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Research Article – Plant Science

NaCl stress causes changes in photosynthetic pigments and accumulation of compatible solutes in *Zea mays* L.

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Abstract

The present study was conducted in order to evaluate the effects of NaCl on photosynthetic pigments and compatible solutes of *Zea mays* under salt stress. Seven NaCl regimes were used, 0mM, 25mM, 50mM, 75mM 100mM, 125mM and 150mM. Plants were analyzed on 15th day after salt treatment. A factorial experiment in a completely randomized block design (CRBD) with seven treatments and three replications were applied. From the data attained, we understand that in accordance with the increase in salinity, photosynthetic pigment content reduced drastically, whereas compatible solutes like proline, glycine betaine and sugar enhanced marginally. The accumulation of compatible solutes makes the plant survive against salinity stress.

Key words: Maize, Salt stress, Photosynthetic pigments, Proline, Glycine betaine

Introduction

Physiological and metabolic processes are harmfully influenced by a chief abiotic stress, soil salinity that leads toward declined growth and yield (Azizspour *et al.,* 2010). Availability of nutrients and water is affected by soil salinity. Moreover, it provokes osmotic stress, the physiological drought, which typically decreases the growth and photosynthesis in plants (Aebi, 1984). Additionally, salt stress is also manifested as an osmotic stress, ion toxicity and oxidative stress (Aghaleh *et al.,* 2009). Ahmed *et al.* (2009) proposed that due to salinity stress growth reduction is also accredited to ion toxicity and nutrient disparity, which causes not only high sodium (Na^+) and chloride (Cl^-) accumulation in plants, but also annihilation affects the uptake of indispensable nutrient elements such as potassium (K^+), calcium (Ca^{2+}) and magnesium (Mg^{2+}) in competition with Na^+ and also nitrate (NO^{3-}) in contrast with Cl^-. Cations for instance, K^+ and Na^+ are recognized to be the main inorganic elements, which make available needed osmotic potential for water uptake by plant cells (Asada, 2000). Regulation of K^+ uptake alongside avoidance of Na^+ entry and efflux of Na^+ from the cell and confiscation of Na^+ in vacuole for osmotic adjustment are the ordinary strategies for continuation of desirable K^+/Na^+ ratios in the cytosol. A soaring K^+ and Na^+ ratio in the cytosol is essential for normal cellular functions of plants (Ashraf, 2009).

Salt stress is a one kind of abiotic stress that can influence the plant growth and physiological activities such as photosynthetic activity and chlorophyll content (Saleh, 2012). Photosynthesis is a vital process that is affected in plants by salinity, which is stomata closure leading to a reduction of intercellular CO_2 concentration. There is strong confirmation that salt affects photosynthetic enzymes, chlorophylls and carotenoids (Stepien and Klobus, 2006), alter in chloroplast ultra structure (Keiper *et al.,* 1998), decrease in rate of photosynthesis (Sixto *et al.,* 2005).

Plants respond to salt stress by activating a complex set of defense pathways that ultimately culminate in tolerance or susceptibility (Zhu, 2002). One of the most ordinary stress responses in plants is overproduction of various types of compatible organic solutes (Ahmad *et al.,* 2010a). Compatible solutes are of low molecular mass and well soluble compounds that are typically nontoxic at high cellular concentrations and also act as osmoprotectants. These solutes include proline, sucrose, polyols, trehalose and quaternary ammonium compounds (QACs) such as glycine betaine, alanine betaine, proline betaine, choline *O*-sulfate, hydroxyl prolinebetaine, and pipecolate betaine (Ahmad and Sharma, 2008). Proline accumulation induced by NaCl has been shown to

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correlate with growth inhibition (Ahmad *et al.,* 2010a). Under stressful conditions, glycine betaine is serving as compatible osmolytes, protectants of macromolecules and also as scavengers of Reactive Oxygen Species (Asharf and Foolad, 2007). Soluble sugar is the main organic osmolyte that could help improve crop tolerance to osmotic stress (Chen *et al*., 2011).

Maize (*Zea mays* L.) is the third most central cereal in the globe after wheat and rice (Khodarahmpour, 2011). It is used as a breakfast cereal crops in the sphere. Maize is the important cereal crop which is the basic need of food and oil for human intake. It is also used as feed for livestock (Hussain *et al.,* 2010). In view of the importance of maize and salinity, current study has been intended to inspect the photosynthetic pigments and compatible solutes indices in salt tolerance of maize (*Zea mays* L.).

