Effects of Different Concentrations of NaCl On Seedling Growth and Physiological Responses in FourVarieties of *Zea mays* L

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ABSTRACT

Studies were planned to analyze the response of *Zea mays* L. genotypes to salt stress (0, 50, 75, 100, 125, 150 and 200 mMNaCl) at seedling stage . Four varieties, (Conquest, Sundance, Reward and Jubliee) were used and data for seedling growth and physiological responses of 14-day old seedling grown in 1/10 strength of Long Ashton were determined. Negative response had happened in length of shoots and roots. In contrast a considerable increase was observed in the total soluble sugar , total amino acids, proline, total organic solutes and osmotic potential in response to various salt treatments . Furthermore , Sodium and chloride content significantly increased in tissues as salinity increased with a high accumulation rate in roots than the translocation to shoots. Meanwhile, anon significant increase in K level in roots. On contrary, a substantial decrease in potassium / sodium ratios in both shoots and roots was observed in all varieties. The highest accumulation of inorganic solutes however, were observed in Jublieevariety shoots and in Conquest roots. Meanwhile the highest accumulation of organic solutes were observed in Sundance variety either in shoots or roots.

Keywords: *Zea may*, salinity, organic solutes, inorganic solutes.

INTRODUCTION

Maize (*Zea mays*L.) is a genus of the family *Graminae* (*Poaceae*), commonly known as the grass family. It occupies a key position as one of the most important cereals both for human and animal consumption and grown under various conditions in different parts of the world (Dowswell*et al*., 1996).It is the third most important cereal after wheat and rice in many countries, it is proudly called as " Queen of Cereals and "king of Fodder" and miracle crop (Rajurkar*et al*. 2011).

Soil salinity continues to be one of the most serious environmental stresses limiting the growth and productivity of most plant species (Shin *et al*., 2000; Pitman and Lauchli, 2002; Musacchi*et al*., 2006) and posing threat to agriculture and food supply (Flowers,2004). Sodium chloride (NaCl) is the most widespread chemical factor causes inhibition of plant growth in nature (Stavark and Rains, 1984). Approximately, 7 % of the world’s land area, 20 % ofthe world’s cultivated land, and nearly half of the irrigated land is affected with highsalt contents (Szabolcs, 1994; Zhu, 2001). In view of another projection, 2.1% of theglobal dry land agriculture is affected by salinity (FAO, 2007).

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Sodium toxicity under saline conditions is particularly common and results in a range of disorders in protein synthesis and enzyme activation (Tester and Davenport, 2003). In general, salinity has been found to induce osmoticstress and physiological drought, which typically reduces photosynthesis in plants (Pasternak, 1987). It inhibits plant growth, lowers external water potentials, causes ion toxicity and ion imbalance (Munns, 1993). It causes a diverse set of physiological, morphological and developmental changes (Anders *et al.*, 1996) and disrupts homeostasis in ion distribution, leading to molecular damage, growth arrest and even death (Zhu, 2001). It is considerable problem adversely affecting physiological and metabolic processes (Ashraf and Harris, 2004).

Salt stress affects plant physiology at whole plant as well as cellular levelsthrough osmotic and ionic stress (Hasegawa *et al*., 2000; Ranjbarfordoei*et al*., 2002; Murphy *and* Durako,2003).Salinityresults in a reduction of K^+ and Ca^{2+} contents and an increased level of Na^+ and Cl^-,which forms its ionic effects. Salt stress induces cellular accumulation of damagingactive oxygen species. Active oxygen species can damage membrane lipids, proteinsand nucleic acids (Mittler, 2002).In addition to causing osmotic andionic stress, salinity causes ionic imbalances that may impair the selectivity of rootmembranes and induce potassium deficiency (Gadallah, 2000).

Two *Zea mays* cultivars, salt sensitive Trihybrid 321 and salt tolerant Giza 2, were studied, their adaptation to salt stress. NaCl decreased fresh mass, dry mass, relative growth rate of shoots and roots, and leaf area ratio in both cultivars.Salt stress induced Na^+ and Cl^- accumulation while it decreased K^+ and Ca^{2+} levels in shoots and roots of both cultivars (Mansour *et al*. 2005).Larino*et al*. (1993) found that salt sensitive cells of maize (*Zea mays* L.) contained higher concentrations of proline than salt resistant cells in salt stressed medium.Moreover, the highest accumulation of amino acids (proline, arginine, alanine and glutamine)was recorded in salt stressed *Atriplexhalimus*(Sangita*et al.*,1999). Johari-Pireivatlou*et al*. (2010) concluded thatproline content increased by water stress in four lines of bread wheat.The osmotic adjustment can usually be accounted by an increase in concentration of a variety of commonsolutes, including sugars, organic acids and ions especially K^+ (Taiz and Zeiger, 1991).

Working with wheat *(cv. Giza* 158) plant, Shukryand Bayerly (2012) noticed that Salt stress treatments resulted in a dramatic decrease in fresh and dry weights of different parts of stress imposedseedlings. Furthermore, a considerable increase was observed in the total soluble sugar levels, total amino acids,proline and total organic solutes in response to various salt treatments. Sodium and chloride content significantlyincreased in tissues as salinity increased with a high accumulation rate in roots than the translocation to shoots.

