Also, Jescke and Wolf (1998) suggested that the association of induced increase in plant Na^+ with a decrease in K^+ content may be due to the competition for sites through which influx of both cations occurs. The retarded growth of salt stressed maize plant in the present work particularly by applying 150 mM of NaCl might be attributed to the increase in the osmotic potential of the saline soil, the toxic effects caused by the accumulation of excessive amounts of Na and / or the deficiency symptoms of Mg and Ca in both shoots and roots of salinized maize plants.

Presoaking *Zea mays* grains in glycine betaine is able to increase ions uptake (K, Mg and Ca) and their maintenance in adequate amounts in salt-stressed plant to enhance the metabolic processes as compared with that of the corresponding stressed plants without glycine betaine. This can be involved in osmotic adjustment and turgor maintenance of stressed cells which improve salt tolerance of *Zea mays* plants cv. Giza 2. On the other hand, glycine betaine is able to decrease the uptake of Na ions in stressed *Zea mays* plants. In this connection, Ahmad *et al.* (1987) reported that glycine betaine may modulate the activity of membrane carrier proteins or channels. It may stimulate the opening of Ca^{2+} and Na^+ / K^+ channels in plants as shown with acetylcholine (Tretyn and Kendrick, 1991).

Amino-n, Proline and Soluble Protein Contents:

The data recorded in Table 6 reveal that the increase in NaCl concentration had a stimulatory effects on amino-N, proline accumulation concurrently with decrease in soluble protein contents in most cases in both shoots and roots of maize plants. The increase in the total free amino acids due to salinity is in accordance with the results obtained by some other authors (Guerier and Bourrgeais-Chaillou, 1994 and Dubey and Pessarakli, 1995). The decrease in soluble proteins in both shoots and roots by salinity indicated that salinity might have promoted hydrolysis of protein resulting in an accumulation of proline particularly at high concentration of NaCl and / or inhibited protein synthesis. These conclusions are in a good agreement with those obtained by Rais *et al.* (1993) on jojoba, by Singh *et al.* (1994) in *Vigna radiata*, by Mahraj and Sudhansha (1995) in pea and by Kasim and Dowidar (2006) in radish seedlings.

The reduction in soluble protein contents in response to treatment of maize plants with 150 mM NaCl were 13.6% and 34.0% in both shoots and roots, respectively below those of the control. These results indicated that the adverse effect of salinity was much more pronounced in roots than in shoots (Table 6). On the other hand, the low concentrations 50 and 100 mM of NaCl increased the protein content of shoots by 9.78% and 8.25% above the control value, respectively. This increase in protein content concurrently with increase in growth of maize plant led to the conclusion that these concentrations of NaCl may enhance protein synthesis which increases the ability of maize plant to cope with salinity (Hassanein, 1999b).

The increase in proline in shoots and roots of salt stressed maize plants was positively correlated to salt concentration used. Moreover, the proline contents were more obvious in shoots than in roots. These results are in good agreement with those obtained by Lin and Kao (1996); Lutts *et al.* (1996) and Hassanein (1999b). Thus the accumulation of proline in salt stressed maize plants is considered as one of the major physiological defense mechanisms of salt stressed maize plant.

Glycine betaine treatment, in the present work was shown to increase amino-N, proline and soluble protein contents concurrently with increased growth rate in unstressed and differently salt-stressed plants. This may be due to the effect of glycine betaine in increasing the amino acids and proteins which can be involved in increasing the ability of *Zea mays* cv. Giza 2 to cope with salinity stress. Also, glycine betaine can be involved in stabilizing the folded protein structure (Low, 1985) and can alter the thermodynamic properties of membranes, perhaps by directly interacting with phosphatidylcholine moieties (Rudolph *et al.,* 1986). In addition, glycine betaine protects proteins against the unfavorable consequences of dehydration-induced thermodynamic perturbation (Paleg *et al.,* 1984).

Protein Electrophoretic Pattern:

It has been observed in the present work that five protein bands (M.wts.: 94, 82, 73, 56 and 46 kDa) disappeared in salt stressed *Zea mays* cv. Giza 2 in response to 24-hours salt treatment, this number decreased to only three bands after 3 weeks of treatment. Levitt (1972) proved that in various crop species, a decrease in the protein level in salt-stressed plants is attributed to a decrease in protein synthesis; the decreased availability of amino acid, and the denaturation of enzyme involved in amino acid and protein synthesis.

