

Study of salt tolerance in wheat (*Triticum aestivum* L.) cultivar Banysoif 1

Amel A Tammam*¹, ²Mona F Abou Alhamd and ²Mabrouka M Hameda

¹Department of Botany, Faculty of Science, South Valley University, Egypt

²Department of Botany, Faculty of Science, Alexandria University, Egypt

*Corresponding author email: amltammam@yahoo.com

Abstract

Salt tolerance of new wheat cultivar Banysoif 1 was investigated to find relevant culturing strategy in saline areas. Wheat cv. Banysoif 1 was grown in clay soil for 7 days in different pots. Then seedlings were irrigated by different saline waters (0, 60, 120, 180, 240 and 320 mM NaCl) near the field capacity. Plants were kept in the natural condition under these saline levels for 155 days. Fresh and dry weight of roots were measured unchanged up to the level of 120 mM NaCl then a significant reduction obtained at 240 and 320 mM NaCl. In shoots and spikes, dry matters were either unchanged or even stimulated to increase toward 180 mM NaCl then a quick reduction was observed. We assumed that it is associated with the increase of leaf area and photosynthetic pigments up to 180 mM NaCl. There was also a remarkable variation in accumulation of different carbohydrate fractions among wheat studied organs. In root, the drastic effect in soluble fraction was accompanied with a remarkable accumulation of insoluble components. In shoots, the production of carbohydrates remained mostly unaffected even at the highest salinity level. In spikes, the soluble fractions were increased significantly by salt stress while the insoluble slightly reduced. Protein content reduced at high levels of salinity in roots while has been increased significantly in shoots and spikes. Amino acid content increased significantly towards 120 mM and 180 mM NaCl then a quick reduction about 55% and 45% recorded in roots and shoots respectively. In spike, there was a significant reduction in amino acids by increasing salt stress. In roots, there was a large accumulation of proline even at the lowest salinity level. Accumulation of proline was failed in spikes and shoots and therefore a non-significant increase in proline content even at the highest salinity level was observed. Sodium content increased significantly in the three organ but the percent of increase was varied considerably among the three organs especially at sever salinity and the opposite pattern was observed in the accumulation and distribution of K^+ , Ca^{+2} and Mg^{+2} . K^+ / Na^+ ratio decreased in response to salt stress in root, shoot and spikes.

Key words: carbohydrate; proline; protein; salinity; spike

Introduction

Soil salinity is one of the most important abiotic stress and limiting factor (De bez et al., 2006 and Koyro, 2006) for worldwide plant production. Up to 20% of the irrigated arable land in arid and semiarid regions is already salt affected and is still expanding (Mühling and Läuchli, 2003). Under salt stress, plants have to cope with stress imposed by the low external

water potential and with ion toxicity due to accumulation of ions inside the plants (Romero-Aranda et al., 2006).

Differences in salt tolerance exist not only among different genera and species, but also within the different organs of the same species (Flowers and Hajibagheri 2001; Ismail, 2003). Comparing the

response of cultivars of one species to salinity provides a convenient and useful tool for unveiling the basic mechanisms involved in salt tolerance.

Salt stress has a different effect on carbohydrate contents. Some authors have reported carbohydrates accumulation in various plants under salinity condition (Abd El- Samad and Azooz, 2002; Parida et al., 2003 and Azooz et al., 2004). On the other hand, Mostafa (2004) observed that at low and moderate salinity levels, sugars and consequently the total carbohydrates are decreased. Soluble protein is generally decreased in response to salinity (Parida et al., 2002 and Abed-Latef, 2005). It has also been reported that high salt concentration either causes an increase in the N-contents and high protein content in some glycophytic plants (Abed El- Baki, 1996; Jones and Mac Millan, 1987) or increase in soluble proteins (Shaddad et al., 2005). The Number of N-containing compounds accumulating in plants subjected to environmental stress (Robe", "1990 and Kuznetsov *et al.*, 2007). The most frequently accumulating compounds include amides (glutamine and asparagines), amino acids (arginine, proline, citrulline and ornithine) and the amine putrescine. Amino acids such as proline, asparagine and amino butyric can play important roles in osmotic adjustment of plant under saline conditions (Gilbert et al., 1998).

