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Salt Pretreatment Enhance Salt Tolerance in *Zea mays* L. Seedlings

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Abstract: Recent molecular studies show that genetic factors of salt tolerance in halophytes exist in glycophytes too, but they are not active. If these plants expose to low level salt stress these factors may become active and cause plants acclimation to higher salt stresses. So because of the importance of these findings in this research the effect of salt pretreatment has been examined in *Zea mays* seedlings. To do the experiment four day old *Zea mays* seedlings (Var. single cross 704) pretreated with 50 mM NaCl for the period of 20 h. Then they were transferred to 200 and 300 mM NaCl for 48 h. At the end of treatment roots and shoots of seedlings were harvested separately. The changes of K^+- leakage, the amount of malondialdehyde, proline, soluble sugars and the Hill reaction rate were analyzed. The results indicated that the amount of K^+- leakage and malondialdehyde (MDA) have been increased because of salt-induced lipid peroxidation and membrane unstability. Soluble sugars and proline as osmoregulators has been increased in stress condition and in pretreated plants with NaCl were the highest. The rate of Hill reaction was reduced significantly in stressed plants. Therefore we concluded that salt stress causes serious physiological and biochemical damages in plants and salt pretreatment enhances tolerance mechanisms of plants and help them to tolerate salt stress and grow on salty environments.

Key words: Hill reaction, malondialdehyde, salt pretreatment, *Zea mays* L.

INTRODUCTION

Most crop plants are glycophytes and sensitive to high soil Na^+ concentration. Soil salinity is one of the major environmental stresses that limits significantly agricultural crop productivity (Gruwel *et al*., 2001). Plant cells must be able to maintain low cytoplasmic Na^+ until to tolerate salt stress. Salt tolerance is a complex mechanism involves many different responses against cellular osmotic and ionic aspects of Na^+. The complexity and polygenic nature of salt tolerance is an important factor that cause many difficulties in breeding salt-tolerant crop varieties (Parida and Das, 2005). Recent molecular studies have shown that glycophytes have also salt tolerance genes. Cell cultures from many different glycophytes have been salt tolerant by exposing to gradually increasing levels of NaCl. Some salt-sensitive plants have also been adapted to grow in the presence of high salinity by this way (Zhu, 2000). Nevertheless, these studies illustrate that all plants have genes for salt tolerance in their genomes, but without adaptation, these genes may not be actively expressed to confer salt tolerance. So, glycophytes such as *Zea mays* may also be expected to contain salt tolerance genes that are not very different from those of halophytes. A better understanding of biochemical and physiological responses of plants under salt pretreatment may help us to realize the important mechanisms that improve the salt tolerance of crop varieties. Therefore the results of this research on *Zea mays* seedlings indicated that salt pretreatment has improved different tolerance mechanisms and has helped the plants to grow better in salty environments.

MATERIALS AND METHODS

This experiment has been done in spring of 2006 at the physiological and biochemical laboratories, in the Department of Biology of Faculty of Science of Urmia University. The seeds of *Zea mays* L. (cv. single cross 704) were cleaned, selected by the size, washed with water and detergent and finally with distilled water and incubated in 25°C to germinate. After 4 days the seedlings with the same size were selected again and 7 seedlings (as a replicate) were pretreated with 50 mM NaCl for 20 h. Then they were transferred to 200 and 300 mM NaCl for 48 h in an aerated and controlled condition (light density 14000 Lux and day/night temperature 24/20°C and humidity 60%). At the end of treatment, the shoots and roots of seedlings were harvested separately. To show the effect of salt pretreatment and salt stress on the plants, the changes of K^+-leakage, malondialdehyde content, proline, soluble sugars and the Hill reaction rate were determined.

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Measurement of K^+-leakage: To do this experiment, 4 day old seedlings of *Zea mays* were pretreated with 50 mM NaCl for 20 h. Then they were transferred to 200 and 300 mM NaCl for 48 h (at above mentioned condition). At the end of treatment the seedlings were harvested and the amount of K^+-leakage in growth solution of each replicate was determined by flame photometer (model 405 made by Fater Electronic com.) (Chen *et al*., 2005).