Materials and Methods

The seeds of *Zea mays* were obtained from Rasi Seed Company, Tamil Nadu, India. The seeds were sown in plastic pots dimensions of 22cm height and 26cm wide and filled with equal amount (1:1:1) of soil, sand and farm yard manure was mixed and mixture was used to fill these pots. The experiment was placed in a Completely Randomized Block Design with three replicates and each replicates consisted of seven pots. Five seeds per pot were used and irrigated with tap water for 20days. After germination the seedlings were thinned to one plant per pot. The salt treatments were started 20days after planting and it consists of 25mM, 50mM, 75mM, 100mM, 125mM and 150mM NaCl and 0mM served as control and imposed on plant to 10days, on 15th day after salt treated samples were collected for further analysis.

Estimation of Photosynthetic pigments

Chlorophyll

Chlorophyll content was estimated as described by Arnon (1949). Five hundred mg of leaf tissue was taken in a pestle and mortar with 10ml of 80 per cent acetone and it was ground well. Then, the homogenate was centrifuged at 800g for 10 minutes and the supernatant was saved. The pellet was re-extracted with 5ml of 80 per cent acetone each time till the pellet become colorless. The absorbance was measured at 663, 645nm with a Spectrophotometer (U-2001, HITACHI). All the extracts were pooled and the chlorophyll content was determined by using the formula.

Total chlorophyll (mg/ml)
$(0.0202) \times (O.D.\ 645) + (0.00802) \times (O.D.\ 663)$

Chlorophyll 'a' (mg/ml)
$(0.0127) \times (O.D.\ 663) - (0.00269) \times (O.D.\ 645)$

Chlorophyll 'b' (mg/ml)
$(0.0229) \times (O.D.\ 645) - (0.00468) \times (O.D.\ 663)$

Carotenoids

Carotenoids content was estimated as described by Davis (1965). Aqueous acetone extracts were shaken three times with an equal volume of hexane in a separating funnel and the combined hexane fractions were washed with equal volume of water to separate carotenes from leaf tissue, the hexane fraction containing the carotenoids was extracted repeatedly with 90 per cent methanol. The hexane fraction containing carotenes was measured by utilizing the values of absorbance at 480nm.

Car A. $480 + (0.114 \times A.\ 663) - (0.638 \times A.\ 645)$

Examine the compatible solutes

Proline (Pro)

Proline accumulation was determined as described by Bates et al. (1973). Five hundred mg of plant tissue was homogenized in 10ml of 3 per cent sulphosalicylic acid. The homogenate was filtered through Whatmann No.42 filter paper. Two ml of acid ninhydrin (1.25g ninhydrin in 30ml of glacial acetic acid and 20ml of 6M phosphoric acid) and 2ml of glacial acetic acid in a test tube was heated for an hour at 100°C. The reaction mixture was extracted with 4ml of toluene and mixed vigorously by using a Vortex mixture for 15-20 seconds. The chromophore containing toluene was aspirated from the aqueous phase. The absorbance of the toluene layer was measured in a Spectrophotometer (U-2001, HITACHI) at 520nm using toluene as blank.

Glycine betaine (Gb)

Glycinebetaine was estimated by the method of Grieve and Grattan (1983). Five hundred mg of finely ground dried plant samples was mechanically shaken with 20ml of de-ionized water for 24hours at 25ºC. Time required for this step was determined by extracting the plant samples for 1, 4, 16, 24 and 48hour. The samples were then filtered and filtrates were stored in the freezer for analysis.

Thawed extracts were diluted with 2N H2SO4 (1:1). The acid potassium tri-iodide solution for total QACs were prepared by dissolving 7.5g resublimed iodine and 10g potassium iodide in 1M HCI and filtered (Speed and Richardson, 1968). Precisely, 0.2ml of acid potassium tri-iodide reagent was added to an aliquot of sample containing between 10-15µg of QACs in water. The mixture was shaken and left for at least 90 minutes in an ice bath with intermittent shaking. Two ml of ice-cold water was

added rapidly to the mixture to reduce the absorbance of blank and to improve replication. This was quickly followed by 10ml of 1, 2-dichloro ethene in ice, and the 2 layers mixed well and kept at 4ºC (Storey and Wyn Jones, 1977). The absorbance of the lower organic layer was measured at 365nm in a Spectrophotometer. The results were expressed as glycinebetaine equivalent by using glycinebetaine for standard value.