The aim of this study was to assess the effects of different levels of salt stress on seedling growth maize under laboratory conditions. Effects of these stress factors on physiological responses were observed. Observations were made on the level of individual corngenotypes

MATERIALS AND METHODS

Four varieties of *Zea Ma*ize with different sensitivity to salt stress were used in this investigation, Conquest, Sundance, Rewards and Jubliee.

Seeds of studied varieties were surface sterilized by soaking in 0.01 M $HgCl_2$ for three minutes, and washed several times with distilled water. Seeds were germinated on moist tissue paper in dark at 25 ºC for three days. Germinated seeds were allowed to grow on nylon mesh suspended on 1/10 strength Long Ashton nutrient solution (Table 1) containing 0.1 mM of Fe-EDTA. Macronutrients and micronutrients were added according to Hoagland and Arnon (1938).

Table 1. Compositions of Long Ashton nutrients solution:

Macro Nutrients		Micro Nutrients	
KNO_3	4.0 mM	$MnSO_4.4H_2O$	10 µM
$Ca(NO_3)_2.4H_2O$	4.0 mM	$ZnSO_4.7H_2O$	1 µM
$MgSO_4.7H_2O$	1.5 mM	$CuSO_4.5H_2O$	1 µM
$NaH_2PO_4.2H_2O$	1.33 mM	H_3BO_3	50 µM
Ferric EDTA mono Na	0.1 mM	$Na_2MoO_4.2H_2O$	0.5 µM
		NaCl	100 µM
		$CoSO_4.7H_2O$	0.2 µM

Different levels of sodium chloride were added respectively (0, 50, 75, 100, 125, 150 and 200 mM). The nutrient solutions were stirred and aerated with moist air. Renewal of nutrient solutions was carried out at 48 hour intervals in a growth chamber. Illumination was provided by white fluorescent tubes supplemented by incandescent bulbs to maintain light intensity at 12000Lux at the top of plants at 25ºC. After 14 days, plants were harvested and data were calculated to estimate growth parameters, organic and inorganic solutes.

Seedlings growth parameters:

Ten seedlings were used for estimation of shoots and roots length.

Estimation of organic solutes:

At time of harvesting, shoots were taken randomly, weighed immediately and extracted in 15 cm^3 of 80% (v/v) ethanol. Roots were dissected from these seedlings, washed quickly with deionized water, blotted, weighed immediately and extracted in 15cm^3 of ethanol. The extraction was carried out at 60 ºC for three hours, and then filtered and the total volume was made up to 20 cm^3 with 80% ethanol. The extraction was stored in a cold room at -4 ºC, and later was used to estimate proline,total α amino acids, and total soluble sugars.

Estimation of proline:

Proline content of plants was determined using procedure of Balts*et al*. (1973).

Estimation of total amino acids:

Total amino acids excluding proline, were measured calorimetrically following the procedure outlined by Rosen (1957).

Estimation of total soluble sugars:

Total soluble carbohydrates were estimated calorimetrically using the procedure outlined by Yemm and Willis (1954).

Estimation of inorganic solutes:

Plant roots were rinsed in distilled water for 30 seconds to remove soil remains from the root surface. Thereafter, plants were separated into shoots and roots and dried in an oven at 80°C until constant weight. Dry weights of samples were recorded. The dry matter was digested in concentrated HNO_3 and made up to volume with deionized distilled water.

Estimation of potassium and sodium:

Potassium and sodium were measured by Atomic Absorption Spectrophotometry (ICP-AES-varian-Liberty series II) according to the method described by Chapman and Pratt (1978).

Estimation of chloride:

The determination of chloride was carried out according to the method described by Silva *et al*. (2005).

Osmotical potential:

Estimation ofosmoticum by using osmometer (π) as osmole recommended by Termaat*et al*., (1985).

Statistical analysis:

The experiment was carried out in completely randomized design with ten replicates for the growth parameters estimations and, three replicates for the organic and inorganic solutes estimations. Data were subjected to statistical analysis of variance (ANOVA).When ANOVA showed a significant ($P < 0.05$) effect, the least significant differences (LSD) where used to compare treatments (Snedecor and Cochran, 1976).

RESULTS AND DISCUSSION:

Shoots and roots length (cm)

The highest length of shoots (12.72 cm) and roots (12.55 cm) was observed in control plants. The gradual increase in salt in concentration resulted in gradual decrease in shoots and roots length (Table 2). Salinity-induced growth reduction has been previously reported in several plant species: maize (Izzo*et al*.,1991; Alberico and Cramer, 1993; Cramer, 1993; Cramer *et al*., 1994).

Reward variety resulted in highest length of shoots and roots comparing with another studied varieties.This might be attributed to the cultivar(Cramer *et al*., 1994) as all plant species and their genotypes differ genetically in their ability to adapt to salt stress environment (Marschner, 1995 and Wahid *et al*., 1997).

Concerning the interaction between varieties and salt treatments, it was cleared that non stressed Reward variety resulted in highest length for shoots (17.80 cm) and roots (19.60 cm) meanwhile, the lowest values were observed when NaCl was added at 200 mM to Jublieeshoots (2.90 cm) and Conquest roots (1.64 cm). Similar conclusion has been reached by Mladenova (1990), Kuiper *et al*. (1988), and Mansour and Salama (1996). They found a greater growth inhibition in different tolerant genotypes relative to sensitive ones under salt stress, which was interpreted by the authors to correlate with salt tolerance.