On the other hand a number of salt-shock proteins appeared in salt stressed *Zea mays* plants after 24 hours of treatment (Plate 1), while seventeen *de novo* synthesized salt-adaptive proteins could be detected after 3 weeks of treatment (Plate 2). In this connection, Bartels *et al.* (1990) assumed that the dehydration process led to a massive synthesis of new transcripts and proteins. among these *de novo* synthesized proteins only one band (Mwt: 89 kDa) could be detected in stressed plants after 24 hours as well as 3 weeks of treatment. This finding would suggest that there may be differences between expression of genes induced by salinity shock and that expressed after adaptation (Hurkman and Tanaka, 1987).

A protein band of molecular weight 31.97 kDa was *de novo* synthesized in response to 3 week treatment with 100 mM NaCl, while another protein band of molecular weight 20.3 kDa was newly synthesized as a

common salt adaptive protein in response to different NaCl concentration used. Similar results were obtained by Ericson and Alfinito (1984) who indicated that tobacco cells adapted to a medium containing NaCl showed two protein bands of 20 and 32 kDa in higher concentration than in unadapted cells.

A salt-adaptive protein band of molecular weight 26.36 kDa newly appeared in response to 100 and 150 mM NaCl treatment for 3 weeks. Since 26 kDa protein is specifically synthesized and accumulated in cell undergoing osmotic adjustment to salt stress, this protein is named osmotin (Singh *et al.,* 1987).

Our results also demonstrated that a protein band of about 28 kDa is intensified in response to treatment with different NaCl concentrations. Such increase could be detected after 24 hours of treatment and continued till the end of the experiment.

In glycine betaine treatment it has been found that, a number of protein bands (M wts: 94.00, 82.02, 73.65, 46.40, 44.14, 40.00 and 36.54 kDa) that disappeared in response to NaCl treatment can be detected in glycine betaine treated *Zea mays* plants exposed to salt stress, suggesting that glycine betaine may protect the plants against severe deterioration of salt stress through preventing the destructive effect of salinity on protein structure. Similar results were obtained by Harinasut *et al.* (1996). Also, ten newly synthesized protein bands of molecular weights 146.33, 140.34, 120.01, 116.31, 113.90, 91.50, 85.13, 43.50, 37.400 and 29.69 kDa were detected in salt stressed plant treated with glycine betaine could not appear in the same plants in absence of glycine betaine. Such results agree with those obtained by Gibon *et al.* (1997) who found that treatment of rape leaf discs with glycine betaine followed by the induction or up regulation of a set of polypeptides, not seen under stress conditions. So, it can be concluded that glycine betaine may enable salt-stressed plants to survive under such circumstances via alteration of gene expression leading to changed protein pattern that may alleviate the harmful effect of salt stress.

Finally, it can be concluded that presoaking *Zea mays* grains in glycine betaine could ameliorate the adverse effects of salinity stress via the enhancement of the hormonal defense systems and the accumulation of organic osmolytes such as soluble sugars, soluble protein and proline and inorganic osmolytes such as mineral ions. Cytoplasmic osmolyte accumulation is believed to reduce cellular water potential below the external water potential, while avoiding deleteriously high ionic strength. This enables water to move into the cell and maintained there. Maintenance of turgor pressure is essential for continued growth. The appearance of new peptides added another protection mechanism to the action of glycine betaine.

Table 1: Effect of post-emergence soil treatment with different levels of NaCl on growth characteristics of *Zea mays* L. cv. Giza 2 in absence or presence of glycine betaine. Each value is a mean of 5 variables.

a- Shoot length (cm)						b- Area of leaves per plant (cm^2)				
GB (mM)	NaCl concentration (mM)					NaCl concentration (mM)				
	0.0	50	100	150	Mean	0.0	50	100	150	Mean
0	48.50	49.97	46.70	36.5	45.42 b	16.62	20.31	15.92	14.13	16.75 b
100	53.56	51.85	49.85	46.7	50.47 a	20.84	21.04	19.51	17.31	19.68 a
Mean	51.03 a	50.91 a	48.23 b	41.60 c		18.73 b	20.68 a	17.72 c	15.72	

