

Physiological Responses of Wheat (*Triticum aestivum* L. cv. Giza 158) Seedlings under Salt Stress Conditions

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ABSTRACT

This investigation was conducted to evaluate the physiological responses of wheat seedlings under salt stress conditions induced by different levels of sodium chloride (NaCl). Growth and physiological parameters were evaluated on 14-day old seedlings of wheat grown in Long Ashton solution containing 0, 50, 75, 100, 125, 150 and 200 mM NaCl. Salt stress treatments resulted in a dramatic decrease in length of shoots and roots, percent of stressed shoots and roots length to control, fresh and dry weights of different parts of stress imposed seedlings. 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 significantly increased in tissues as salinity increased with a high accumulation rate in roots than the translocation to shoots. On contrary, a substantial decrease in potassium and total inorganic solutes contents in both roots and shoots was observed.

Keywords: *Triticum aestivum*, Salinity, Total organic solutes, Total inorganic solutes.

INTRODUCTION

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). The excess of salt in the soil or in the irrigation water is one of the biggest problems in agriculture since almost all cultivated plants are sensitive (Jóse et al., 2000 ; Stefano, 2001). Sodium chloride (NaCl) is the most widespread chemical factor causes inhibition of plant growth in nature (Stavark and Rains, 1984).

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 osmotic stress 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).

Wheat (*Triticum* spp.) is a grass, originated from the Fertile Crescent region of the Near East, but now

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cultivated world wide (Belderok et al., 2000). In 2007 world production of wheat was 607 million tons, making it the third most-produced cereal after maize (*Zea mays* L.) (784 million tons) and rice (*Oryza sativa* L.) (651 million tons) (FAO, 2007). Wheat is an important cereal crop and a salt-sensitive glycophyte (Xue et al., 2004). Growth and grain yield of wheat decreased significantly by salinity (Saqib et al., 2004 2005). Growth response to salinity has also been confirmed for wheat as wheat genotypes differing in rates of Na^+ accumulation and salt resistance did not differ in growth reduction in the first phase of salt stress (Munns et al., 1995). Munns and James (2003) reported that Na^+ accumulation in leaves was the most important character that correlated with the salt tolerance of wheat.

In the field where the salinity rises to 100 mM NaCl (about 10 dS m^{-1}), rice (*Oryza sativa*) will die before maturity, while wheat will produce a reduced yield. Even barley (*Hordeum vulgare*), the most-tolerant cereal, dies after extended periods at salt concentrations higher than 250 mM NaCl (equivalent to 50% seawater). The halophytic relative of wheat, tall wheat grass (*Thinopyrum ponticum*, syn. *Agropyron elongatum*), is grown for forage on saline soils. Distant halophytic relatives of barley, such as sea barleygrass (*Hordeum marinum* L.), are even more salt tolerant but are not useful for forage (Garthwaite et al., 2005),

Free proline in two lemon (*Citrus aurantifolia*) scions increased with increasing salt levels from 40-80 mol NaCl/ m^3 (Nieves et al., 1991). In addition, 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, Rus and Guerrier (1994) showed that proline concentration increased markedly in tomato (*Solanum lycopersicum* L.) calluses (salt sensitive) treated with 140 mM NaCl. Moreover, El-Sayed et al. (1995)

reported that proline accumulation of olives (*Olea europea* L.) was increased when irrigated with saline water at 6000 ppm. Moreover, the highest accumulation of amino acids (proline, arginine, alanine and glutamine) was recorded in salt stressed *Atriplex halimus* (Sangita et al., 1999). Johari-Pireivatlou et al. (2010) concluded that proline content increased by water stress in four lines of bread wheat.

Singh et al. (2000) recorded gradually increases in total soluble sugars and proline concentrations with increasing NaCl level of six genotypes of *Vitis vinifera*. The osmotic adjustment can usually be accounted by an increase in concentration of a variety of common solutes, including sugars, organic acids and ions especially K^+ (Taiz and Zeiger, 1991). Johari-Pireivatlou et al. (2010) concluded, content of total soluble sugars increased by water stress in four lines of bread wheat.