Nieman*et al*. (1988) clarified that The reduction of growth under salt stress conditions might be attributed to loosing of water and turgor pressure. Another suggestion is the reduction in RNA, DNA and protein synthesis (Streb and Feierabend, 1996) or the decrease in IAA (Dunlap and Binzel,1996). Moreover, Fortmeer and Schubert (2006) investigated The influence of NaCl and Na_2SO_4 on growth of two maize cultivars (Pioneer 3906 and Across 8023). Na^+ treatment with different accompanying anions (Cl^-/SO_4^{2-}) showed that ion toxicity was caused by Na^+.

Table 2. Effect of different NaClconcentrations in culture medium on shoots and roots length (cm) of 14-days old four *Zea maize*varieties.

NaCl Conc. (mM)	Shoots					Roots				
	Conquest	Sundance	Reward	Jubliee	Mean	Conquest	Sundance	Reward	Jubliee	Mean
0	9.51	10.98	17.80	12.60	**12.72**	7.73	9.47	19.60	13.40	**12.55**
50	8.73	8.13	14.30	10.60	**10.44**	4.98	11.22	15.80	13.00	**11.25**
75	7.87	7.51	13.30	9.00	**9.42**	**5.70**	11.27	8.20	8.70	**8.47**
100	7.21	6.81	10.90	8.10	**8.26**	4.79	10.22	6.80	7.70	**7.38**

NaCl Conc. (mM)	Shoots					Roots				
	Conquest	Sundance	Reward	Jubliee	Mean	Conquest	Sundance	Reward	Jubliee	Mean
125	7.09	5.53	8.90	6.20	**6.93**	3.82	6.25	6.20	5.40	**5.42**
150	5.23	5.35	8.80	4.30	**5.92**	3.19	5.33	5.20	4.20	**4.48**
200	3.42	4.43	5.70	2.90	**4.11**	1.64	4.32	3.30	3.80	**3.27**
Mean	**7.01**	**6.96**	**11.39**	**7.67**	**8.26**	**4.55**	**8.30**	**9.30**	**8.03**	**7.55**

L.S.D. Treatment = 1.23 L.S.D. Treatment = 1.40

L.S.D. Varieties= 0.93 L.S.D. Varieties = 1.06

L.S.D. Interaction = 1.1439 L.S.D. Interaction = 1.1439

Inorganic solutes concentration:

The gradual increase in NaCl concentration resulted in gradual increase in the inorganic solutes and the highest values (314.91 mMg^{-1} D.W. in shoots and 331.46 mMg^{-1} D.W. in roots) were observed when NaCl was added at 200 mM.(Table 3). Jublieevarietyresulted in highest concentration of inorganic solutes for shoots (249.47 mMg^{-1} D.W.). Meanwhile, Conquest variety resulted in highest concentration of inorganic solutes for roots (252.76 mMg^{-1} D.W.). The interaction between salt treatments and varieties also show that non treated Sundance variety resulted lowest concentrations of inorganic solutes for shoots and roots. Application of NaCl at 200 mM resulted in highest concentration of the inorganic solutes in Jubliee shoots and Conquest roots.

Table 3. Effect of different NaClconcentrations in culture medium on inorganic solutes concentration (expressed as mM g^{-1} D.W.) of 14-days old four*Zeamaize* variety.

NaCl Con. (mM)	Shoots					Roots				
	Conquest	Sundance	Reward	Jubliee	Mean	Conquest	Sundance	Reward	Jubliee	Mean
0	113.81	94.05	119.60	134.30	**115.44**	70.92	69.24	81.17	131.80	**88.28**
50	173.36	205.12	180.60	209.80	**192.22**	206.38	170.00	134.38	181.10	**172.97**
75	205.15	228.09	195.20	235.00	**215.86**	220.69	187.73	184.44	162.90	**188.94**
100	224.71	261.79	205.70	241.30	**233.38**	251.27	272.61	191.50	212.70	**232.02**
125	240.07	265.18	217.70	249.10	**243.013**	274.74	277.28	206.50	213.40	**242.98**
150	256.80	268.10	233.50	248.20	**251.65**	298.61	296.22	223.10	254.80	**268.18**
200	323.86	272.39	234.80	428.60	**314.91**	446.70	360.53	267.90	250.70	**331.46**
Mean	**219.68**	**227.82**	**198.16**	**249.47**		**252.76**	**233.37**	**184.14**	**201.06**	

L.S.D. Treatment = 15.48 L.S.D. Treatment = 12.10

L.S.D. Varieties= 11.70 L.S.D. Varieties = 9.14

L.S.D. Interaction = 181.116 L.S.D. Interaction = 110.594

Chloride concentration:

Effect of different NaCl treatments on chloride concentration has been illustrated in Table 4. Sundance varietyresulted in highest chloride concentration either in shoots (227.82 mMg^{-1} D.W.) or in roots (233.37 mMg^{-1} D.W.). On the other side, regardless to studied varieties, NaCl application increased chloride concentration and the highest values were observed when NaCl was added to 200 mM. In this respect Higher cytoplasmic Cl- concentrations were found in maize and barley salt sensitive cultivars than in salt tolerant ones (Hajibagheri*et al*., 1988; Flowers and Hajibagheri, 2001).