Changes of lipid peroxidation: Lipid peroxidation was determined by measuring the amount of MDA produced by the thiobarbituric acid reaction as described by Heath and Packer (1968). The crude extract was mixed with the same volume of a 0. 5% (w/v) thiobarbituric acid solution containing 20% (w/v) trichloroacetic acid. The mixture was heated at 95°C for 30 min and then quickly cooled in an ice-bath. The mixture was centrifuged at 3000 g for 10 min and the absorbance of the supernatant was monitored at 532 and 600 nm. After subtracting the non- specific absorbance (600 nm), the MDA concentration was determined by its molar extinction coefficient (155 mM^{-1} cm^{-1}) and the results expressed as MDA. g^{-1} FW.

Measurement of proline content: To show the changes of proline, the roots and shoots of seedling that were pretreated and treated as above mentioned condition, were dried separately at 60°C for three days. 0.04 g of every sample was grinded in glass powder. The proline content was determined by the method of Bates *et al.* (1973) using 3% aqueous sulphosalicylic acid for preparing the leaf homogenate. The homogenate was centrifuged at 1000 rpm for 15 min. Two milliliter of the supernatant was mixed with an equal volume of glacial acetic acid and acid ninhydrin and incubated for 1 h at 100°C. Then 4 mL of toluene was added to mixture tube were put in ice and the chromatophore containing fraction was then aspirated from the aqueous phase and its absorbance was determined at 520 nm on a spectrophotometer.

Measurement of soluble sugars: Ten milliliter of 80% ethanol was added to 0.01 g of the powdered root and shoot of each sample to extract the soluble sugars. After one week the amount of soluble sugars was determined by the method of Dubois *et al.* (1956). Two milliliter of the supernatant of aliquot was mixed with 1 mL of 5% phenol and 5 mL of sulfuric acid. The mixtures incubated for 30 min, after cooling the changes in absorbance were estimated at 485 nm on a spectrophotometer.

Assay of hill reaction rate: To determine the rate of Hill reaction, the shoots of *Zea mays* seedlings prepared at the same condition were harvested. Leaf spieces were placed in a prechilled blender cup containing 10 mL of ice-cold 0. 5 M sucrose. Then was blended for 15 sec at top speed, the resulting homogenate was squeezed through four layers of prechilled cheesecloth into a prechilled beaker. Equal amounts of this green filtrate were centrifuged at 200 g for 3 min. The supernatant was decanted into additional cold centrifuge tubes and centrifuged again at 1000 g for 7 min. The supernatant was discarded and the chloroplasts precipitates were resuspended immediately in 10 mL of cold 0.5 M sucrose. This process repeated again. At last, it was resuspended in 25 mL of cold 0.1 M phosphate buffer at pH 6.5. 0.5 mL of the chloroplast suspension was placed in a spectrophotometric cuvette and mixed with 0.2 mL of DCPIP (electron acceptor) prepared in extraction buffer; then the absorbance of this solution was determined at 600 nm and repeated every 20 sec after the solution were illuminated with a 100-watt incandescent lamp Choudhury and Biswal (1980).

RESULTS AND DISCUSSION

K^+-leakage: Analysis of the results has indicated that the K^+-leakage has been increased significantly by NaCl treatments, its amount in plants treated with 300 mM NaCl was 9.3 times higher than the controls and 2.16 times higher than the plants pretreated with 50 mM NaCl and treated with 300 mM NaCl (Fig. 1). K^+ is one of the most important and abundant cation in plant cells. There is also a negative correlation between magnitude of K^+ efflux and membrane stability and salt tolerance of a particular cultivar (Chen *et al.* 2005; Kronzucker and Szczerba, 2003). Therefore we conclude that the salt stress has caused membrane unstability and this effect has also been confirmed by producing of induced level of MDA in this research.

