Degree of salt tolerance in some newly developed maize (*Zea mays* L.) varieties

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Abstract

Salinity is a major abiotic-stress worldwide which decreases crop growth productivity. The objective of the present study was to investigate whether salt stress has adverse effects on growth, photosynthetic efficiency, biochemical properties and nutrient status of maize. An experiment was carried out with seeds of four varieties of maize which were allowed to germinate for one week. Afterwards, one-week old maize seedlings of each variety were subjected to 0, 150 mM NaCl for four weeks. Significant decreasing effect by salinity was found in shoot-root fresh and dry biomass, chlorophyll contents, total soluble proteins and macronutrients of all maize varieties. From the results, it is obvious that amino acids concentration increased under salinity as compared to control in all maize varieties.

Keywords: salt tolerance; protein; amino acids; macronutrients; salinity; abiotic stress

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Introduction

Salinity is one of the major abiotic-stresses for agriculture worldwide (Munns, 2009; Ashraf et al., 2010). Out of current 230 Mha of irrigated land, 45 million ha are salt-affected soils (19.5%) and of the almost 1,500 million ha of dry land agriculture, 32 million ha are salt affected (2.1%), to varying degrees by human induced processes (Munns and Tester, 2008). Total area of Pakistan is 79.61 Mha out of which 21 Mha is cultivated and 17 Mha is irrigated land. Salinization of irrigated lands causes annual global income loss of about US$ 12 billion (Ghassemi et al., 1995). Salinity decreases the osmotic potential in the soil solution (Ψs) and increases ionic components, which is related to the accumulation of toxic ions and to decrease of essential elements, such as K^+ and Ca^+. Munns (1993) stated that increased sodium chloride salinity causes decrease in vegetation growth and the rate of photosynthesis. The large amount of salt can cause various modifications in plant metabolism such as inhibition of enzyme activity, changes in phosphorylation state and production of reactive oxygen species (Allakhverdiev et al., 2000, Blumwald et al., 2000).

Various strategies are used to improve the salinity tolerance of crops. These include screening and selection, conventional breeding, development of transgenic plants for salt tolerance, exogenous use of osmoprotectants, growth regulators and antioxidants.

All over the world as well as in Pakistan, maize is the third most important cereal after wheat and rice. The major

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objective of the present study was to find whether genotypic responses at different salinity levels can be effective to improve the salinity tolerance of maize. Secondly it was also aimed to find out the maize variety that can perform better under salt stress in terms of some biochemical attributes.

Materials and Methods

Seeds of four maize varieties (Desi, Nelum, Pak. F, Sultan) were obtained from New Punjab Farm Services, Khanewal, Pakistan. The experiment was conducted in a naturally lit greenhouse with average photoperiod 8h and day/night temperature 26±6 °C and 16±4 °C during November - February 2009-2010. The relative humidity ranged from 34.5 to 46.5 percent. The aluminum trays of 150cm of length and 70cm of width were filled with river sand thickness of 5 inches thoroughly washed with tap water. The experiment was arranged in completely randomized design with two treatments (0, 150 mM NaCl) and four replicates. Seeds of maize varieties were sown in rows at 20cm distance from each other. Treatments were started after the establishment of seedlings. Salinity concentration was increased stepwise in aliquots of 50 mM to avoid the salt shock (Chartzoulakis and Loupassaki, 1997). Adequate amount of water was applied to each tray on alternate days to minimize evapo-transpiration loss. The appropriate treatment solution was applied every week to each tray to regularly maintain the salinity level in sand. After 4 weeks growth, plants were harvested, and shoots and roots were separated. After recording the fresh weights of shoots and roots, they were dried in oven at 80 ^{o}C for four days and dry weights of shoots and roots were measured. Before harvesting chlorophyll (Witham et al., 1971), total free amino acids (Hamilton and Van Slyke, 1943) and total soluble proteins (Bradford, 1976) were also measured. Macronutrients (N, P, K) of leaf and root were determined by the method described by Allen et al. (1986). One hundred mg of ground dry plant leaf material was digested in 2ml of sulphuric peroxide digestion mixture until a clear and almost colorless solution was obtained. After digestion, the volume of the sample was made to 100ml with deionized water. K was determined with a flame photometer (Jenway PFP7, Dunmow, Essex, UK). Phosphorous was estimated by the method described by Jackson (1958) using a spectrophotometer (Hitachi U-2000, Tokyo, Japan) and N by titration method following Allen et al. (1986).