L.S.D. (0.05) GB = 0.613   NaCl = 0.867 GB x NaCl = 1.227   L.S.D. (0.05) GB = 0.613   NaCl = 0.867 GB x NaCl = 1.22

c- Root length (cm.)						d- Number of adventitious roots				
0	16.14	18.22	18.00	16.50	17.22 b	12.0	14.0	10.2	8.4	11.51 b
100	17.68	20.00	19.00	18.20	18.72 a	12.8	14.4	12.0	10.8	12.50 a
Mean	16.91 c	19.11 a	18.50 b	17.35 c		12.4 b	14.2 a	11.1 c	9.6 d	

L.S.D. (0.05) GB = 0.387   NaCl = 0.547 GB x NaCl = 0.774   L.S.D. (0.05) GB = 0.544   NaCl = 0.769 GB x NaCl = 1.089

e- Fresh weight of shoot (g)						f- Fresh weight of root (g)				
0	2.15	2.40	1.97	1.55	2.02 b	1.16	1.52	1.13	0.93	1.19 a
100	2.89	2.80	2.71	2.10	2.63 a	1.22	1.54	1.34	1.14	1.31 a
Mean	2.52 a	2.60 a	2.34 b	1.83 c		1.19 bc	1.53 a	1.24 b	1.04 c	

L.S.D. (0.05) GB = 0.091   NaCl = 0.128 GB x NaCl = 0.180   L.S.D. (0.05) GB = 0.134   NaCl = 0.189 GB x NaCl = 0.268

g- Fresh weight of shoot/fresh weight of root ratio						h- Dry weight of shoot (g)				
0	1.94	1.66	1.75	1.67	1.76 b	0.26	0.27	0.24	0.20	0.24 b
100	2.4	1.83	2.13	1.9	2.07 a	0.30	0.30	0.29	0.25	0.29 a
Mean	2.17 a	1.75 b	1.94 ab	1.79 b		0.28 a	0.29 a	0.27 b	0.23 c	

L.S.D. (0.05) GB = 0.241   NaCl = 0.341 GB x NaCl = 0.483   L.S.D. (0.05) GB = 0.007   NaCl = 0.009 GB x NaCl = 0.013

i- Dry weight of root (g)						j- Dry weight of shoot/dry weight of root ratio				
0	0.16	0.17	0.13	0.10	0.14 b	1.65	1.58	1.78	1.50	1.63 b
100	0.16	0.17	0.16	0.13	0.16 a	1.84	1.79	1.84	1.91	1.85 a
Mean	0.16 b	0.17 a	0.15 c	0.12 d		1.75 ab	1.69 b	1.81 a	1.71 ab	

L.S.D. (0.05) GB = 0.003   NaCl = 0.004 GB x NaCl = 0.006   L.S.D. (0.05) GB = 0.076   NaCl = 0.108 GB x NaCl = 0.152

Means with the same letter are not significantly different

Table 2: Effect of post-emergence soil treatment with different levels of NaCl on acidic hormones, ethylene and cytokinin contents in *Zea mays* L. cv. Giza 2 in absence or presence of glycine betaine.

Treatment (mM NaCl)	Acidic hormones (μg 100 g^{-1} f.wt.)			Ethylene n mole/g/hr	Cytokinins (μg 100 g^{-1} f.wt.)	
	IAA	GA3	ABA		Zeatin	Zeatin riboside
0.0 (control)	5.8	196	2.0	0.2	116.0	155.0
50	1.28	114.4	3.9	0.45	125.0	226.0
100	0.91	91.5	7.7	4.20	178.2	329.0
150	0.56	34.7	13.8	10.7	308.0	383.0
0.0 + GB	6.4	234.0	1.4	0.07	156.4	171.9
50 + GB	5.8	152.0	2.6	0.29	146.5	279.0
100 + GB	3.4	142.3	5.5	0.57	214.0	380.0
150 + GB	0.7	83.36	8.8	0.75	417.3	643.5

Table 3: The effect of post-emergence soil treatment with different levels of NaCl on photosynthetic pigments in *Zea mays* L. cv. Giza 2 in absence or presence of glycine betaine. Results are expressed as μg g^{-1}f.wt. Each value is a mean of 3 variables.