Lipid peroxidation: The MDA content in the shoots of seedlings was measured as index of the rate of lipid peroxidation. Its amount has been increased significantly in plants treated with 300 mM NaCl (2.9 times higher than the plants pretreated with 50 mM NaCl and treated with 300 mM NaCl and 3.3 times higher than the controls) (Fig. 2). Therefore, these results indicated that salt stress has caused an extensive lipid peroxidation, which has often been used as an indicator of salt induced oxidative damage in membranes (Hernandez and Almansa, 2002). MDA levels have decreased in pretreated plants, reduction of MDA in these plants was probably due to increased antioxidative enzymes activities, which has reduced H_2O_2 levels and membrane damages (Shalata *et al.*, 2001). MDA as a lipid peroxidation product, has been used widely to assess the levels of free

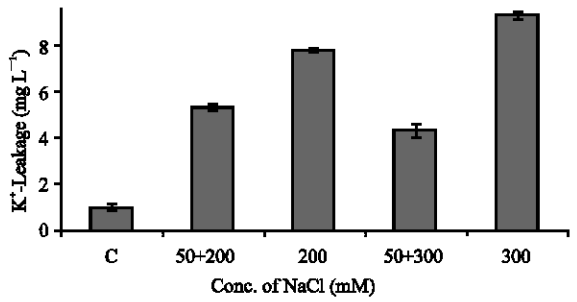

Fig. 1: The changes of K^+-leakage of *Zea mays* seedlings roots exposed to different concentrations of NaCl (200 and 300 mM) with and with out salt pretreatment (50 mM NaCl) during the period of 48 h. The values represent the mean of three replicates ±SE

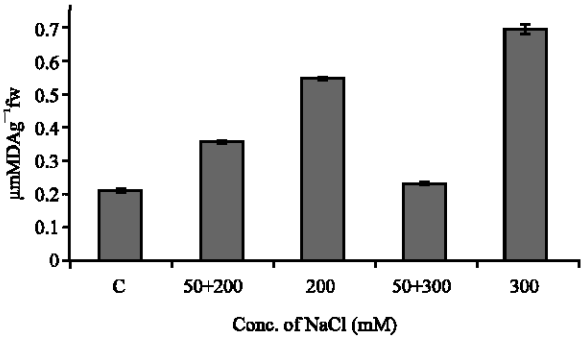

Fig. 2: The changes of total malondialdehyd of *Zea mays* seedlings shoots exposed to different concentrations of NaCl (200 and 300 mM) with and with out salt pretreatment (50 mM NaCl) during the period of 48 h. The values represent the mean of three replicates ±SE

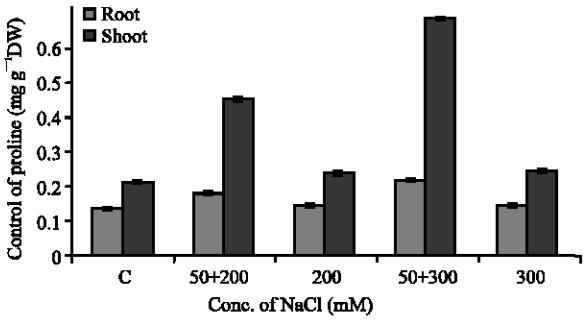

Fig. 3: The changes of proline content of *Zea mays* seedlings roots and shoots exposed to different concentration of NaCl (200 and 300 mM) with and with out salt pretreatment (50 mM NaCl) during the period of 48 h. The values represent the mean of three replicates ±SE

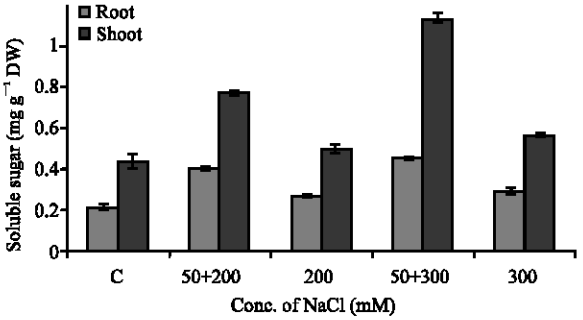

Fig. 4: The changes of soluble sugars of *Zea mays* seedlings roots and shoots exposed to different concentrations of NaCl (200 and 300 mM) with and with out salt pretreatment (50 mM NaCl) during the period of 48 h. The values represent the mean of three replicates ±SE

radicals in living cells (Hong *et al*. 2000). Therefore in this research pretreated plants has been better protected from oxidative damage under salt stress.