Proline accumulation might be used as an indicator in selection for withstanding saline stress through the involvement in osmoregulation (Haroun, 2002 and Ueda et al., 2007). Expression of one or more additional genes for proline accumulation can be induced by stress (Stewart et al., 1986). Additionally, proline accumulation under stress conditions may be caused by induction of proline biosynthesis enzymes, reduction the rate of proline oxidation conversion to glutamate, decrease utilization of proline in proteins synthesis and enhancing proteins turnover (Claussen, 2005). The process of accumulation of ions such as sodium (Na^+) potassium (K^+), calcium (Ca^{2+}) and magnesium (Mg^{2+}) in plants are different and depend on variation in the external environment. Werner and Finkelstein (1995) reported that plants tend to take up more Na^+ and exclude K^+ with increasing NaCl concentration in the *in vitro* conditions. Parida et al., (2004) have reported a significant increase in Na^+ and Cl⁻ contents in leaf, stem and root of mangrove *Bruguiera parviflora* without any significant endogenous alteration of K^+ and Fe^{2+} . Decrease in Ca^{2+} and Mg^{2+} content of leaves has also been reported by salt accumulation in this species.

The K^+/Na^+ ratio has been used as an index for sodium toxicity in plant tissues, because it is assumed that activity of K^+ requires some enzymes (Cramer et al., 1994), and higher K^+/Na^+ ratio indicates less Na^+ toxicity. Some authors reported that K^+/Na^+ ratio is decreased under salt stress (Gadallah, 1999; Haroun, 2002). In this study, the common physiological behavior of a new wheat cultivar Banysoif 1 (derived from breeding programs) under salt stress and effect of salt tolerance on different organs is being studied.

Materials and Methods

Culture Techniques

Wheat (*Triticum aestivum* L.) cv. Banysoif 1 was obtained from the breeding program of Agricultural Research Center, Dokky, Cairo, Egypt. Two kilogram of soil composed of dried clay was put into the pots. Ten grains were sowed in each pot. They were daily irrigated with water until appearance of seedlings (7 days). Thereafter, the pots were then watered to the saline levels (0, 60, 120, 180, 240 and 320, mM NaCl) by adjustment the water content of soil to near the field capacity. Pots were daily irrigated with tap water to reach the soil filed capacity. The plants were kept to grow in the natural conditions under the saline levels for 155 day.

Measurement of variables

Leaf area, fresh weight and dry matter yields of the different organs (roots, shoots and spikes) were measured after 155 days. Leaf area was measured by a Planimeter (SOKKIA PLANIMELER KP-90 UK). To determine the dry matter yields, the freshly harvested organs (roots, shoots and spikes) were dried in an aerated oven at 80°C.

Successive weighting was carried out until the constant dry weight, chlorophyll a, chlorophyll b and total carotenoids were extracted and determined according to Moran (1982). Soluble and total carbohydrates were also extracted by anthrone sulphuric acid method as described by Badour (1959). Total and soluble proteins were determined according to Bradford (1976) and also proline content measured (Bates et al., 1973). Free amino acids were extracted and determined according to Moore and Stein (1948). The mixed acid digestion method of Allen et al. (1974) was used to prepare samples for determination

Table 1. Effect of salinity treatment on fresh and dry matter (g plant⁻¹) in the root, shoot and spike of 155-day old wheat of cv.Banysoif 1

Treatment (NaCl)	Fresh matter (Root)	Dry matter (Root)	Fresh matter (Shoot)	Dry matter (Shoot)	Fresh matter (Spikes)	Dry matter (Spikes)
Control	2.45	0.45	7.16	2.25	1.87	0.63
60 mM	2.42	0.42	8.73	1.98	2.13	0.67
120 mM	2.38	0.44	8.01	2.23	2.36*	0.74*
180 mM	2.08	0.35*	6.50	2.01	2.85**	1.29**
240 mM	0.74**	0.15**	3.18**	0.92**	0.82**	0.25**
320 mM	0.65**	0.14**	2.90**	0.84**	0.86**	0.26**
LSD at p ≤0.05	0.39	0.08	2.33	0.30	0.44	0.10
LSD at p ≤0.01	0.54	0.28	3.31	0.39	0.65	0.32

of minerals. The amount of sodium, potassium, calcium and magnesium concentration were estimated using a flame photometer (CORN NG 400 USA) All determinations were done in duplicate and three plants were taken for each determination .Experimental data were subjected to one way analysis of variance (ANOVA) and the means were separated by the least significant difference LSD.

Results

The fresh and dry matter yields of root remained unchanged up to the level of 120m NaCl then a highly significant reduction was obtained which was much more expressed at the level of 240 and 320 mM NaCl salinity (Table 1). In shoots, the salt stress, up to 120 mM NaCl enhanced the fresh and dry matter yield then these growth parameters remained mostly unchanged up to 180 mM NaCl thereafter a highly significant reduction was observed. In spikes, the fresh and dry matter yield was stimulated by salinity stress, up to 180mM NaCl then a quick reduction exhibited. Accordingly, there was a major difference in the salt tolerance of the three wheat organs, root was the most salt sensitive organ while spikes was the most salt tolerant and shoot was intermediate. This was associated with an increase in leaf area and photosynthetic pigments up to the level of 180mM NaCl (Table 3).