Total Sugar

Sugar content was estimated as described by Nelson (1944). One ml of ethanol extract taken in the test tubes was evaporated in a water bath. To the residue, 1ml of distilled water and 1ml of 1N sulphuric acid were added and incubated at 49°C for 30 minutes. The solution was neutralized with 1N sodium hydroxide using methyl red indicator. One ml of Nelson reagent was added to each test tube. The test tubes were heated for 20 minutes in a boiling water bath, cooled and 1ml of arsenomolybdate reagent was added. The solution was thoroughly mixed and diluted to 25ml and read at 495 nm in a Spectrophotometer (U-2001, HITACHI). The reducing sugar content of the unknown samples was calculated from glucose standards.

Data analysis

The experiment was placed in a completely randomized black design (CRBD) with three replications of the each treatment. The results were analyzed by one-way ANOVA with the help of SPSS 16.0 software package. Means and standard deviation were calculated from three replications.

Results

Photosynthetic pigments

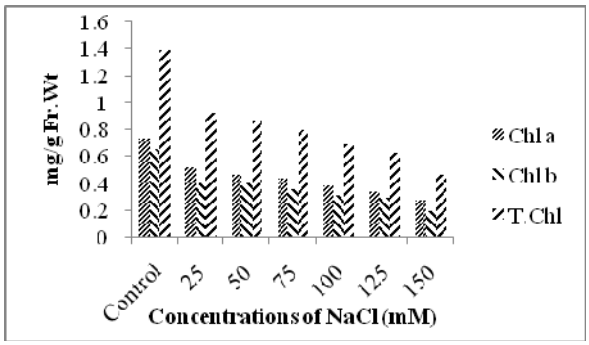
Figure1 and 2 show the effect of salt stress, using different concentrations of NaCl, on the chlorophyll content of the maize plants under study, including chlorophyll a, b, total chlorophyll and carotenoids. The chlorophyll and carotenoids content decreased with increasing NaCl concentrations. The highest accretion of photosynthetic pigments was documented at respective control plants.

Compatible solutes

Effect of NaCl on Proline content of maize

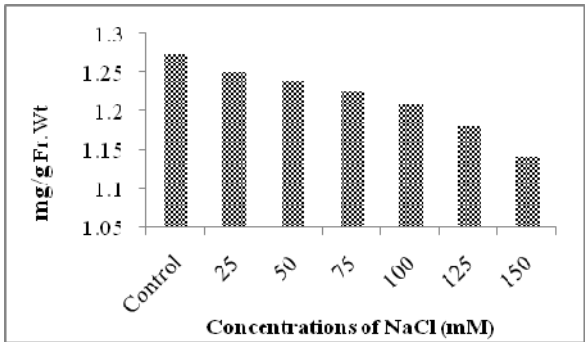
Proline, which is regarded as a non-toxic solute, increased under NaCl stress and was found to be concentration dependent. There was a steady raise in the level of proline in all the three tissues with increasing NaCl concentrations up to 150mM. Although, leaf had more proline content than that of shoot and root (Fig. 3).

Figure. 1. Effect of NaCl on chlorophyll 'a' chlorophyll 'b' and total chlorophyll content (mg/g Fr. Wt.) of the leaves of *Zea mays* on 15th day after salt treatment



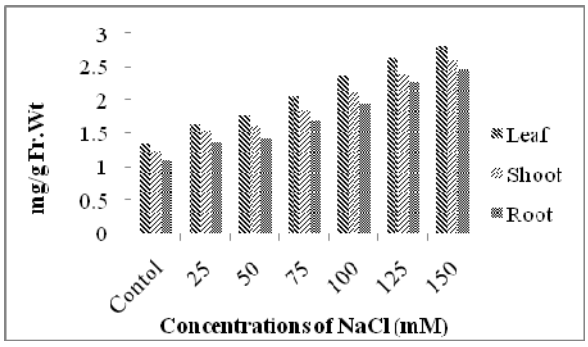
(Values are mean ± S.D. of 3 samples, n-3 and expressed in mg/g fresh weight)

Figure. 2. Effect of NaCl on carotenoid content (mg/g Fr. Wt.) in the leaves of *Zea mays* on 15th day after salt treatment



(Values are mean ± S.D. of 3 samples, n-3 and expressed in mg/g fresh weight)