Statistical analysis

The data obtained were subjected to 3-way ANOVA using the statistical computer package COSTAT (Cohort software, Berkeley, USA) and mean values were compared with least significance difference test following Snedecor and Cochran (1980).

Table 1
Analysis of variance of data for shoots and root fresh and dry weight of four maize varieties (V) when four week old plants grown under non-saline or saline (S) conditions in sand culture in full strength Hoagland's nutrient solution.

SOV	df	Shoot f. wt.	Shoot d. wt.	Root f. wt.	Root d. wt
V	3	1.020***	0.005***	0.248***	0.001**
S	1	9.523***	0.023***	5.189***	0.028***
V x S	3	0.159***	6.84×10^{-4}**	0.015ns	1.89×10^{-4}ns
Error	24	0.015	1.09×10^{-4}	0.031	2.53×10^{-4}

, *, significant at 0.01, and 0.001 levels, respectively.
ns = non-significant

Results

Effect of salt stress on maize varieties at the initial growth stages

Imposition of salt stress significantly reduced the fresh and dry weights of all maize varieties. However, varieties differed significantly in their shoot fresh and dry biomass (Table 1). Variety "Pak F" had maximum shoot fresh and dry weight under both non-saline and saline conditions, whereas var. "Sultan" was minimal in its shoot fresh and dry weight. In addition, remaining two varieties were intermediate in their shoot fresh and dry weight under both non-saline and saline conditions (Fig. I).

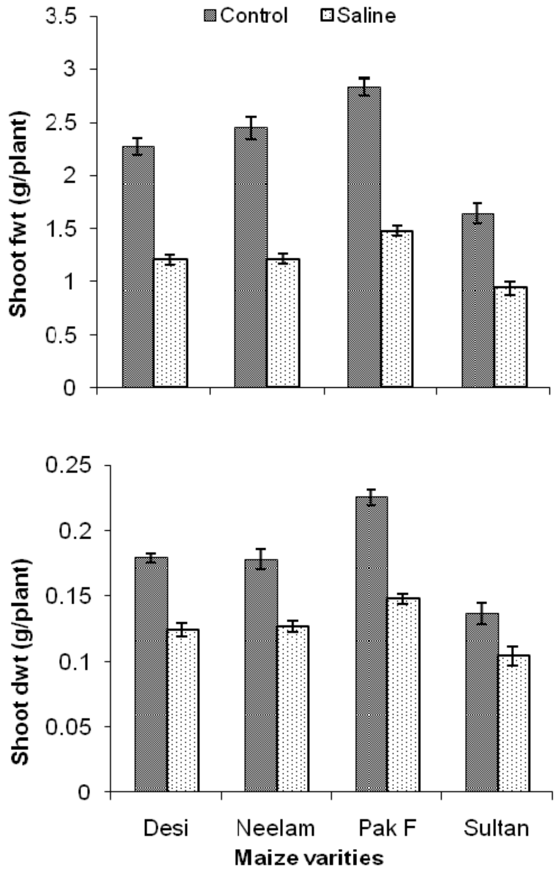

Fig. I. Fresh and dry weight of shoot (g/plant) of four maize varieties grown for four weeks under non-saline or saline conditions in sand culture in full strength Hoagland's nutrient solution

Root fresh and dry weights decreased significantly due to the addition of 150 mM NaCl in the growth medium. Varieties also differed in their root fresh and dry weights. Maximum root fresh and dry biomass was found in both non-stressed and salt stressed plants of var. "Pak F", whereas the reverse was true for var. "Sultan" (Fig. II).

From the results, it is obvious that salt stress had inhibitory effect on chlorophyll "a", "b" and total chlorophyll (Table 2). Varieties did not differ significantly in their chlorophyll "a" contents. Only stressed var. "Neelam" showed a maximum increase in chlorophyll whereas var. "Desi" decreased in chlorophyll under saline conditions (Figs. III, IV).

Salinity had a significant increasing effect on amino acids and the effect of their interaction was also significant (Table 3; Fig. V). Maximum total free amino acids were found in stressed var. "Neelam".