The accumulation of carbohydrates and nitrogen compounds varied among the three wheat organs (roots, shoots and spikes) In roots, the drastic effect in soluble fractions was accompanied with a remarkable accumulation of insoluble compounds; consequently the total carbohydrates remained mostly unchanged

even at the highest salinity level. In shoots, the production of carbohydrates remained mostly unaffected up to the highest salinity level. In spikes, while the soluble fraction increased significantly by salt stress, the insoluble slightly reduced and the total carbohydrates decreased significantly (Table 4).

However, protein content varied among the three wheat organs so that, generally reduced in roots (the reduction approached 30% at the level of 320mM NaCl) and increased in shoots and spikes significantly, at severe salinity conditions (Table 5) Roots maintained their protein content around the control, up to the level of 120mM NaCl. Amino acids increased significantly in roots, up to the level of 120 mM and 180 mM NaCl in shoots then a sharp reduction about 55% and 45% in root and shoot was recorded respectively. In spikes, there was a significant reduction in amino acids by increasing salinity level in the soil. There was a big variation in the accumulation of proline among the three wheat organs. In roots, there was a large accumulation of proline even at the lowest salinity level. The accumulation of proline content was about 169.66% and 788% at the level of 60 mM and 120mM NaCl respectively. Interestingly spikes failed to accumulate proline at any NaCl levels. In shoots, there was insignificant increase in proline content even at the highest salinity level (Table 6).

This irregular behavior of proline content among the three wheat organs is problematic and needs further studies to follow up complicated events of proline in salt affected glycophytes. An important question has come out about the relationships between unexpected behavior of glycophytes and proline content in Banysoif wheat breeders (Sakha station) which might

Table 2. Effect of salinity treatment on water content (WC) of root, shoot and spike of 155 days old wheat cultivar Bansoif

Treatment (NaCl)	WC% (Root)	WC% (Shoot)	WC% Spike
Control	81.63	68.57	66.31
60 mM	82.64	70.57*	86.54*
120 mM	81.51	72.15*	68.64**
180 mM	83.17	69.07*	66.49
240 mM	79.72	71.06*	69.51**
320 mM	78.55*	71.03*	69.76**
LSD at $p \leq 0.05$	1.99	1.55	1.02
LSD at $p \leq 0.01$	3.19	2.87	2.25

Table 3. Effect of Salinity treatment on chlorophyll a (Chl a), chlorophyll b (Chl b) carotenoid (Car) pigments (mg g^{-1} FW), leaf area ($\text{cm}^2 \text{ plant}^{-1}$) transpiration ($\text{g plant}^{-1} \text{ day}^{-1}$) of 155-day old wheat of cv.Banysoif 1

Treatment (NaCl)	Chl a	Chl b	Carot.	Leaf area	Transpiration
Control	1.66	1.22	0.52	19.02	106.23
60 mM	2.02**	1.44*	0.59*	22.15*	86.55**
120 mM	2.08**	1.48*	0.60*	19.81	92.37**
180 mM	1.77	0.94**	0.62*	19.55	52.81**
240 mM	1.03**	0.85**	0.41**	10.38**	64.90**
320 mM	1.09**	0.73**	0.34**	9.22**	60**
LSD at $p \leq 0.05$	0.12	0.19	0.05	2.25	8.71
LSD at $p \leq 0.01$	0.25	0.29	0.11	4.73	11.32

be, somehow, because of effect of different chromosomal changes in wheat as a result of different techniques, applied during breeding programs.

Rate of mineral content was varied considerably among the three wheat organs even in accumulation of Na^+ . At the level of 320 mM NaCl, the percent increase of Na^+ was 138.27, 39.71 and 58.33 mg g^{-1} dry matter in roots, shoots and spikes respectively, indicating that root is the highest Na^+ accumulator organ. Interestingly the opposite pattern was exhibited in the accumulation and distribution of K^+ , Ca^{2+} and Mg^{2+} , where shoots and spikes accumulated a large amount of ions in comparison to the roots. At the level of 320 mM NaCl, K^+ content was 3.5 and 4 times higher in shoots and spikes respectively in

comparison with roots. The same pattern also was seen relatively in distribution of Ca^{2+} among three studied organs. K^+ / Na^+ ratio decreased in response to salt stress in root, shoot and spike.

Discussion

The most important feature in wheat cultivar Banysoif 1 is that the drastic effect of salinity stress during the reproductive stage was obvious only at the highest salinity level at all tested organs. Additionally, this effect seems to be more expressed in root than in stem and spikes. Thus we have suggested that this cultivar tolerates moderate salt stress. NaCl salinity enhanced the biosynthesis of

Table 4. Effect of salinity treatment on carbohydrates content (mg g⁻¹ DW) in the roots, shoots and spikes of 155-day old wheat cv. Banysoif 1.