Figure. 3. Effect of NaCl on proline content (mg/g Fr. Wt.) of the leaf, shoot and root of *Zea mays* on 15th day after salt treatment



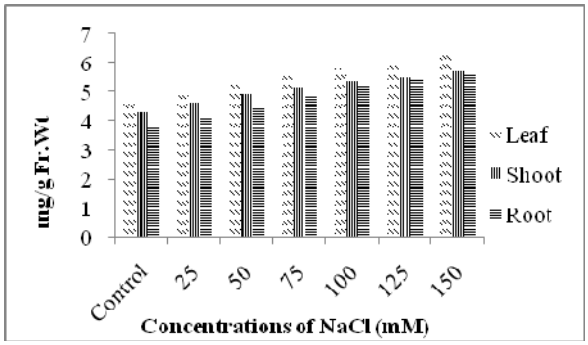
(Values are mean ± S.D. of 3 samples, n-3 and expressed in mg/g fresh weight)

Effect of NaCl on Glycine betaine content of maize

Salt stress caused an increase in the glycine betaine content in leaf, shoot and root of maize studied when compared to non saline plants. There was a significant increase in the accumulation of

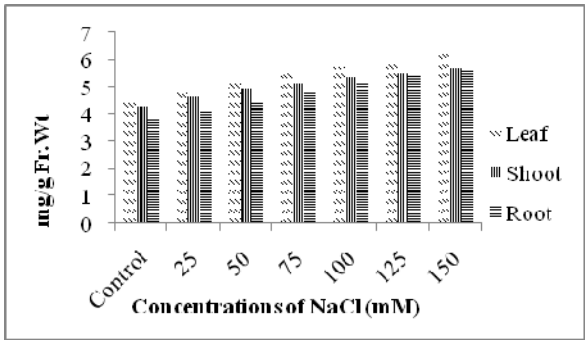
glycine betaine with increasing salinity up to 150mM (Fig. 4).

Figure. 4. Effect of NaCl on glycine betaine content (mg/g Fr. Wt.) of the leaf, shoot and root of *Zea mays* on 15th day after salt treatment



(Values are mean ± S.D. of 3 samples, n-3 and expressed in mg/g fresh weight)

Figure. 5. Effect of NaCl on total sugar content (mg/g Fr. Wt.) of the leaf, shoot and root of *Zea mays* on 15th day after salt treatment



(Values are mean ± S.D. of 3 samples, n-3 and expressed in mg/g fresh weight)

Effect of NaCl on total Sugar content of maize

Sugar content augmented in all the treatments when compared to non-treated plants. Among all treatments, 150mM showed the higher total sugar level when compared with the others (Fig. 5).

Discussion

A decrease in photosynthetic pigments such as chlorophyll a and b and carotenoids is also associated with a decline in the net photosynthesis rate in maize under salt stress (El Sayed, 2011; Qu *et al.,* 2012). Cha-um and Kirdmanee (2009) observed a linear reduction in chlorophyll a and b, total chlorophyll, and carotenoid contents in maize with increased salt stress. Moreover, due to degradation of chlorophyll and carotenoid contents the maximum quantum and photon yield of photosystem II and net photosynthetic rate also decreased.

Reduction in chlorophyll content with increased salt concentration in the present study accordance with *Brassica juncea* (Ahmad, 2010). Harinasut *et al.* (2000) also were observed the decrease in chlorophyll content in *Morus alba* with elevating concentrations of salt. (Parida *et al.,* 2004) reported that total chlorophyll content has also been observed to decrease significantly by NaCl treatment in *A.corniculatum*.

Salt-induced degradation of photosynthetic pigments could be attributed to develop activity of specific enzymes namely chlorophyllase enzyme (Ashraf and Ashraf, 2012). The reduction in leaf chlorophyll content under NaCl stress has been attributed to the destruction of chlorophyll pigments and the unsteadiness of the pigment protein complex (Levit, 1980). Carotenoids is responsible for quenching of singlet oxygen (Koyro, 2006), the decrease in Car under salinity stress leads to degradation of β-carotene and formation of zeaxanthins, which are apparently concerned in fortification against photoinhibition (Sultana *et al.,* 1999).