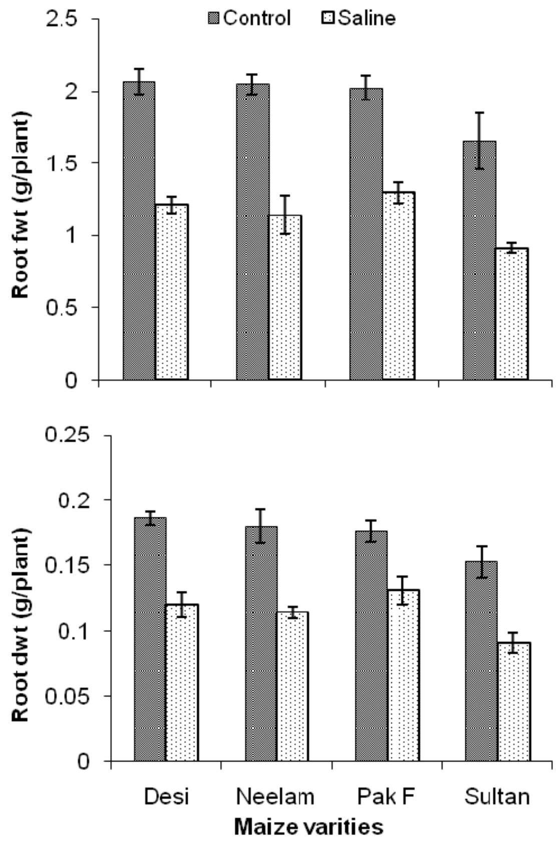

Fig. II. Fresh and dry weight of root (g/plant) of four maize varieties grown for four weeks under non-saline or saline conditions in sand culture in full strength Hoagland's nutrient solution

Analysis of variance of the data showed that salt stress caused a significant decreasing effect on total soluble proteins in the leaves of all maize varieties. Non-significant varietal

Table 2
Analysis of variance of data for chlorophyll "a", "b", a/b ratio and total chlorophyll of four maize varieties grown for four weeks under non-saline or saline conditions in sand culture in full strength Hoagland's nutrient solution

SOV	df	Chlorophyll a	Chlorophyll b	Chlorophyll a/b	Total Chlorophyll
V	3	0.005ns	0.010***	0.062ns	0.026ns
S	1	2.545***	0.494***	1.197***	5.283***
V x S	3	0.071*	0.005**	0.138ns	0.114**
Error	24	0.016	0.001	0.054	0.023

*, **, ***, significant at 0.05, 0.01, and 0.001 levels, respectively.
ns = non-significant

Table 3
Analysis of variance of data for total free amino acids and total soluble proteins of four maize varieties grown for four weeks under non-saline or saline conditions in sand culture in full strength Hoagland's nutrient solution.

SOV	df	Total Free Amino Acids	Total Soluble Proteins
V	3	2.986***	6.422ns
S	1	21.19***	208.6***
V x S	3	1.244*	1.996ns
Error	24	0.391	2.863

*, ***, significant at 0.05, and 0.001 levels, respectively.
ns = non-significant

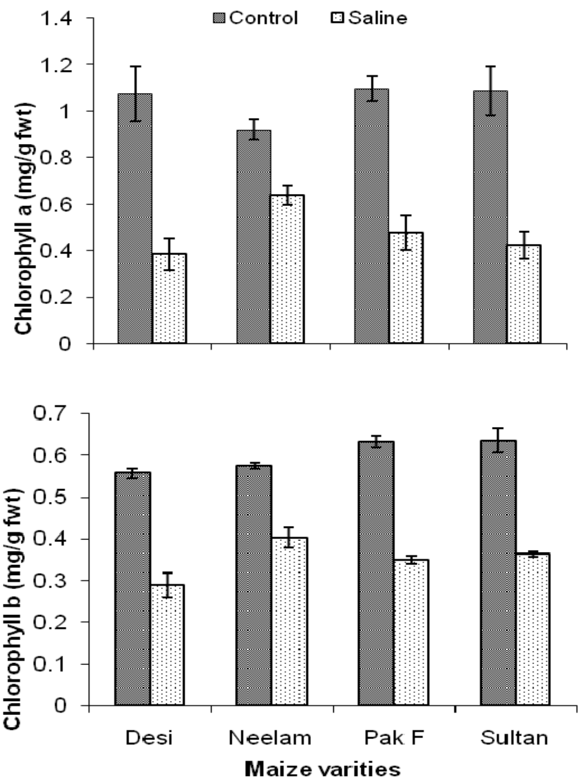

Fig. III. Chlorophyll "a", "b" (mg/g f. wt.) of four maize varieties grown for four weeks under non-saline or saline conditions in sand culture in full strength Hoagland's nutrient solution

difference was also found in their protein attributes (Table 3). Protein contents were high in var. "Neelam" under non-saline and saline conditions (Fig. VI).