Non stressed Conquest variety resulted in lowest concentration of chloride and the heist concentration however were observed when NaCl was added at 200 mM to Conquest shoots and Sundance roots. Ullah*et al*. (1993) pointed that the increment in chloride concentration under salt stress condition might be due to the key role Cl in the ion equilibrium consequently, The presence of chloride in the culture soils might increase the absorption and the accumulation rates of chloride in salt stressed plants (Maynard and David ,1987).

Table 4. Effect of different NaCl Concentrations in culture medium on Cl concentration (expressed as mM g^{-1} D.W.) of for-days old four *Zea Maize* varieties.

NaCl Conc. (mM)	Shoots					Roots				
	Conquest	**Sundance**	**Reward**	**Jubliee**	**Mean**	**Conquest**	**Sundance**	**Reward**	**Jubliee**	**Mean**
0	43.08	45.20	56.38	64.89	**52.39**	13.48	23.05	36.17	72.34	**36.26**
50	73.22	104.25	68.44	77.66	**80.89**	89.72	80.95	54.61	81.56	**76.71**
75	90.78	122.20	71.28	94.68	**94.74**	112.06	95.04	80.14	91.01	**94.56**
100	101.77	143.26	80.50	103.90	**107.36**	126.09	154.25	83.33	101.42	**116.27**
125	113.50	144.32	91.84	107.06	**114.18**	134.40	156.03	96.45	103.90	**122.70**
150	125.53	147.82	95.39	109.19	**119.48**	168.50	174.82	108.51	110.64	**140.62**
200	177.34	151.42	97.87	181.60	**152.06**	184.40	219.15	133.69	119.86	**164.28**
Mean	**103.60**	**122.64**	**80.24**	**105.57**		**118.38**	**129.04**	**84.70**	**97.254**	

L.S.D. Treatment = 6.96 L.S.D. Treatment = 12.39

L.S.D. Varieties = 5.28 L.S.D. Varieties= 9.37

L.S.D. Interaction = 36.7488 L.S.D. Interaction = 116.0943

Sodium concentration:

The highest concentration of Na in shoots (89.17 mMg^{-1} D.W.) and in roots (139.86 mMg^{-1} D.W.) were observed when NaCl was applied at 200 mM. Moreover, the highest recorded values of Na were observed in Jubliee shoots (70.55 mMg^{-1} D.W.) and Conquest roots (93.02 mMg^{-1} D.W.). Table 5.It has been reported that maize cultivars with low concentrations of Na^+ in the shoot are more salt tolerant, suggesting that Na^+ exclusion may be positively correlated with salt tolerance (Fortmeier and Schubert, 1995). Roots appear more resistant than shoots (Munns, 2002).

Application of NaCl at 200 mM resulted in highest concentration of Na in Jubliee shoots and Conquest roots. Meanwhile, the lowest concentrations of Na were observed in non stressed Rewards shoots and Conquest roots.Some authors stated that, Salt tolerance is not negatively correlated with Na^+ accumulation in different plant species (Lauchli, 1984; Hajibagheri and Harvey, 1987; He and Cramer, 1994; Alberico and Cramer, 1993; Cramer, 1993; Cramer, 1994; Essah*et al*., 2003; Flowers, 2004). Flowers and Hajibagheri (2001) found that salt tolerant barley had lower cytoplasmic Na^+ than sensitive cultivar.

Table 5. Effect of different NaClconcentrations in culture medium on Na concentration (expressed as mM g^{-1} D.W.) of 14-days old four *Zea maize*varieties.

NaCl Conc. (mM)	Shoots					Roots				
	Conquest	**Sundance**	**Reward**	**Jubliee**	**Mean**	**Conquest**	**Sundance**	**Reward**	**Jubliee**	**Mean**
0	16.40	8.33	4.52	6.77	**9.01**	15.97	20.10	29.51	38.22	**25.95**
50	48.53	47.42	61.13	66.61	**55.92**	66.66	59.45	63.87	73.71	**65.92**
75	58.42	51.97	66.94	67.42	**61.19**	77.31	67.69	84.67	79.29	**77.24**
100	62.79	57.21	67.74	75.00	**65.69**	85.05	89.69	87.09	85.48	**86.83**
125	66.25	61.16	68.61	77.42	**68.36**	88.14	92.78	90.64	86.87	**89.61**
150	71.13	65.93	69.35	81.29	**71.93**	101.20	97.93	96.45	113.54	**102.28**
200	98.96	68.04	70.32	119.35	**89.17**	216.80	116.83	109.51	116.29	**139.86**
Mean	**60.35**	**51.44**	**58.37**	**70.55**		**93.02**	**77.78**	**80.25**	**84.77**	

L.S.D. Treatment = 6.23 L.S.D. Treatment = 0.30

L.S.D. Varieties = 4.71 L.S.D. Varieties = 7.03

L.S.D. Interaction = 29.34 L.S.D. Interaction = 2.109

Potassium concentration:

Data presented in Table 6 show that the highest concentration of potassium was observed in Jubliee shoots (81.05 mMg^{-1} D.W.) and Conquest roots (33.17 mMg^{-1} D.W.). NaCl application at 200 mM resulted in highest concentrations of K for shoots (74.28 mMg^{-1} D.W.). On the other side, no significant differences were observed in K concentration of roots for most treatment with the priority to treatment with NaCl at 50 mM.