Proline, an amino acid is well known to get accumulated in a large assortment of organisms ranging from bacteria to higher plants on exposure to abiotic stress (Ahmad, 2010). *Harinasut et al.* (2000) showed that proline content in leaves of *Morus alba* increases at 150mM NaCl conditions over control. The present results are in accordance with our earlier findings in *Pisum sativum* with NaCl stress (Ahmad *et al.,* 2008b). Under stress conditions Pro accumulation may either be caused by induction or activation of enzymes of proline biosynthesis or a declined proline oxidation to glutamate, decreased utilization of proline in protein synthesis and improved protein turnover (Delauney and Verma, 1993). The expression of genes encoding key enzymes of proline synthesis (P5C synthase, P5C reductase) and proline oxidation (proline dehydrogenase) is controlled by osmotic and salinity stress and proceeds the increase or decrease of proline concentration in plant tissue (Strizhov *et al.,* 1997). Proline improves the salt tolerance by protecting protein turnover machinery and up regulating stress protective proteins (Thakur and Sharma, 2005), and interacts with enzymes to not only maintain protein structure and activities but also reduces enzyme denaturation caused due to salt stress, moreover acts as a reserve source of carbon, nitrogen, and energy during recovery from stress (Sairam and Tyagi, 2004).

GB, a water-soluble quaternary ammonium compound, is known to play a important role in effective protection of cells against abiotic stresses (Shirasawa *et al.,* 2006). Apparently it protects the plant cells exposed to salinity stress by osmotic adjustment and protein stabilization and acts as a ROS scavenger. Ahmad *et al.* (2013) proposed that glycine betaine is synthesized within the cell from

either choline or glycine. Synthesis of glycine betaine from choline is a 2-step reaction involving two or more enzymes. In the first step choline is oxidised to betaine aldehyde which is then again oxidized in the next step to form glycine betaine. In higher plants the first conversion is carried out by the enzyme choline monooxygenase (CMO), whereas the next step is catalysed by betaine aldehyde dehydrogenase (BADH). In the present study, an increase in GB concentration was examined in the maize when exposed to different salt concentrations. These results are parallel to some earlier reports for *Zea mays* (Yang and Lu, 2005) and other crop species wheat (Sairam *et al.,* 2002) and eggplant (Abbas *et al.,* 2010).

Soluble sugar is the major organic osmolyte in the leaves (Wang *et al*., 2011), which plays important role in osmotic adjustment and protects against photo damage (Rodríguez-Calcerrada *et al*., 2011). In this study, the increase of soluble sugar led by salt at grain filling stage was observed, which could improve the permeability and keep a balance of water metabolism (Liao and Chen, 2007). Larger increase percentage of soluble sugar was observed in salt tolerant cultivar, indicating that oat could improve salt tolerance by increasing soluble sugar. The increment of soluble sugar contents induced by salt has been detected in wheat (Li *et al*., 2009).

Conclusion

In the present study, various levels of salt treatment alone decreased the photosynthetic pigments, while the compatible solutes may have contributed to the reduced oxidative damage and osmotic adjustment in the cytoplasm under salinity stress.

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References

Abbas, W, Ashraf, M and Akram, N.A. (2010). Alleviation of salt-induced adverse effects in eggplant (*Solanum melongena* L.) by glycine betaine and sugar beet extracts. *Scientia Horticulturae,* 125, 188-195.

Aebi, H. (1984). Catalase in Vitro. *Methods of Enzymology,* 105, 121-126.

Aghaleh, M, Niknam, V and Ebrahimzadeh, H. (2009). Salt Stress Effect on Growth, pigments, Proteins and Lipid Peroxidation in *Salicornia persica* and *S. europaea*. *Biologia Plantarum,* 53 (2), 243-248.

Ahmad, P. (2010). Growth and antioxidant responses in mustard (*Brassica juncea* L.) plants subjected to combined effect of gibberellic acid and salinity, *Archives of Agronomy and Soil Science,* 56, 575–588.

Ahmad, P, Jaleel, C.A and Sharma, S. (2010a). Antioxidant defense system, lipid peroxidation, proline metabolizing enzymes, and biochemical activities in two *Morus alba* genotypes subjected to NaCl stress. *Russian Journal of Plant Physiology,* 57, 509–517.

Ahmad, P, Jhon, R, Sarwat, M and Umar, S. (2008b). Responses of proline, lipid peroxidation and antioxidative enzymes in two varieties of *Pisum sativum* L. under salt stress. *International Journal of Plant Production,* 2, 353–366.