Organ	Treatment (NaCl)	Soluble	Insoluble	Total
Root	Control	79.73	174.90	254.64
	60 mM	57.77**	163.16**	220.93**
	120 mM	59.71**	164.74**	224.45**
	180 mM	30.61**	212.89**	243.50**
	240 mM	26.37**	224.03**	250.40
	320 mM	16.33**	237.94**	254.27
	LSD at p ≤0.05	4.43	6.30	5.55
	LSD at p ≤0.01	7.86	9.71	6.30
Shoot	Control	150.94	192.70	343.64
	60 mM	160.02	188.05	348.07
	120 mM	158.76	190.36	342.13
	180 mM	148.39	196.98	344.37
	240 mM	149.54	200.73	349.27
	320 mM	148.04	200.00	348.04
	LSD at p ≤0.05	13.02	7.03	12.35
	LSD at p ≤0.01	16.52	10.77	15.35
Spikes	Control	134.31	440.31	574.62
	60 mM	140.02**	428.07**	568.09**
	120 mM	160.13**	423.82**	563.95**
	180 mM	192.87**	320.05**	512.92**
	240 mM	180.20**	315.50**	495.70**
	320 mM	167.58**	321.37**	488.96**
	LSD at p ≤0.05	5.95	3.88	2.73
	LSD at p ≤0.01	9.33	6.54	4.54

soluble carbohydrates, proteins and even amino acids in shoots especially at mild salinity. Enhancement of inorganic solutes can be used as a suitable trait to discriminate genotypes for salt tolerance and osmotic stresses. Mohammed (2007) reported that it is important to know how the sink source relationships are affected in plant growth under salt stress conditions, because the efficient use of assimilate may be a limiting factor to plant growth under salinity.

The relative ability of the plant or plant organ to stimulate the accumulation of cytosolutes in its tissues (osmotic adjustment) will partially determine its tolerance to stress conditions (Karimi et al., 2005 and Kukreja et al., 2005). The marked increase in soluble saccharides as well as soluble protein and tissue water contents in shoots might indicate the superiority of shoots and even spikes over roots to

alleviate the imposed salt stress, either via osmotic adjustment (Romero–Aranda et al., 2006) or by conferring desiccation resistance to plant cells (Handa et al., 1983 and Erdei and Taleisnik, 1993).

Also, the increase in protein content at mild salinity associated with the increase in growth while at severe salinity accompanied with a significant reduction in growth. This effect is considered to be important in salt adaptation of wheat Banysoif 1 which accumulated protein to maintain osmotic adjustment and growth at moderate salinity. The opposite effect on severe salinity could be due to the ability of shoots and even spikes and consequently the whole plant in triggering the transition of cells and tissues from a state of active growth to a state of salt tolerance. In addition, the observed accumulation of protein is linked with a reduction of carbohydrates. Ability of the moderate salt–adapted wheat to divert most of the

Table 5. Effect of salinity treatment on protein contents (mg g⁻¹ DW) in the roots shoots and spikes of 155-day old wheat cv. Banysoif 1.

Organ	Treatment(NaCl)	Soluble	Insoluble	Total
Root	Control	68.04	89.88	157.92
	60 mM	60.48	90.96	151.44**
	120 mM	59.64	91.52	151.16**
	180 mM	49.56**	58.80**	108.36**
	240 mM	48.72**	38.64**	87.36**
	320 mM	48.72**	40.32**	89.04**
	LSD at p ≤0.05	3.52	4.44	10.26
	LSD at p ≤0.01	4.31	6.03	12.15
Shoot	Control	116.76	100.00	216.76
	60 mM	120.84	110.71**	231.55**
	120 mM	120.32	115.88**	236.20**
	180 mM	120.28	116.23**	237.51**
	240 mM	120.24	122.52**	242.76**
	320 mM	120.72	112.76**	233.48**
	LSD at p ≤0.05	9.94	6.66	7.73
	LSD at p ≤0.01	10.87	9.34	10.15
Spikes	Control	69.30	90.72	160.02
	60 mM	69.55	90.56	160.11
	120 mM	69.72	90.04	159.76
	180 mM	70.96	95.04*	166.00
	240 mM	73.50*	96.68	160.18
	320 mM	75.00*	99.48**	174.48
	LSD at p ≤0.05	6.32	3.33	12.13
	LSD at p ≤0.01	10.15	7.42	15.14