Data also show the interaction effect between salt treatments and studied varieties in K concentration. The highest value was observed in Jubliee variety shoots when NaCl was added at 200 mM and in Conquest variety roots when NaCl was added at 50 mM. The lowest concentrations of K were observed in non stressed Sundance shoots and Rewards roots.

K has been known for its particular role in enhancing salt tolerance of various crops (Mengel and Kirkby, 2001; Liang *et al*., 2007). It is a major osmoticum, which contributes to osmotic adjustment, stomatal movement and restriction of Na+ uptake under salinity (Mengel and Kirkby, 2001). Maintenance of adequate levels of K is important for plant survival in saline conditions (Chow *et al*., 1990). Numerous studies have shown that K mitigates the adverse effects of salinity on plant growth (Marschner, 1995 and Sanjakkara*et al*., 2001) by regulating desirable K+/Na+ ratio, a good indicator of salt tolerance (Zhu, 2001).

Table 6. Effect of different NaClconcentrations in culture medium on K concentration (expressed as mM g^{-1} D.W.) of14-days old four *Zea maize*varieties.

NaCl Conc. (mM)	Shoots					Roots				
	Conquest	Sundance	Reward	Jubliee	Mean	Conquest	Sundance	Reward	Jubliee	Mean
0	54.33	40.51	58.67	62.60	**54.03**	41.47	26.09	15.49	21.28	**26.08**
50	51.61	53.45	51.03	65.49	**55.40**	50.00	29.70	15.90	25.83	**30.36**
75	55.59	53.96	57.02	72.93	**59.88**	31.32	28.75	19.63	25.59	**26.32**
100	60.15	61.32	57.43	80.37	**64.82**	29.70	28.67	19.57	25.83	**25.94**
125	56.32	59.70	59.29	80.58	**63.97**	27.20	28.47	19.42	25.62	**25.18**
150	60.14	57.35	68.80	84.71	**67.75**	26.97	25.97	18.18	24.98	**24.03**
200	57.35	52.94	66.12	120.70	**74.28**	25.50	24.55	17.79	24.58	**23.11**
Mean	**56.50**	**54.18**	**59.77**	**81.05**		**33.17**	**27.46**	**18.00**	**24.82**	

L.S.D. Treatment = 7.76 L.S.D. Treatment = 4.70

L.S.D. Varieties= 5.87 L.S.D. Varieties = 3.55

L.S.D. Interaction = 45.55 L.S.D. Interaction = 16.69

K/Na ratio:

Control plants resulted in highest values of K/Na ratio either in shoots (7.60 mMg^{-1} D.W.) or roots (1.25 mMg-1 D.W.). On the other side and regardless to the salt treatments, the highest K/Na ratio was observed in Jubliee shoots (3.45 mMg-1 D.W.) and Conquest roots (0.69 mMg-1 D.W.)(Table 7). In this respect (Fortmeier and Schubert, 1995), stated that , maize is as a salt-sensitive plant, although this sensitivity is dependent on the cultivar (Cramer *et al*., 1994). Various approaches are advocated to improve plant survival under salt stress (Marschner, 1995). Nevertheless, plant species and their genotypes differ genetically in their ability to adapt to salt stress environment (Wahid *et al*., 1997).

Concerning the interaction between salinity and varieties on K/Na ratio, Data in Table (7) also show that NaCl application at 200 mM resulted in lowest concentration of K/Na in Conquest shoots and roots. Highest concentrations of K/Na were observed in non treated Rewards shoots and Conquest roots.

K^+/Na^+ ratio have been considered as a useful guide to assess salt tolerance and selection of genotypes as a strategy to minimize growth reduction in saline soils (Santa-Maria and Epstein, 2001). According to Qadir and Schubert (2002), the degree to which different plant species/genotypes can resist soil salinity may partly be related to their abilities to selective absorption of K^+ over Na^+.Cicek and Cakirlar (2008) found a considerable decline caused by salt stress treatments in K^+: Na^+ ratio, plant height, fresh and dry biomass of the shoot in soybeans cultivars. The adverse correlation between K and Na ions refer to the antagonism between these two ions. In contrary, A high K^+: Na^+ ratio maintained by roots shows the selectivity for K^+ over Na^+ and the preferential loading of K^+ rather than Na^+ into xylem (Carden*et al*., 2003).

Table 7. Effect of different NaClconcentrations in culture medium on K/ Na ratio (expressed as mM g^{-1} D.W.) of 14-day old four *Zea maize*varieties.

NaCl Conc. (mM)	Shoots					Roots				
	Conquest	Sundance	Reward	Jubliee	Mean	Conquest	Sundance	Reward	Jubliee	Mean
0	3.31	4.86	12.98	9.25	**7.60**	2.60	1.30	0.52	0.56	**1.25**
50	1.06	1.13	0.83	9.67	**3.17**	0.75	0.50	0.25	0.35	**0.46**
75	0.95	1.04	0.85	1.08	**0.98**	0.41	0.42	0.23	0.32	**0.35**
100	0.95	1.07	0.84	1.07	**0.98**	0.35	0.32	0.22	0.30	**0.30**
125	0.85	0.98	0.86	1.04	**0.93**	0.31	0.31	0.21	0.29	**0.28**
150	0.84	0.87	0.99	1.04	**0.94**	0.27	0.27	0.19	0.22	**0.24**
200	0.57	0.78	0.94	1.01	**0.83**	0.12	0.21	0.16	0.21	**0.18**
Mean	**1.22**	**1.53**	**2.61**	**3.45**		**0.69**	**0.48**	**0.25**	**0.32**	