Ahmad, P and Sharma, S. (2008). Salt stress and phyto-biochemical responses of plants- a review. *Plant, Soil and Environment,* 54, 89–99.

Ahmad, R, Lim, C.J and Kwon, S.Y. (2013). Glycine betaine: a versatile compound with great potential for gene pyramiding to improve crop plant performance against environmental stresses. *Plant Biotechnology Reports,* 7, 49–57.

Ahmed, P, Jaleel, C, Azooz, M and Gowher, N. (2009). Generation of ROS and Non-Enzymatic Antioxidants during Abiotic Stress in Plants. *Botany Research International*, 2 (1), 11-20.

Arnon, D.I. (1949). Copper enzymes in isolated chloroplasts. Polyphenol oxidase in *Beta vulgaris. Plant physiology*, 24, 1-15.

Asada, K. (2000). The Water-Water Cycle as Alternative Photon and Electron Sinks. *Philogical Transactions of the Royal Society Biological sciences,* 355 (1402), 1419- 1431.

Ashraf, M. (2009). Biotechnological Approach of Improving Plant Salt Tolerance Using Antioxidants as Markers. *Biotechnology Advances,* 27 (1), 84-93.

Ashraf, M and Foolad, M.R. (2007). Role of glycine betaine and proline in improving plant abiotic stress resistance. *Environmental and Experimental Botany,* 59, 206-216.

Ashraf, M.A and Ashraf, M. (2012). Salt-induced variation in some potential physiochemical attributes of two genetically diverse spring wheat (*Triticum aestivum* L.) cultivars: photosynthesis and photosystem II efficiency. *Pakistan Journal of Botany,* 44 (1), 53–64.

Azizpour, K, Shakiba, M.R, Sima, K.N, Alyari, H, Moghaddam, M, Esfandiari, E and Pessarakli, M. (2010). Physiological Response of Spring Durum Wheat Genotypes to Salinity. *Journal of Plant Nutrition,* 33 (6), 859-873.

Bates, S, Waldren, R.P and Teare, I.D. (1973). Rapid determination of the free proline in water stress studies. *Plant and Soil,* 39, 205–208.

Cha-um, S and Kirdmanee, C. (2009). Effect of salt stress on proline accumulation, photosynthetic ability and growth characters in two maize cultivars. *Pakistan Journal of Botany,* 41, 87–98.

Chen, W, Feng, C, Guo, W, Shi, D and Yang, C. (2011). Comparative effects of osmotic, salt-and alkali stress on growth, photosynthesis and osmotic adjustment of cotton plants. *Photosynthetica,* 49, 417-425.

Davis, R.F. (1965). Salinity effects on the electrical and ionic parameters of *Atriplex gmelinii.* In: plant membrane transport current conceptual tissues. Spanwich, R. M., W.J. Lucos and J. Dainty (Ed.), (pp. 407-408). North Holland, Amsterdam: Elsevier.

Delauney, A.J and Verma, D.P.S. (1993). Proline biosynthesis and osmoregulation in plants. *Plant Journal,* 4, 215–223.

El Sayed, H.E.S.A. (2011). Influence of salinity stress on growth parameters, photosynthetic activity and cytological studies of *Zea mays*, L. plant using hydrogel polymer. *Agriculture and Biology Journal of North America*, 2, 907–920.

Grieve, C.M and Grattan, S.R. (1983). Rapid assay for determination of water soluble quaternary ammonium compounds. *Plant and Soil,* 70, 303–307.

Harinasut, P, Srisunak, S, Pitukchaisopol, S and Charoensataporn, R. (2000). Mechanism of adaptation to increasing salinity of mulberry: Proline content and ascorbate peroxidase activity in leaves of multiple shoots. *Science Asia,* 26, 207–211.

Hussain, K, Majeed, A, Nawaz, K and Nisar, M.F. (2010). Changes in morphological attributes of maize (*Zea mays* L.) under NaCl salinity. *American Eurasian Journal of Agricultural Environmental Science,* 8 (2), 230-232.

Keiper, F.J, Chen, D.M and DeFilippis, L.F. (1998). Respiratory, photosynthetic and ultra structural changes accompanying salt adaptation in culture of *Eucalyptus microcorys*. *Journal of Plant Physiology,* 152, 564-573.

Khodarahmpour, Z. (2011). Screening maize (*Zea mays* L.) hybrids for salt stress tolerance at germination stage. *African Journal of Biotechnology,* 10 (71), 15959-15965.