The content of total N in leaves decreased at the high salinity treatments, but the significant varietal difference was not found under both non-stressed and stressed conditions (Table 4). Variety "Sultan" had lower concentration of N in its leaves under stressed conditions. Maximum root N was observed in non-stressed var. "Sultan" while minimum was recorded in non-stressed var. "Desi". Under stressed condition var. "Pak F" showed low N contents while high content was found in var. "Desi" (Fig. VII).

Analysis of variance showed that in leaves and roots phosphorus the difference between four maize varieties was only marginal and, in most cases, non-significant under both non-saline and saline conditions (Table 4). Phosphorus concentration in the leaves of non-stressed and stressed var. "Neelam" was considerably high while it was lowest in var. "Sultan". Root phosphorus content was relatively high in non-stressed var. "Sultan" whereas it was low in non-stressed var. "Pak F". Similarly, it was lowest in stressed var. "Sultan" (Fig. VIII).

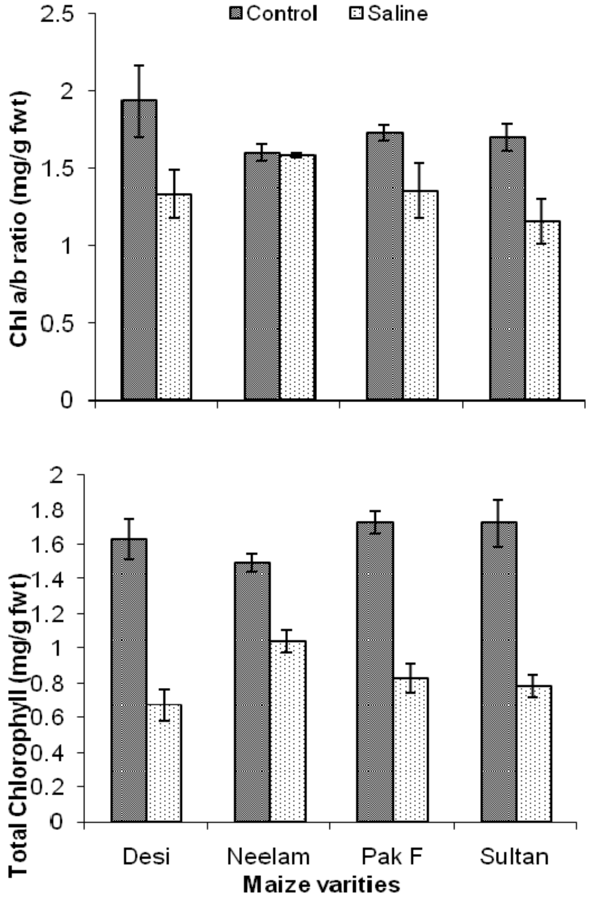

Fig. IV. Chlorophyll a/b and total chlorophyll (mg/g f. wt.) of four maize varieties grown for four weeks under non-saline or saline conditions in sand culture in full strength Hoagland's nutrient solution

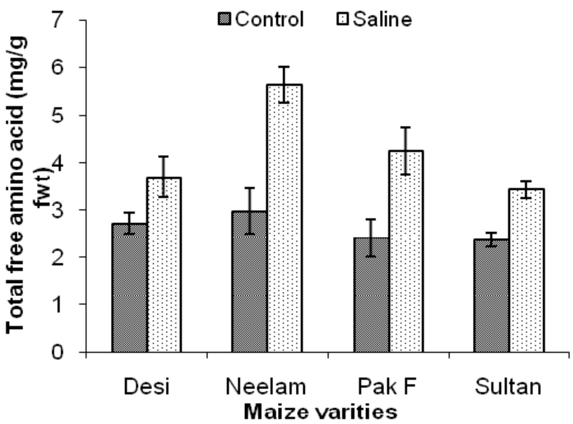

Fig. V. Total free amino acids (mg/g f. wt.) of four maize varieties grown for four weeks under non-saline or saline conditions in sand culture in full strength Hoagland's nutrient solution