Chlorophyll a						Chlorophyll b				
GB(mM)	NaCl concentration (mM)					NaCl concentration (mM)				
	0.0	50	100	150	Mean	0.0	50	100	150	Mean
0	854.0	804	506	427.8	647.95 b	256.62	218.48	136.32	120.44	182.97 b
100	872.0	844	751.2	596.0	765.80	259.52	239.77	215.37	156.84	217.88 a
Mean	863.0 a	824.0 b	658.6 c	511.9 d		258.07 a	229.13 b	175.85 c	138.64 d	
L.S.D. (0.05) GB = 6.265	NaCl = 8.860 GB x NaCl = 12.53					L.S.D. (0.05) GB = 1.791	NaCl = 2.533 GB x NaCl = 3.582			
Chlorophyll a / chlorophyll b ratio						Carotenoid				
0	3.33	3.68	3.71	3.55	3.57 a	164.50	167.0	141.10	138.10	152.68 b
100	3.36	3.52	3.49	3.80	3.54 a	226.20	224.0	205.00	184.00	209.80 a
Mean	3.345 c	3.60 b	3.60 b	3.675 a		195.35	195.5 a	173.05 b	161.05 c	
L.S.D. (0.05) GB = 0.032	NaCl = 0.046 GB x NaCl = 0.065					L.S.D. (0.05) GB = 1.588	NaCl = 2.245 GB x NaCl = 3.176			

Table 4: Effect of post-emergence soil treatment with different levels of NaCl on soluble sugars, polysaccharides and total carbohydrate contents in *Zea mays* L. cv. Giza 2 in absence or presence of glycine betaine. Values are expressed as μg 100 g^{-1} dry weight. Each value is a mean of 3 variables.

a- In shoot						b- In root				
Soluble sugars						Soluble sugars				
GB(mM)	NaCl concentration (mM)					NaCl concentration (mM)				
	0.0	50	100	150	Mean	0.0	50	100	150	Mean
0	2933.33	2744.33	1777.67	1560.00	2253.83 b	2175.00	2350.00	1750.00	1500.00	1943.75 b
100	3555.67	2850.00	2100.00	2050.00	2638.92 a	2300.00	2600.00	2125.00	2000.00	2256.25 a
Mean	3244.5 a	2797.17 b	1938.84 c	1805.00 d		2237.5 b	2475.00 a	1937.5 c	1750.00 d	
L.S.D. (0.05) GB = 68.463 NaCl = 96.821GBxNaCl = 136.932						L.S.D. (0.05) GB = 126.42 NaCl = 178.78 GBxNaCl = 252.85				
Polysaccharides						Polysaccharides				
0	10900	9833	7500	4572	8201.25 b	7622.00	9050.00	8742.00	6724.00	8034.5 b
100	12631	12871	13369	15182	13513.25 a	10277.00	11310.00	12000.00	12654.33	11560.33 a
Mean	11765.5 a	11352.00 b	10434.5 c	9877.00 d		8949.5 c	10180 ab	10371 a	9689.2 b	
L.S.D. (0.05) GB = 145.58 NaCl = 205.88 GBxNaCl = 291.16						L.S.D. (0.05) GB = 374.08 NaCl = 529.03 GBxNaCl = 748.2				
Total carbohydrate						Total carbohydrate				
0	13833.00	12677.67	9278.33	6145.33	10483.58 b	9797.0	11700.0	10492.0	8224.0	10053.25 b
100	16186.67	15621.00	15469.00	17232.00	16127.17 a	12577.0	13610	14125.0	14654.0	13741.5 a
Mean	15010.00 a	14149.33 b	12373.67 c	11688.67 d	11187.0 b	12655.0 a	12308.5 a	11439.0 b		
L.S.D. (0.05) GB = 108.99 NaCl = 154.13 GBxNaCl = 217.98						L.S.D. (0.05) GB = 344.73 NaCl = 487.53 GBxNaCl = 689.50				

Means with the same letter are not significantly different.