L.S.D. Treatment = 0.32 L.S.D. Treatment = 0.17 L.S.D. Varieties = 0.24 L.S.D. Varieties = 0.14

L.S.D. Interaction = 0.077 L.S.D. Interaction = 0.024

Organic solutes concentration:

Application of NaCl at 200 mM resulted in highest accumulation of organic solutes in roots (19.54 mMg^{-1} D.W.). Meanwhile, the highest accumulation of organic solutes in shoots (41.98 mg g^{-1} D.W.) was observed when NaCl was applied at 150 mM. Sundance variety resulted in highest accumulation of organic solutes either in shoots (52.79 mg g^{-1} D.W.) or in roots (26.75 mg g^{-1} D.W.) (Table 8).The highest concentration of organic solutes was observed in Sundance variety when NaCl was added at 200 mM. Meanwhile the lowest concentration was recorded in non stressed Reward variety.

Table 8. Effect of different NaClconcentrations in culture medium on organic solutes (expressed as mg g^{-1} D.W.) of 14-days old four *Zea maize*varieties.

NaCl Conc. (mM)	Shoots					Roots				
	Conquest	Sundance	Reward	Jubliee	Mean	Conquest	Sundance	Reward	Jubliee	Mean
0	12.00	27.35	4.94	8.83	**13.28**	7.97	16.28	3.46	5.38	**8.27**
50	12.81	34.16	13.79	9.63	**17.60**	8.22	16.81	7.24	7.95	**10.06**
75	15.86	39.18	14.00	10.39	**19.86**	8.51	25.35	8.64	12.61	**13.78**
100	17.80	51.97	15.13	11.02	**23.98**	8.75	27.62	11.47	12.98	**15.21**
125	26.17	69.25	20.66	11.11	**31.80**	12.40	31.92	11.50	13.43	**17.31**
150	29.29	73.47	23.18	11. 25	**41.98**	12.84	33.67	11.91	13.55	**17.99**
200	31.35	74.13	26.20	32.32	**41.00**	14.21	35.63	14.20	14.11	**19.54**
Mean	**20.75**	**52.79**	**16.84**	**13.88**		**10.41**	**26.75**	**9.77**	**11.43**	

L.S.D. Treatment = 2.79 L.S.D. Treatment = 1.63 L.S.D. Varieties = 2.11 L.S.D. Varieties = 1.24

L.S.D. Interaction = 5.89 L.S.D. Interaction = 2.02

Proline concentration:

The highest accumulation of proline (1.440 µg g^{-1} D.W. and 0.711 µg g^{-1} D.W. in shoots and roots respectively) was observed when NaCl was added at 200 mM.

On the other side, Sundance variety resulted in highest accumulation of proline either in shoots (1.483 µg g^{-1} D.W.) or in roots (0.715 µg g^{-1} D.W.) (Table 9). We infer that proline accumulation in Sundance might have a role in its salt tolerance.Data also show that the highest accumulation of proline was observed in Sundance variety when NaCl was applied at 200 mM and the lowest accumulation was recorded in non treated Rewards shoots and Jubliee roots.Salinity increased markedly the Proline content in different salt sensitive and tolerant species/genotypes: with more Proline accumulation in salt tolerant ones, which is supposed to correlate with the adaptation to salinity (Wyn Jones *et al*., 1984; Rains,1989; Ashraf, 1994; Lutts*et al*., 1996; Hare and Cress, 1997; Mansour, 2000; Hien*et al*., 2003; Ashraf and Harris, 2004). Our results implicate that NaCl stress increasesPro accumulation in the shoots and roots of the four maize varieties. Compatible solutes are overproduced under osmotic stress aiming to facilitate osmotic adjustment (Zhu, 2000; Shao *et al*., 2005).

It has been shown that, proline also have a key role in stabilizing cellular proteins and membranes in presence of high concentrations of osmoticum (Errabii*et al*., 2006).

Table 9. Effect of different NaClconcentrations in culture medium on proline concentration (expressed as µg g^{-1} D.W.) of 14-days old four *Zea maize*varieties.

NaCl Conc. (mM)	Shoots					Roots				
	Conquest	Sundance	Reward	Jubliee	Mean	Conquest	Sundance	Reward	Jubliee	Mean
0	0.085	0.044	0.015	0.059	**0.051**	0.125	0.154	0.059	0.022	**0.090**
50	0.412	0.404	0.037	0.059	**0.228**	0.169	0.272	0.074	0.044	**0.140**
75	0.835	0.444	0.057	0.107	**0.361**	0.176	0.323	0.096	0.066	**0.165**
100	1.191	1.308	0.066	0.109	**0.669**	0.323	0.484	0.096	0.066	**0.242**
125	1.297	2.213	0.110	0.259	**0.970**	0.330	0.632	0.125	0.074	**0.290**
150	1.389	2.727	0.294	0.381	**1.198**	0.345	0.985	0.139	0.081	**0.388**
200	1.669	3.242	0.364	0.485	**1.440**	0.419	2.154	0.147	0.125	**0.711**
Mean	**0.983**	**1.483**	**0.135**	**0.208**		**0.270**	**0.715**	**0.105**	**0.068**	