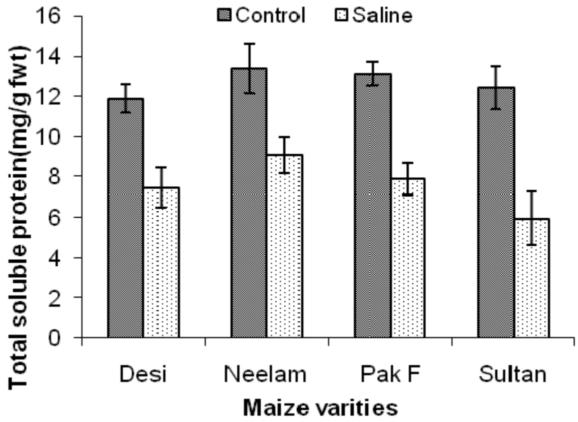

Fig. VI. Total soluble protein (mg/g f. wt.) of four maize varieties grown for four weeks under non-saline or saline conditions in sand culture in full strength Hoagland's nutrient solution

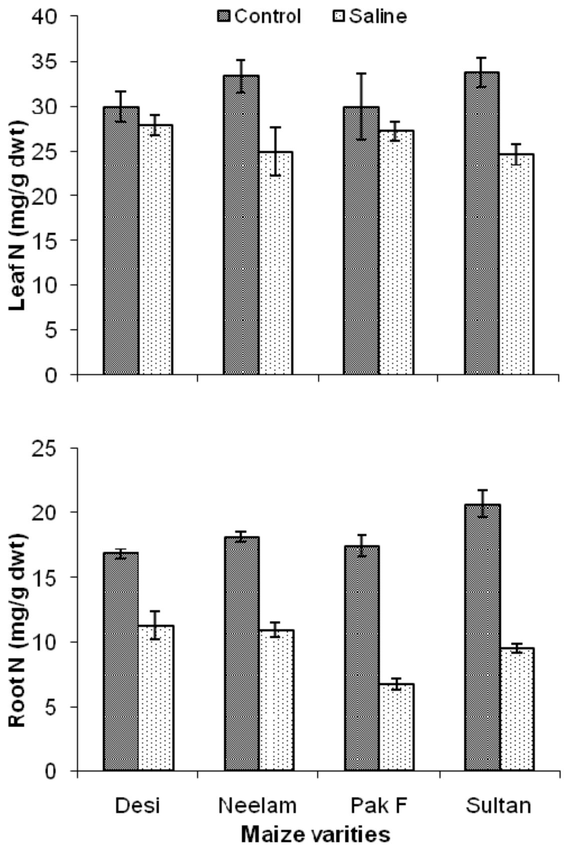

Fig. VII. Leaf and root N (mg/g d. wt.) of four maize varieties grown for four weeks under non-saline or saline conditions in sand culture in full strength Hoagland's nutrient solution

Salinity caused dramatic decrease in both leaf and root K^+ content. In leaves and roots the difference between four maize varieties was significant (Table 4). In term of genotypic difference, the K^+ results appear to be much

Table 4
Analysis of variance of data for N, P, K^+ of shoots and roots of four maize varieties grown for four weeks under non-saline or saline conditions in sand culture in full strength Hoagland's nutrient solution

SOV	df	Leaf N	Root N	Leaf P	Root P	Leaf K^+	Root K^+
V	3	0.620ns	13.70***	1.212ns	0.731ns	35.42*	82.88***
S	1	250.3***	604.8***	9.595**	10.66**	3945.1***	1920.1***
V x S	3	27.42ns	14.80***	0.512ns	7.610**	9.995ns	12.99ns
Error	24	12.47	1.528	1.214	1.359	10.05	7.772

*, **, ***, significant at 0.05 0.01, and 0.001 levels, respectively.
ns = non-significant

more sensitive indicators of salt tolerance. Potassium concentration in stressed leaves of var. "Neelam" was the lowest whereas it was high in stressed var. "Desi". Similarly, potassium concentration in non-stressed roots of var. "Pak F" and stressed roots of var. "Desi" was the lowest, whereas it was high in non-stressed and stressed var. "Sultan" (Fig. IX).