Table 5: The effect of post-emergence soil treatment with different levels of NaCl on ion contents of *Zea mays* L. cv. Giza 2 in absence or presence of glycine betaine. Results are expressed as mg 100 g^{-1} dry weight. Each value is a mean of 3 variables.

a- In shoot						b- In root				
Sodium						Sodium				
GB (mM)	NaCl concentration (mM)					NaCl concentration (mM)				
	0.0	50	100	150	Mean	0.0	50	100	150	Mean
0	76.80	180.03	189.83	230.80	169.37 a	163.00	184.00	192.00	215.33	188.58 a
100	41.67	131.97	165.30	186.00	131.24 b	160.67	170.33	178.00	183.00	173.00 b
Mean	59.24 d	156 c	177.57 b	208.4 a		161.84 d	177.17 c	185.00 b	199.17 a	
L.S.D. (0.05) GB = 1.221	NaCl = 1.73 GB x NaCl = 2.44					L.S.D. (0.05) GB = 1.06	NaCl = 1.45 GB x NaCl = 2.12			
Potassium						Potassium				
0	464.00	290.9	228.9	107.4	272.8 a	392.00	482.33	767.00	1028.00	667.33 b
100	546.50	455.6	277.4	126.4	351.48 b	515.33	870.00	820.00	1370.00	818.83 a
Mean	505.25 a	373.25 b	253.15 c	116.90 d		453.67 d	526.17 c	793.50 b	1199.00 a	
L.S.D. (0.05) GB = 1.575	NaCl = 2.23 GB x NaCl = 3.15					L.S.D. (0.05) GB = 1.93	NaCl = 2.73 GB x NaCl = 3.85			

Table 5: Cont

Magnesium						Magnesium				
0	486.67	470.67	399.67	273.00	407.5 b	169.5	152.50	95.00	50.50	116.88 b
100	554.00	524.67	478.00	402.67	489.84 a	260.67	167.00	146.00	103.00	169.17 a
Mean	520.34 a	497.67 b	438.83 c	337.84 d		215.09 a	159.75 b	120.5 c	76.75 d	
L.S.D. (0.05) GB = 1.609		NaCl = 2.276 GB x NaCl = 3.22				L.S.D. (0.05) GB = 1.37		NaCl = 1.947 GB x NaCl = 2.75		
Calcium						Calcium				
0	891.00	1089.67	790.00	733.67	876.09 b	810.00	661.67	506.67	320.00	574.59 b
100	993.33	1176.67	870.00	783.00	955.57 a	829.67	751.67	633.67	403.00	654.5 a
Mean	942.17 b	1133.17 a	830.00 c	758.34 d		849.84 a	706.67 b	570.17	361.50 d	
L.S.D. (0.05) GB = 1.713		NaCl = 2.422 GB x NaCl = 3.43				L.S.D. (0.05) GB = 1.45		NaCl = 2.06 GB x NaCl = 2.914		

Means with the same letter are not significantly different

Table 6: The effect of post-emergence soil treatment with different levels of NaCl on amino nitrogen, proline and total soluble protein contents in *Zea mays* L. cv. Giza 2 in absence or presence of glycine betaine. Results listed are expressed as mg g^{-1} fresh weight in case of total soluble protein, as mg 100 g^{-1} dry weight in case of amino-N and protein. Each value is a mean of 3 variables.

a- In shoot						b- In root				
Amino-N						Amino-N				
GB (mM)		NaCl concentration (mM)				NaCl concentration (mM)				
	0.0	50	100	150	Mean	0.0	50	100	150	Mean
0	460.67	466.67	505.33	652.33	521.25 b	194.33	530.00	596.00	1272.33	648.17 a
100	480.00	596.00	538.00	728.33	585.58 a	168.33	353.00	391.67	469.00	345.50 b
Mean	470.34 c	531.34 b	521.67 b	690.33 a		181.33 d	441.5 c	493.84 b	870.67 a	
L.S.D. (0.05) GB = 29.073 NaCl = 41.115 GBxNaCl = 58.15						L.S.D. (0.05) GB = 35.514 NaCl = 50.225 GBxNaCl = 71.03				
Proline						Proline				
0	34.40	42.40	46.90	58.60	45.58 b	3.13	7.0	8.60	9.4	7.03 b
100	46.20	62.50	69.60	51.60	57.48 a	3.9	9.1	12.5	17.8	10.83 a
Mean	40.30 d	52.45 c	58.25 a	55.1 b		3.52 d	8.05 c	10.55 b	13.60 a	
L.S.D. (0.05) GB = 1.267		NaCl = 1.791		GBxNaCl = 2.53		L.S.D. (0.05) GB = 1.074		NaCl = 1.52		GBxNaCl = 2.15
Total soluble protein						Total soluble protein				
0	30.67	33.67	33.20	26.50	31.01 b	21.8	21.97	20.20	14.37	19.59 b
100	33.80	35.60	35.00	31.20	33.90 a	28.47	29.13	26.37	19.00	25.74 a
Mean	32.24 b	34.64 a	34.10 a	28.85 c		25.14 a	25.55 a	23.29 b	16.69 c	
L.S.D. (0.05) GB = 0.41		NaCl = 0.58		GBxNaCl = 0.82		L.S.D. (0.05) GB = 0.419		NaCl = 0.593		GBxNaCl =0.84