synthesized carbohydrates into distinct types of building materials (insoluble protein) and osmoregulation (soluble protein) is also confirmed particularly at moderate salinity. This leads us to conclude that salt tolerance of wheat. Banysoif 1 at moderate salinity seems to be linked with an equilibrium and inter-conversion between carbohydrate and nitrogen metabolism, whereas saline injury leads to metabolic disturbances in both components. It is important to note that, although carbohydrates, proteins and even amino acids (including proline) enhanced remarkably up to the level of 180mM NaCl but in sever salinity the opposite effect occurred with sever reduction in dry matter yields except for proline, which increased significantly. Therefore, proline is the only organic cytosolute which able to make the major contribution

for osmotic adjustment at sever salinity in roots, while in shoots and spikes the contribution of proline in osmoregulation might be reduced. We concluded that there is no stable situation in usage of organic or inorganic soluble components in osmotic adjustment in the cultivars and lines on different salinity levels. This is happened not only in different cultivars but also in different organs which conferring the contrasting opinions about the physiological significance of proline which has remained controversial among physiologists. Many reports have pointed out that proline is mostly accumulated when plants growth ceased (Lutts *et al.*, and 1996 and Joly *et al.* 2000). In spikes significant reduction in proline has been shown in most salinization levels, while the growth criteria of plant organ enhanced up to the 180m M NaCl.

Table 6. Effect of salinity treatment on amino acid and proline contents (mg g⁻¹ DW) in the roots, shoots and spikes of 155-day old wheat cv. Banysoif 1.

Organs	Treatment(NaCl)	Amino Acids	Proline
Root	Control	13.76	0.89
	60 mM	23.84**	2.40*
	120 mM	23.12**	3.31*
	180 mM	9.81**	5.29*
	240 mM	6.08**	7.23**
	320 mM	6.09**	7.9**
	LSD at p ≤0.05	5.22	0.25
	LSD at p ≤0.01	8.66	5.9
Shoot	Control	40.53	7.21
	60 mM	45.6*	8.37*
	120 mM	45.*	8.26
	180 mM	46.9**	9.46**
	240 mM	22.06**	9.23**
	320 mM	25.60**	7.30
	LSD at p ≤0.05	3.68	1.22
	LSD at p ≤0.01	5.71	2.03
Spikes	Control	109.74	19.26
	60 mM	93.12**	23.56**
	120 mM	77.02**	18.90
	180 mM	64.25**	19.83
	240 mM	76.77**	19.68
	320 mM	66.12**	17.26*
	LSD at p ≤0.05	6.34	1.98
	LSD at p ≤0.01	8.55	2.52

Table 7. Effect of salinity treatment on mineral composition (mg g⁻¹ DW) in the root of 155-day old wheat cv. Banysoif 1.

Treatment (NaCl)	Na ⁺	K ⁺	Ca ²⁺	Mg ²⁺
Control	10.66	38.40	4.53	2.80
60 mM	12.00	35.20*	3.33**	3.68**
120 mM	12.80*	36.66	3.20**	2.96
180 mM	16.30**	29.86**	3.06**	2.40**
240 mM	20.70**	21.33**	3.06**	2.24**
320 mM	25.00**	20.26**	2.93**	2.08**
LSD at p ≤0.05	1.50	2.20	0.88	0.18
LSD at p ≤0.01	2.03	4.11	1.03	0.35

Table 8. Effect of salinity treatment on mineral composition (mg g⁻¹ DW) in the shoot of 155-day old wheat cv.Banysoif 1.

Treatment (NaCl)	Na ⁺	K ⁺	Ca ²⁺	Mg ²⁺
Control	9.06	81.06	7.46	2.64
60 mM	9.33	85.33*	7.46	3.44**
120 mM	9.60	78.40*	7.46	2.92*
180 mM	10.53	70.70**	6.42**	3.42
240 mM	11.06	58.13**	5.60**	3.92**
320 mM	12.50**	69.86**	6.93*	3.44**
LSD at p ≤0.05	2.87	2.52	0.70	0.22
LSD at p ≤0.01	3.33	4.30	1.03	0.40

Table 9. Effect of salinity treatment on mineral composition (mg g⁻¹ DW) in the spike of 155-day old wheat of cv.Banysoif 1

Treatment(NaCl)	Na ⁺	K ⁺	Ca ²⁺	Mg ²⁺
Control	9.60	70.96	4.13	3.84
60 mM	12.26**	84.26**	4.83*	4.24
120 mM	13.33**	68.80**	5.33**	5.44**
180 mM	14.40**	54.93**	6.00**	2.92**
240 mM	15.10**	57.60**	6.90**	2.16**
320 mM	15.20**	82.20**	7.73**	2.96**
LSD at p ≤0.05	1.11	5.91	0.63	0.42
LSD at p ≤0.01	2.32	7.93	0.95	0.60