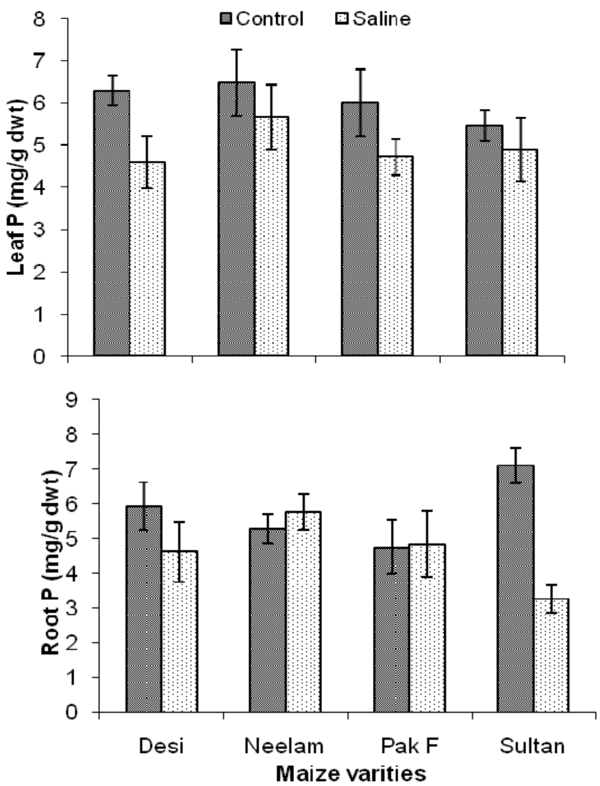

Fig. VIII. Leaf and root P (mg/g d. wt.) of four maize varieties grown for four weeks under non-saline or saline conditions in sand culture in full strength Hoagland's nutrient solution

Discussion

In the present study, salt stress caused a decrease in growth of all maize varieties. These results can be explained in view of already published literature in which it was generalized that under saline stress accumulation of Na^+ in the leaves and roots of plants causes specific ion toxicity thereby resulting in reduced growth, e.g., canola (Ulfat et al., 2007), sunflower (Ashraf and McNeiley, 1987) and wheat (Ashraf and Khanum, 1997; 2000). Likewise,, Shannon et al. (2000) also reported that salinity reduced fresh weight of all nine leafy vegetables. In the present study, maize varieties differed in their growth under saline conditions. For example, variety "Pak F" showed better performance in terms of shoot, root growth, and proved to be tolerant to higher level of salinity. In our experiment, variety "Sultan" appeared to be more salt sensitive as compared to other maize varieties, confirming the finding from earlier studies that growth of maize is highly sensitive to salt stress (Rodriguez et al., 2004; Schmidhalter et al., 1998; Studer, 1993). Meloni et al. (2001) reported similar genotypic difference in salt tolerance of cotton cultivars. While examining genotypic variation for salt tolerance in nine hexaploid wheat lines Sarwer and Ashraf (2003) found two salt tolerant lines. Moreover, a significant amount of genetic variability for salinity tolerance has already been reported in a number of crops such as mungbean, maize, cotton, sorghum, canola etc. (Ashraf and Akram, 2009; Munns, 2009).

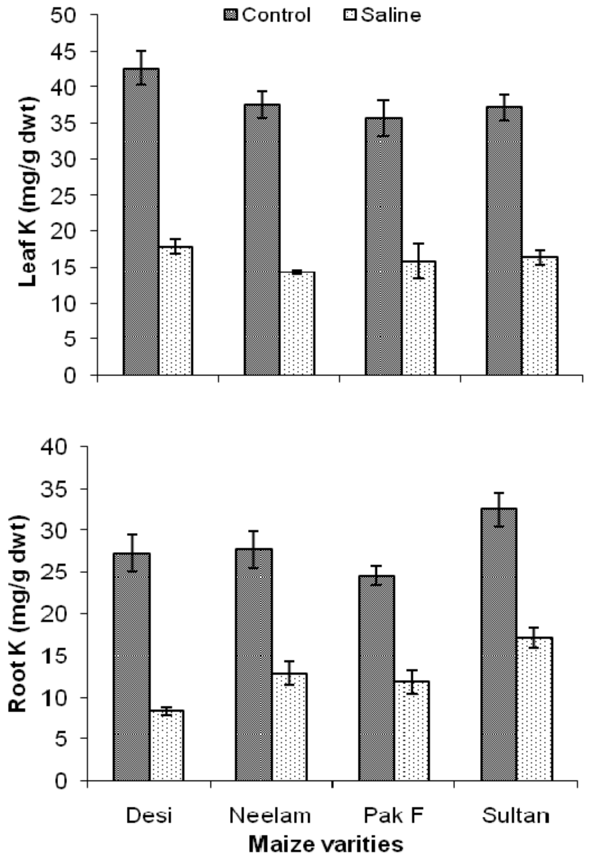

Fig. IX. Leaf and root K (mg/g d. wt.) of four maize varieties grown for four weeks under non-saline or saline conditions in sand culture in full strength Hoagland's nutrient solution