L.S.D. Treatment = 0. 232 L.S.D. Treatment = 0.166

L.S.D. Varieties= 0.175 L.S.D. Varieties = 0.125

L.S.D. Interaction = 0.041 L.S.D. Interaction = 0.021

Total soluble sugars (TSS):

Data in Table 10 show that the lowest concentrations of TSS were observed in control plants either in shoots or roots. The gradual increase in salt concentration resulted in gradual increase in TSS and the highest values (38.65 mg g^{-1} D.W. and 18.92 mg g^{-1} D.W. for shoots and roots respectively) were observed when NaCl was added at 200 mM. On the other side, the highest accumulation of TSS was observed in shoots and roots of Sundance variety.Moreover, regarding to the interaction between varieties and salt treatments, Data in Table 10 also show that application of NaCl at 200 mM resulted in highest accumulation of TSS in Sundance variety and the lowest values however were recorded in the non treated Reward variety.

In general, the culture under salt stress causes increase in total soluble sugars meanwhile no changes of starch level occurs (Munns*et al*., 1982).

In Maize (Zea*mays* L.) plants which have grown under different salt stress conditions, salt sensitive lines accumulate higher concentrations of total soluble sugars compared with salt tolerate lines (Larino*et al*., 1993).

Sugars and proline have equivalent responses to salinity, proline acts as cyto-osmotic and sugar acts as vacuole osmotic, consequently, both have direct effect in protection without inhibition to the enzymes activities in salt stressed plants and in osmotic adjustment which refers specifically to a net increase in solute concentration due to metabolic process triggered by stress (Krist, 1990).

Table 10. Effect of different NaClconcentrations in culture medium on TSS concentration (expressed as mg g^{-1} D.W.) of 14-days old four *Zea maize* varieties.

NaCl Conc. (mM)	Shoots					Roots				
	Conquest	Sundance	Reward	Jubliee	Mean	Conquest	Sundance	Reward	Jubliee	Mean
0	11.46	26.73	4.76	8.43	**12.85**	6.46	15.05	3.06	4.92	**7.37**
50	12.28	33.54	13.35	9.57	**17.19**	7.22	16.08	6.57	7.82	**9.42**
75	14.83	36.14	13.46	10.23	**18.67**	7.66	24.79	8.21	12.26	**13.23**
100	16.30	47.82	14.39	10.89	**22.35**	8.10	26.70	10.94	12.48	**14.56**
125	24.78	64.40	20.41	11.05	**30.16**	11.66	28.18	11.05	12.97	**15.97**
150	27.91	67.80	22.65	11.10	**32.37**	11.98	32.39	11.38	13.19	**17.24**
200	28.56	68.29	25.99	31.74	**38.65**	13.24	34.80	14.01	13.63	**18.92**
Mean	**19.45**	**49.25**	**16.43**	**13.29**		**9.47**	**25.43**	**9.32**	**11.04**	

L.S.D. Treatment = 2.32 L.S.D. Treatment = 1.74

L.S.D. Varieties= 1.76 L.S.D. Varieties = 1.32

L.S.D. Interaction = 4.08 L.S.D. Interaction = 2.30

Total amino acids concentration (TAA):

The increase in Total amino acid accumulation was due to the increase of salt concentration and the highest values (1.56 µg g^{-1} D.W. and 0.996 µg g^{-1} D.W. for shoots and roots respectively) were observed when NaCl was applied at 200 mM. Sundance variety resulted in highest accumulation of amino acids either in shoots or roots(Table 11).Regarding the interaction between salt treatments and salt application, It was obvious that lowest accumulation of TAA was observed in non stressedJubliee variety and, the highest accumulation was observed in Sundance variety when NaCl was added at 200 mM.Willadino*et al.* (1996) observed that total amino acids in salt stressed maize (Zea m*ays* L.) line was decrease, or did not increase in salt stressed sorghum (Sorghum L.) lines(Yang *et al*., 1990). In both stressed plant (*Zea mays* and *sorghum*) proline content was increased.

Table 11. Effect of different NaClconcentrations in culture medium on TAA concentration (expressed as µg g^{-1} D.W.) of 14-days old four *Zea maize* varieties.

NaCl Conc. (mM)	Shoots					Roots				
	Conquest	Sundance	Reward	Jubliee	Mean	Conquest	Sundance	Reward	Jubliee	Mean
0	0.120	0.216	0.096	0.048	**0.120**	0.432	0.192	0.048	0.048	**0.180**
50	0.192	0.576	0.096	0.048	**0.228**	0.480	0.960	0.288	0.228	**0.489**
75	0.336	2.592	0.144	0.048	**0.780**	0.528	0.288	0.336	0.336	**0.372**
100	0.360	2.593	0.240	0.096	**0.822**	0.576	0.432	0.336	0.336	**0.420**
125	1.056	2.833	0.240	0.096	**1.056**	0.624	0.576	0.432	0.348	**0.495**
150	1.200	3.121	0.528	0.144	**1.248**	0.624	0.960	0.480	0.432	**0.624**
200	1.776	3.457	0.672	0.336	**1.560**	1.440	1.584	0.528	0.432	**0.996**
Mean	**0.720**	**2.198**	**0.288**	**0.117**		**0.672**	**0.713**	**0.350**	**0.309**	