Koyro, H.W. (2006). Effect of salinity on growth, photosynthesis, water relations and solute composition of the potential cash crop halophyte *Plantago coronopus* (L.). *Environmental and Experimental Botany,* 56, 136–146.

Levit, J. (1980). Responses of Plants to Environmental Stresses, Academic Press, Vol. II, New York.

Li, X.Y, Liu, J.J, Zhang, Y.T, Lin, J.X and Mu, C.S. (2009). Physiological responses and adaptive strategies of wheat seedlings to salt and alkali stresses. *Soil Science and Plant Nutrition,* 55, 680-684.

Liao, Y and Chen, G.Z. (2007). Physiological adaptability of three mangrove species to salt stress. *Acta Ecologica Sinica,* 27, 2208-2214.

Nelson, N. (1944). A photomorphic adaptation of the somogyi's method for the determination of reducing sugar. *Analytical Chemistry,* 31: 426-428.

Parida, A.K, Das, A.B, Sanada, Y and Mohanty, P. (2004). Effects of salinity on biochemical components of the mangrove, *Aegiceras corniculatum*. *Aquatic Botany,* 80, 77–87.

Qu, C, Liu, C, Gong, X, Li, C, Hong, M, Wang, L and Hong, F. (2012). Impairment of maize seedling photosynthesis caused by a combination of potassium deficiency and salt stress. *Environmental and Experimental Botany,* 75, 134–141.

Rodríguez-Calcerrada, J, Shahin, O, Carmen del Rey, M.D and Rambal, S. (2011). Opposite changes in leaf dark respiration and soluble sugars with drought in two Mediterranean oaks. *Functional Plant Biology,* 38, 1004-1015.

Sairam, R.K, Rao, K.V and Srivastava, G.C. (2002). Differential response of wheat genotypes to long term salinity stress in relation to oxidative stress, antioxidant activity and osmolyte concentration. *Plant Science,* 163, 1037–1046.

Sairam, R.K and Tyagi, A. (2004). Physiology and molecular biology of salinity stress tolerance in plants. *Current Science,* 86, 407–421.

Saleh, B. (2012). Effect of salt stress on growth and chlorophyll content of some cultivated cotton varieties grown in Syria. *Communications in Soil Science and Plant Analysis,* 43, 1976-1983.

Shirasawa, K, Takabe, T, Takabe, T and Kishitani, S. (2006). Accumulation of glycine betaine in rice plants that over express choline monooxygenase

from spinach and evaluation of their tolerance to abiotic stress. *Annals of Botany,* 98, 565–571.

Sixto, H, Grau, J.M, Alba, N and Alia, R. (2005). Response to sodium chloride in different species and clones of genus *Populus* L. *Forestry,* 78, 93-104.

Stepien, P and Klobus, G. (2006). Water relations and photosynthesis in *Cucumis sativus* L. leaves under salt stress. *Biologia Plantarum,* 50 (40), 610-616.

Strizhov, N, Ábrahám, E, Ökrész, L, Blickling, S, Zilberstein, A, Schell, J, Koncz, C and Szabados, L. (1997). Differential expression of two P5CS genes controlling proline accumulation during salt-stress requires ABA and is regulated by ABA1, ABI1 and AXR2 in *Arabidopsis*. *Plant Journal,* 12, 557–569.

Sultana, N, Ikeda, T and Itoh, R. (1999). Effect of NaCl salinity on photosynthesis and dry matter accumulation in developing rice grains. *Environmental and Experimental Botany,* 42, 211–220.

Thakur, M and Sharma, A.D. (2005). Salt stress induced proline accumulation in germinating embryos: Evidence suggesting a role of proline in seed germination. *Journal of Arid Environment,* 62, 517– 523.

Wang, X.P, Geng, S.J, Ri, Y.J, Cao, D.H, Liu, J, Shi, D.C and Yang, C.W. (2011). Physiological responses and adaptive strategies of tomato plants to salt and alkali stresses. *Scientia Horticulturae,* Amsterdam, 130, 248-255.

Yang, X and Lu, C. (2005). Photosynthesis is improved by exogenous glycine betaine in salt-stressed maize plants. *Physiologia Plantarum,* 124, 343–352.

Zhu, J.K. (2002). Salt and drought stress signal transduction in plants. *Annual Review of Plant Biology,* 53,