Proline content: Proline content in the shoots has been increased in pretreated plants with 50 mM NaCl and treated with 300 mM NaCl (it was 3.3 times higher than control and 2.8 times higher than plants treated only with 300 mM NaCl), also its content in roots pretreated with 50 mM NaCl and treated with 300 mM NaCl was the highest (it was 1.6 times higher than control and 1.4 times higher than plants treated only with 300 mM NaCl). Content of proline in the shoots of seedlings was higher than the roots (Fig. 3). Proline is known to play an important role as an osmoprotectant in plants subjected to hyperosmotic stresses such as drought and soil salinity (Hong *et al*., 2000; Arshi *et al*., 2005). Stewart *et al*. (2004) suggested that the capacity to accumulate proline is correlated with salt tolerance. Hong *et al*. (2000) suggested that increased resistance to oxidative stress is due to some indirect metabolic or physiological consequence of the accumulation of proline and other metabolites. Accumulation of proline in shoots and roots of pretreated plants increased (Fig. 3). Therefore in these plants proline acts as a free radical scavengering and increases salt tolerance of these plants.

Soluble sugars: Soluble sugars content in the shoots of plants have been increased by the salt pretreatment; its amount in shoots of plants pretreated with 50 mM NaCl and treated with 300 mM NaCl was 2 times higher than the shoots of plants only were treated with 300 mM NaCl and 3 times higher than the controls (Fig. 4). The amount of

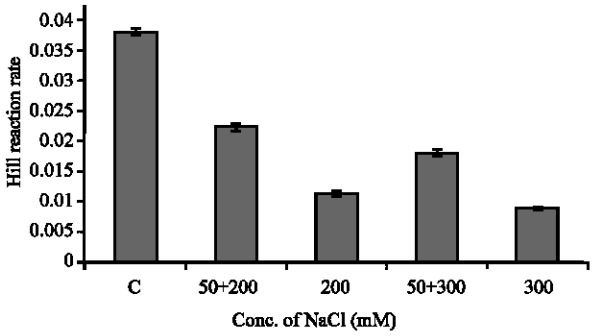

Fig. 5: Effects of different concentration of NaCl (200 and 300 mM) with and with out salt pretreatment (50 mM NaCl) on Hill reaction rate on *Zea may* seedlings leaves during the period of 48 h. The values represent the of three replicates ±SE mean

this component in the roots pretreated with 50 mM NaCl and treated with 300 mM NaCl was the highest (it was 2 times higher than control and 1.5 times higher than plants treated only with 300 mM NaCl). Content of soluble sugar in the shoots of seedlings was higher than the roots (Fig. 4). Carbohydrate changes has direct relationship with such physiological processes as photosynthesis, translocation and respiration. Adaptation to salt and water stress has been attributed to the stress-induced increase in carbohydrate levels (Ashraf and Garris, 2004; Naeini *et al*., 2004). Soluble sugars play an important role in osmotic regulation of cells during germination and strees conditions (Gill *et al.* 2002).

Rate of Hill reaction: Rate of Hill reaction in plants treated with 300 mM NaCl and 200 mM NaCl was, respectively 4.2 and 3.4 times lower than control (Fig. 5). Reduced rate of Hill reaction was because of salt stress damages on photosynthetic photosystems and oxygen evolving complex (Allakhverdiev *et al*., 2000). Salt stress imposes a water deficit because of osmotic effects on a wide variety of metabolic activities (Parida and Das, 2005). Krieger-Liszkay (2004) proposed that the active oxygen forms destroy the proteins, lipids and important cell components which can secondarily lead to damage in PSII machinery too. Hence, it can expected that Hill reaction is affected by salt stress indirectly. Hill reaction in *Zea mays* seedlings decreased by 200 and 300 mM NaCl (Fig. 5). This in turn decreases reduction of $NADP^+$and phosphorylation of ADP, which will result in a strong inhibition of CO_2 assimilation.

CONCLUSION

We concluded that in pretreated plants, K^+-leakage and the amount of MDA was decreased in comparison to unpretreated-stressed plants. In contrast amount of proline, soluble sugars and Hill reaction rate was increased. These results indicated the induced tolerance of pretreated plants to salt stress. A better understanding of these mechanisms under salt stress may help us to improve salt tolerance of crop varieties by gradually adaptation of them to saline soils.

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