When tissue water content was increased at sever salinity in shoots and spikes, proline content increased insignificantly in shoots and decreased in spikes (Table 2). while the opposite events exhibited in roots simultaneously. Therefore, it seems that contribution of proline in osmotic adjustment is negligible. Lutts et al., (1996) found that the accumulation of proline in salt sensitive cultivars is higher than in salt tolerant rice cultivars. They concluded that there is no relation between osmotic potential and proline accumulation. Distribution of the two compounds with the wheat organs should be similar if proline dose act as a compatible solute in response to Na⁺ vacuolar accumulation. Obviously, this is not applied among the different organs of wheat Banysoif 1 where the proline content of shoots remained mostly unchanged and decreased in spikes. Since, the Na⁺ content increased significantly in shoots and spikes it is assumed that Na⁺ and proline accumulation negatively correlated. On the other hand, there is a positive correlation in the accumulation of Na⁺ and proline in roots which both

increased progressively. The function of this osmoprotectant is presumed to be protective, with a role in scavenging free radicals (Mansour, 2000). Minimization of reactive oxygen as a result of inhibition of photosynthesis and maximization of their removal (scavenging) is likely to be an important response to high salinity, among other stresses (Zhu, 2001).

Lutts et al. (1996) recorded that rice salt-sensitive cultivars accumulate more Na⁺ and proline than salt-tolerant. They also reported that proline accumulation is not related to proteolysis and could not be explained by stress-induced modifications in Δ-pyrroline 5-carboxylase reductase or proline dehydrogenase activities recorded *in vitro*. Therefore, they suggested that proline accumulation is a symptom of salt-stress injury in rice and that accumulation in salt sensitive plant results from an increase in ornithine α-aminotransferase activity and increase in the endogenous pools of its precursor glutamate.

Table 10. Effect of Salinity treatment on K⁺/Na⁺ ratio in the roots, shoots and spikes 155-day old wheat of cv.Banysoif 1

Treatment (NaCl)	Root	Shoot	Spikes
Control	3.60	8.94	7.38
60 mM	2.93*	9.14	6.87
120 mM	2.83**	8.16*	5.16**
180 mM	1.83**	6.71**	3.81**
240 mM	1.08**	5.01**	3.81**
320 mM	0.8**	5.58**	5.40**
LSD at p ≤0.05	0.55	0.23	0.54
LSD at p ≤0.01	0.71	4.01	0.99

Results obtained from three wheat organs have shown the lack of consistent correlation between salinity tolerance and criteria of proline. Nevertheless, the accumulation of proline and Na⁺ in wheat roots might be protected the dehydration of the cytoplasm as a result of the accumulation of Na⁺. Proline also serve as a sink for energy to regulate redox potentials, as a hydroxyl radical scavenger, a solute that protects molecules against denaturation, and means reducing the acidity in the cell (Tammam, 2003).

In confirmatory to the above results and discussions Na⁺ in shoots and spikes is considerably lower than in roots. Conversely potassium accumulation in shoots and spikes is higher than in roots (3.5- fold in shoots and 4-fold in spike). The clear restriction of Na⁺ content in shoots and spikes may be a suitable index of salt stress adaptation. Zhu *et al.*, (2001) found that plants are able to tolerate moderately saline environments with a greater ability to exclude Na⁺ from shoot or at least the leaf blade and concurrently maintain high level of K⁺. Similarly the K⁺ content in shoots and spikes is an index of osmotic adjustment, consequently the higher K⁺/Na⁺ ratios in shoots and spikes as compared with roots could attribute to the operation of enhanced K⁺/Na⁺ discrimination traits (Gorham, 1994). Cuin *et al* (2003) recorded that high K⁺/Na⁺ ratio is more important for many species than simply maintaining a low concentration of Na⁺, which makes sense given that much of the basis for Na⁺ toxicity is due to competition with K⁺ for K⁺ binding sites. Thus the salt tolerance of this wheat cultivar as well as the difference in salt tolerance among the three wheat organs might be closely related to its ability to enhance K⁺/Na⁺ discrimination traits which enhanced the machinery of water flow. This was supported by

the relative highest water content in shoots. In severe salinity plant tissues are able to maintain their water content much, for more tolerance to dehydration (Flower and Hajiabagheri, 2001). In wheat, extracellular Ca²⁺ inhibits unidirectional Na⁺ influx and also inhibits Na⁺ influx through a non selective cation channel, isolated in planar lipid bilayers, suggesting that the effect of Ca²⁺ on Na⁺ influx might be direct and cytosolic signaling for modification of ion channel activity is not required (Davenport *et al.*, 1997).

This study on effect of NaCl wheat organs showed a general relationship between low shoot content of Na⁺ and salt tolerance in term of dry matter production and tissue water content. The potency of proline and its mode of action in stress tolerance remains controversial, mainly because of inconsistent results, suggesting that stress induced proline accumulation is not essential for tolerance in plants. External supply of proline is toxic to plants in spite of its protective functions under stress condition (Deuschle *et al.*, 2001).