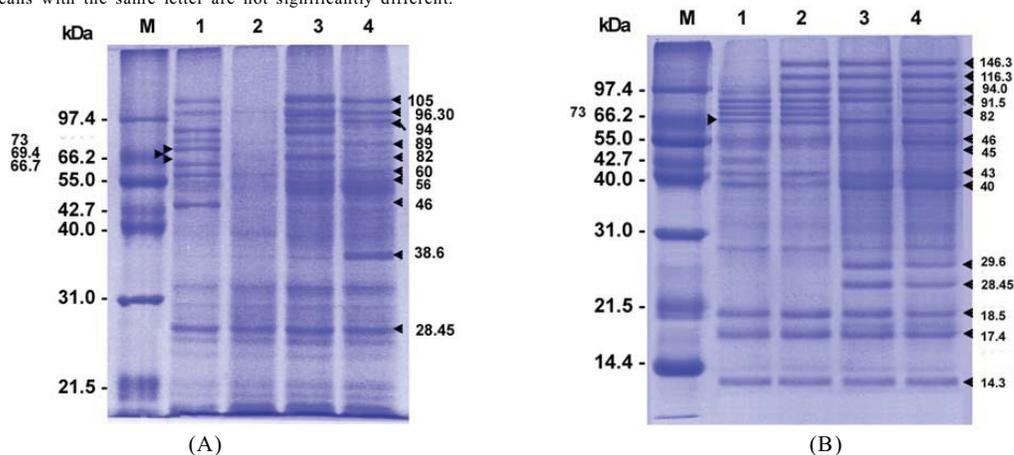Means with the same letter are not significantly different.

Plate 1: Analysis of protein pattern of *Zea mays* L.cv. Giza 2 plants treated with 0.0, 50, 100 and 150 mM NaCl for 24 hours in absence or presence of glycine betaine using one-dimensional SDS-PAGE.

(A) Plants produced from grains presoaked in tap water and treated with NaCl.
Lane (M)Protein marker.
Lane (1)0.0 mM NaCl (control).
Lane (2)50 mM NaCl.
Lane (3)100 mM NaCl.
Lane (4)150 mM NaCl.

(B) Plants produced from grains presoaked in glycine betaine (GB) and treated with NaCl.
Lane (M)Protein marker.
Lane (1)0.0 mM NaCl + GB
Lane (2)50 mM NaCl + GB.
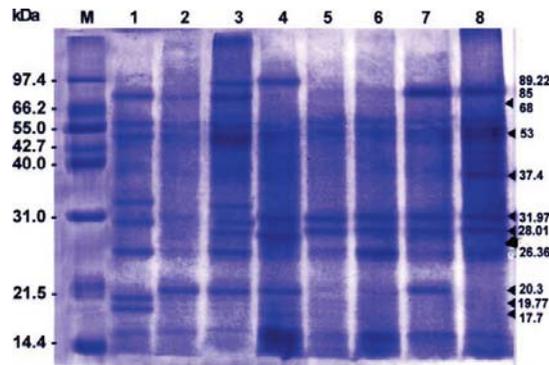Lane (3)100 mM NaCl + GB.
Lane (4)150 mM NaCl + GB.

Plate 2: Analysis of protein pattern of *Zea mays* L.cv. Giza 2 plants treated with 0.0, 50, 100 and 150 mM
NaCl for 3 weeks in absence or presence of glycine betaine using one-dimensional SDS-PAGE.

Lane (M)Protein marker.
Lane (1)0.0 mM NaCl (control).
Lane (2)50 mM NaCl.
Lane (3) 100 mM NaCl.
Lane (4)150 mM NaCl.
Lane (5) 0.0 mM NaCl +GB.
Lane (6) 50 mM NaCl +GB.
Lane (7)100 mM NaCl +GB.
Lane (8)150 mM NaCl +GB.

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