The decrease in photosynthesis under salt stress can also be attributed to a reduction in chlorophyll content (Delfine et al., 1999). The results from our experiment show that salinity causes a significant reduction in chlorophyll "a", "b" and total chlorophyll of all maize varieties. Reduction in all maize varieties due to salt stress is in agreement with some previous studies on different crops, e.g., alfalfa (Winicov and Seemann, 1990), sunflower (Ashraf and Sultana, 2000), and wheat (El-Hendawy et al., 2005). The reduction in chlorophyll "a", "b" contents due to salinity may have been due to either slow synthesis or fast breakdown of chlorophyll pigments.

The accumulation of organic compounds, such as amino acids, in the cytoplasm plays an important role in the osmotic balance of plants (Morgan, 1992). Higher amounts of total free amino acids were observed in maize varieties when exposed to the salinity. From the results, it is obvious that amino acids concentration increased under salinity as compared to control. It is important to note that, although amino acids enhanced remarkably up to the level of 180 mM NaCl but in severe salinity the opposite effect occurred with severe reduction in dry matter yields (Tammam et al., 2008).

In the present study, imposition of NaCl decreased the protein in all maize varieties which differed in their protein concentration under saline conditions. Demiral and Turkan (2006) detected that total soluble protein content of sensitive rice cultivar (*Oryza sativa* cv. IR-28) decreased under salt stress. Similar results were reported in barley, sunflower and rice plants (Parvaiz and Satyavati, 2008). Protein synthesis is affected by the exposure of plant to sodium chloride and sometimes protein hydrolysis occurs which releases and accumulates free amino acids in the plant tissues (Levit, 1980; Neumann, 1997; Orcutt and Nilsen, 2000). In the cell, many of the protein synthesis steps are very sensitive to alterations in the ionic equilibrium, which may result in a complete blockage of protein metabolism (Levit, 1980; Neumann, 1997; Singh and Chatrath, 2001). These results on salinity stress show that the responses to salt stress depends on plant species even in varieties of the same plant species, plant developmental stage, duration and severity of the salt application (Parvaiz and Satyavati, 2008).

In most cases an increase in salinity levels in a plant's surrounding environment leads to a decrease of nitrogen uptake and accumulation by the root (Orcutt and Nilsen, 2000). The N concentration (mg N per kg dry weight) increases or remains unchanged under optimal N conditions (Munns and Termaat, 1986; Hu and Schmidhalter, 1998). These findings suggest that the reduced growth rate of crop plants under salinity might prevent the dilution effect of nutrient elements and that the N concentration in plants might not be associated with salt injury for plant growth. The results showed that content of total N in leaves and root decreased at the high salinity treatments and significant varietal difference was not found under both non-stressed and stressed conditions. In our study, stressed var. “Pak F” showed low N contents in roots.

Saline conditions have been found to inhibit phosphorous uptake in most of the agricultural species, e.g., cotton (Martinez and Lauchli, 1994), lupins (Treeby and Van Steveninck, 1988) and melons (Navarro et al., 2001). This effect however seems to be more effective at low, rather than high substrate P concentrations.

Potassium nutrition is disturbed under salt stress due to reduction in deposition rate in growing cell (Bernstein et al., 1995) and change in transcript level of several K^+ transporter genes (Su et al., 2002). Tammam et al. (2008) reported that shoot had higher concentration of K^+ than roots while Na^+ accumulation was low in shoot than in roots. The reason for reduced K^+ uptakes is its competition with Na^+ uptakes through Na^+-K^+ co-transporters, which may also block K^+ transporters of root cell under salinity (Zhu, 2002). In the present study, appraisal of K^+ of the non-stressed and stressed leaves of all maize varieties showed a differential pattern of accumulation of this nutrient.

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