References

- Abdel-Baki GK, (1996). Response of some plants to the interactive effect of salinity and organic acids. M.Sc. Thesis, El-Minia Univ., El-Mina, Egypt. 1-187.
- Abdel-Samad HM, and Azooz MM, (2002). Salt tolerance of maize cultivars Bull. Fac. Sci., Univ., Assuit, Egypt. 31: 27-34.
- Abdel-Latef AA, (2005). Salt tolerance of some wheat cultivars. Ph.D.Thesis, South valley Univ. Qena, Egypt. 1-159.

- Allen S, Grimshaw HM, Parkinson JA, Quarmby C, (1974). Chemical Analysis of Ecological Materials. Blackwell Sci. Publ. Oxford, London.
- Azooz MM, Shaddad MA, Abdel-Latef AA, (2004). The accumulation and compartmentation of proline in relation to salt tolerance of three sorghum cultivars. *Ind. J. Plant Physiol.* 9:1-8.
- Badour SSA, (1959) Analytical chemische untersuchung des kaliummangles bei chlorella in vergleich mit anderen Mangelzuständen Ph.d. Dissertation Göttingen.
- Bates LS, Waldren RP, Tear LD, (1973). Rapid determination of free proline for water-stress studies. *Plant Soil.* 39: 205-207.
- Bradford MM, (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein – dye binding. *Anal. Biochem.* 72: 248-254.
- Claussen W, (2005). Proline as measure of stress in tomato plants. *Plant Sci.* 168: 241-248.
- Cramer GR, Alberico GJ, Schmidt C, (1994). Salt tolerance is not associated with the sodium accumulation of two maize hybrids. *Aust. J. Plant Physiol.* 21: 672-692.
- CuinTA, MillerAJ, Laurie SA, Leigh RA, (2003). Potassium activities in cell compartments of salt – grown barley leaves. *J. Exp. Bot.* 54:657-661.
- Davenport RJ, Reid RJ, Smith FA, (1997). Sodium – Calcium interactions in two wheat species differing in salinity tolerance. *Physiol. Plant.* 99: 233–237.
- Debez A, Saadaoui D, Ramani B, Ouerghi Z, Koyro HW, Huchzermeyer B, Abdelly C, (2006). Leaf H⁺ - ATPase activity and photosynthetic capacity of *Cakile maritima* under increasing salinity. *Environ. Exp. Bot.* 57: 285-29
- Deuschle K, Funck D, Hellmann H, Däschner K, BinderS, Frommer WB, (2001). A nuclear gene encoding mitochondrial Δ- pyrroline – S- carboxylate dehydrogenase and it's potential role in protection from proline toxicity. *Plant J.* 27: 345-355
- Erdei L, Taleisnik E, (1993). Changes in water relation parameters under osmotic and salt stresses in maize and sorghum. *Physiol. Plant.* 89: 381-387.
- Flowers TJ, Hajiabagheri MA, (2001). Salinity tolerance in *Hordeum vulgare*: ion concentration in root cells to cultivars differing in salt tolerance. *Plant Soil.* 231: 1-9.
- Gadallah MAA, (1999). Effects of proline and glycinebetaine on *Vicia faba* responses to salt stress. *Biol. Plant.* 42: 249-257.
- Gilbert G, Gadush M, Wilson C, Madore M, (1998). Amino acid accumulation in Sink and source tissues of *Coleus blumei* Benth during salinity stress. *J. Exp. Bot.* 49: 107-114.
- Gorham J, (1994). Salt tolerance in the triticeae K/Na discrimination in some perennial wheat grasses and their amphiploids with wheat. *J. Exp. Bot.* 273: 441-447.
- Handa S, Bressan RA, Handa AK, Carpita NC, Hasegawa PM, (1983). Solutes contributing to osmotic adjustment in cultured plant cells adapted to water stress. *Plant Physiol.* 73: 834-843.
- Haroun SA, (2002) . Fenugreek growth and metabolism in response to gibberellic acid and sea water. *Assuit Univ., J. Bot.* 31: 11-12.
- Ismail AM, (2003). Effect of salinity on physiological responses of selected lines / variety of wheat .*Acta Agron.* 51 : 1-9.
- Joly RJ, Maggio A, Reddy MP, (2000). Leaf gas exchange and solute accumulation in the halophyte *Salvadora persica* grown at moderate salinity. *Env. Exp. Bot.* 44. 31-38.
- Jones RL, Mac Millan J, (1987). Gibberellins. In: "Advanced plant physiology" (Ed) Wilkins. M.B. The Bath press. Avon. Pp.1-52 .
- Karimi G, Ghorbanli M, Heidari H, Khavari Nejad RA, Assareh MH, (2005). The effect of NaCl on growth water relations, osmolytes and ion content in *Kochia prostrata*. *Biol. Plant.* 49: 301-304
- Koyro HW, (2006). Effect of salinity on growth, photosynthesis, water relations and solute composition of the potential cash crop halophyte *Plantago coronopus* (L-). *Environ. Exp. Bot.* 56: 136-146.
- Kukreja S, Nandwal AS, Kumar N, Sharma SK, Unvl V, Sharma PK, (2005). Plant water status, H₂O₂ scavenging enzymes, ethylene evolution and membrane integrity of *Cicer arietinum* roots as affected by salinity. *Biol. Plant.* 49: 305-308.
- Kuznetsov V, Shorina M, Aronova E, Stetsenko L, Rakitin V, Shevyakov N, (2007). NaCl and ethylene dependent cadaverine accumulation and its possible protective role in the adaptation of the common ice plant to salt stress. *Plant Sci.* 172: 363-370.
- Lutts S, Kinet JM, Bouharment J, (1996). Effect of salt stress on growth mineral nutrition and proline accumulation in relation to osmotic adjustment in rice (*Oryza sativa* L.) cultivars differing in salinity resistance. *Plant Growth Regul.* 19: 207-218.