L.S.D. Treatment = 0.229   L.S.D. Treatment = 0.230

L.S.D. Varieties = 0.170   L.S.D. Varieties = 0.174

L.S.D. Interaction = 0.039   L.S.D. Interaction= 0.04002

Osmoticum:

Sundance variety resulted in highest values of osmotic potential either in shoots (502.97 m osmol kg-1) or roots (426.29 mosmol kg-1) (Table 12). Moreover, the increase in salt concentration causes significant increase in osmotical potential either in shoots or roots of all varieties under study.Carcelleret *al*. (1999) have reached the same conclusion in other all studied maize cultivars. Application of NaCl at 200 mM resulted in the highest values of osmotical potential in Sundance shoots and Conquest roots. The lowest values were observed in non treatedConquest variety. The significant increament in osmotical potential either in shoots or roots of all varieties under study is in agreement with previous reports (Kingsbury *et al*., 1984;Kingsbury and Epstein, 1986; Mansour *et al*., 1993b; Mansour and Salama, 1996).

It has been widely reported that plant cells achieve their osmotic adjustment by the accumulation of some kinds of compatible solutes such as proline, betaine, and polyols to protect membranes and proteins (Delauney and Verma, 1993). Compatible solutes are overproduced under osmotic stress aiming to facilitate osmotic adjustment (Zhu, 2000; Shao *et al*., 2005).

Table 12. Effect of different NaClconcentrations in culture medium on osmotic potential (expressed as m osmol Kg^{-1})of 14-days old four *Zea maize*varieties.

NaCl Conc. (mM)	Shoots					Roots				
	Conquest	Sundance	Reward	Jubliee	Mean	Conquest	Sundance	Reward	Jubliee	Mean
0	212.00	249.00	256.00	284.00	**250.25**	127.00	185.00	171.00	166.00	**162.25**
50	286.00	407.00	294.00	371.00	**339.50**	293.00	313.00	248.00	288.00	**285.50**
75	332.00	436.00	330.00	399.00	**374.25**	345.00	362.00	302.00	305.00	**328.50**
100	346.00	577.00	370.00	444.00	**434.25**	378.00	437.00	370.00	360.00	**386.25**
125	384.00	595.00	385.00	476.00	**460.00**	488.00	474.00	400.00	383.00	**436.25**
150	469.00	598.00	425.00	494.00	**496.50**	540.00	577.00	434.00	569.00	**530.00**
200	632.00	656.00	501.00	624.00	**603.25**	702.00	636.00	540.00	605.00	**620.75**
Mean	**380.14**	**502.57**	**365.86**	**441.71**	**422.55**	**410.43**	**426.29**	**352.14**	**382.29**	**392.79**

L.S.D. Treatment= 37.86 L.S.D. Treatment= 46.37

L.S.D. Varieties = 28.64 L.S.D. Varieties= 35.05

L.S.D. Interaction= 1084.3104 L.S.D. Interaction= 1625.2685

CONCLUSION

In conclusion, biochemical responses to NaCl stress were significantly different in four cultivars of maize differing in salt tolerance , where the Reward variety resulted in highest length of shoots and roots comparing with another studied varieties. (non sensitive to salinity). Meanwhile, the three maize varieties were more sensitive to salinity ,Sundance variety will adapted to salinity in highest accumulation of organic solutes either in shoots or in roots in the form of total amino acids or total soluble sugars .For Jubilee and Conquest variety resulted in highest concentration of inorganic solutes for shoots and roots respectively

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تأثير تراكيز مختلفة من كلوريد الصوديوم في نمو البادرات و الإستجابة الفيزيولوجية لأربعة أصناف من الذرة

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ملخص

تهدف هذه الدراسة إلى تحديد الإستجابات الفيزيولوجية لأربعة أصناف من نبات الذرة (كونكويست – ساندانس– ريوورد– جبلي) تحت ظروف الإجهاد الملحي (0, 50, 75, 100, 125, 150, 200 ميللي مولار كلوريد الصوديوم) في مرحلة نمو البادرات. جمعت البيانات أثناء نمو البادرات وحددت الإستجابات الفيزيولوجية لبادرات بعمر 14 يوماً مزروعة على بيئة 1/ 10 لونغ – أشتون. بينت النتائج تأثر طول الأفرع والجذور سلبياً بجميع معاملات الملوحة مقارنة بالشاهد. على العكس من ذلك أدت معاملات الملوحة المختلفة إلى زيادة ملحوظة في السكريات الذائبة الكلية والأحماض الأمينية الكلية والبرولين والذائبات العضوية الكلية والضغط الأسموزي. إضافة إلى ذلك، زاد محتوى الصوديوم والكلور مع زيادة نسبة الملوحة في أنسجة البادرات حيث تراكمت في الجذور بمعدل أكبر من الساق. بينما لم تكن الزيادة معنوية في عنصر البوتاسيوم على مستوى الجذور. من جهة أخرى انخفضت نسبة البوتاسيوم / الصوديوم في جذور وأفرع جميع الأصناف المدروسة. بالمقابل لوحظ أعلى تراكم للمركبات اللا عضوية في أفرع الصنف جبلي و جذور الصنف كونكويست. بينما لوحظ أعلى تراكم للذائبات العضوية في صنف ساندانس سواء في الجذور أو الأفرع.

الكلمات الدالة: ذرة سكرية – ملوحة – ذائبات عضوية – ذائبات لا عضوية.

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