- Mansour MMF, (2000). Nitrogen containing compounds and adaptation of plants to salinity stress. *Biol. Plant.* 43: 491-500.
- Mohammed HAM, (2007). Physiological studies on the antioxidative responses and some related metabolites of Lupin and Sorghum plants grown under sea water M.Sc. thesis, South Vally. Univ. Quena, Egypt. 1-184.
- Moore, Sand Stein, WH (1948). Photometric ninhydrin method for use in the chromatography of amino acids. *J. Biol. Chem.* 176:367-388.
- Moran R, (1982). Formulae for determination of chlorophyllous pigments extracted with N-N Dimethylformamide. *Plant Physiol.* 69: 1376-1381.
- Mostafa DM, (2004). Metabolic imbalance and salinity tolerance of two maize cultivars. M.Sc. Thesis. El-Minia Univ. Elminia, Egypt 1-195.
- Mühling KH, Läuchli A, (2003). Interaction of NaCl and Cd stress on compartmentation pattern of cations, antioxidant enzymes and proteins in leaves of two wheat genotypes differing in salt tolerance. *Plant Soil* 253: 219-231.
- Parida A, Das AB , Das,P (2002). NaCl stress causes changes in photosynthetic pigments, proteins and other metabolic components in the leaves of a true mangrove, *Bruguiera parviflora* in hypotonic cultures. *J.Plant Biol.* 45: 28-36.
- Parida AK, Das AB, Mitra B, (2003). Effect of NaCl stress on the structure, pigment complex composition and photosynthetic activity of mangrove *Bruguiera parviflora* chloroplasts. *Photosynth.* 41: 191-200.
- Parida AK, Das AB, Mitra B, (2004). Effects of salt on growth ,ion accumulation photosynthesis and leaf anatomy of the mangrove, *Bruguiera parviflora*. *Trees- Struct. Funct.* 18: 167-174.
- Robe E, (1990). Stress Physiology : The functional significance of the accumulation of nitrogen containing compound. *J. Hot.Sci.* 65: 231-243.
- Romero-Aranda MR, Jurado O, Cuartero J, (2006). Silicon alleviates the deleterious salt effect on tomato plant growth by improving plant water status *J. Plant Physiol.* 163: 847-855.
- Shaddad MA, Ismail AM, Azooz MM, Abdel- Latef A, (2005). Effect of salt stress on growth and some related metabolites of three wheat cultivars. *Assuit Univ. J. Bot.* 34: 477-491.
- Stewart CR, Voetberg G, Rayapati PJ, (1986). The effects of benzyladenine, cycloheximide and cordycepin on wilting – induced abscisic acid accumulations and induced proline accumulations in barley leaves. *Plant Physiol.* 82: 703-707.
- Tammam AA, (2003). Response of *Vicia faba* plants to the interactive effect of sodium chloride salinity and salicylic acid treatment. *Acta Agron. Hung.* 51: 239-248.
- Ueda A, Yamamoto-Yamane Y, Takabe T, (2007). Salt stress enhances proline utilization in the apical region of barley roots. *Biochem. Biophys. Res. Comm.* 355: 61-66.
- Werner JE, Finkelstein RR, (1995). Arabidopsis mutants with reduced response to NaCl and osmotic stress. *Physiol. Plant.* 93: 659-666.
- Zhu C, Kinet JM, Lutts S, (2001). Characterization of rice (*Oryza sativa* L.) F-3 populations selected for salt resistance. I-Physiological behavior during vegetative growth. *Euphytica* 121: 251-263