Taher (1983) revealed that Na concentrations in leaves of three citrus rootstocks (*C. aurantium*, *C. sinensis* and *C. aurantifolia*) was increased by increasing of salinity levels (0, 1000, 3000, 5000 and 7000 ppm) of NaCl and CaCl_2 at 1:1 ratio. In addition, Sherif (1985) on 1-year old citrus rootstocks seedlings (*C. aurantium*, *C. lemon* and *C. aurantifolia*), found that irrigation the plants with saline water using NaCl, CaCl_2 or Na_2SO_4 (2000, 3000 and 4000 ppm) reduced N and increased Na concentrations in the leaves. El-Hefnawy (1986) who has worked on guava (*Psidium guajava*) mentioned that increasing level of soil salinity caused clear reduction in leaf K (4000 and 6000 ppm). A gradual decrease in K, and a gradual increase in Na and Ca concentrations were observed in mango (*Mangifera indica*) seedlings leaves when irrigated with tap water containing different concentrations of chloride salts of Na, Ca and Mg at the ratio of 3 NaCl : 1 (3CaCl_2 : 1 MgCl_2) at 0, 1000 to 4000 ppm (Abd El-Karim, 1991). Moreover, Adding NaCl at 50 or 100 mol/ m^3 to 10% Hogland's solution increased K

and Na concentrations in seedling shoots of eight citrus rootstocks (Zekri, 1993). The effect of salinity levels (2, 10, 25 and 40 meq/l) on the composition of banana (*Musa accuminata*) leaves were studied in greenhouse experiment by Santos et al. (1994). They found that the concentration of K, Na and Cl increased significantly by increasing salt levels. In addition, El-Hammady et al. (1995) has been reported that irrigation with Salinized water at 1500 or 3000 ppm of salt mixture (NaCl, CaCl₂ and MgCl₂) markedly increased leaf concentration of Na and Cl regardless of rootstock kind of citrus seedlings. Leaves content of Na and Ca concentrations of 14 olive (*Olea europea* L.) cultivars showed an increment when treated plants with saline water at 6000 ppm, meanwhile, N and K concentrations decreased markedly and Mg concentration was not affected by salt treatments (El-Sayed et al., 1995). Recently, Kambiz et al. (2010) observed that salinity stress decreased relative water content (RWC), potassium content, potassium/sodium ratio in two genotypes, 'Turkey 506' (salt tolerant) and 'Egypt 557' (salt sensitive), were grown in hydroponic conditions, exposed to various salt levels (0, 50, 100, 150 and 200 mmol NaCl). Therefore, this study is conducted to evaluate the physiological responses of wheat seedlings under salt stress conditions induced by different levels of sodium chloride (NaCl).

Material and Methods:

Grains of wheat (*Triticum aestivum* L.cv.Giza 158), were surface sterilized by soaking in 0.01M HgCl₂ for three minutes, washed several times thoroughly with distilled water. They were germinated on moist tissue paper in dark at 25°C for three days. Germinated grains were allowed to grow on nylon mesh suspended on half strength Long Ashton nutrient solution (Hewitt,1952) (Table 1) containing 0.1mM of Fe-EDTA. Macronutrient and micronutrient were added according to Hoagland

and Arnon (1938).

Table (1): Composition of Long Ashton Nutrient Solution

Macro Nutrients

KNO ₃	4.0 mM
Ca(NO ₃) ₂ .4H ₂ O	4.0 mM
MgSO ₄ .7H ₂ O	1.5 mM
NaH ₂ PO ₄ .2H ₂ O	1.33 mM
Ferric EDTA mono Na	0.1 mM

Micro Nutrients

MnSO ₄ .4H ₂ O	10 µM
ZnSO ₄ .7H ₂ O	1 µM
CuSO ₄ .5H ₂ O	1 µM
H ₃ BO ₃	50 µM
Na ₂ MoO ₄ .2H ₂ O	0.5 µM
NaCl	100 µM
CoSO ₄ .7H ₂ O	0.2 µM

Different levels of sodium chloride were added respectively (0, 50,75,100,125,150 and 200 mM). The nutrient solution was 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 12000 Lux at the top of plants at 25°C. After 14 days, plants were harvested and data were calculated to estimate growth parameters, organic and inorganic solutes .

Seedlings growth parameters:

Ten seedling were used for estimation of shoots and roots length, fresh and dry weight and percent of stressed shoots and roots length to control.

Plant length:

Roots were rinsed in distilled water for 30 seconds to

remove culture solution from the root space. The length of shoots and roots was measured.

Plant fresh and dry weight:

Plants were separated into shoots and roots. Fresh weight was determined immediately for each sample of shoots and roots. Plants were dried to a constant weight at 80 °C for 48 hours to determine the dry weight of shoots and roots.

Shoots and roots length:

Stressed shoots and roots length was estimated in percentage according to the following equations:

$$\% \text{ of stressed shoots length to control} = \frac{\text{Shoot length of stressed plant}}{\text{Shoot length of control plant}} \times 100$$

$$\% \text{ of stressed roots length to control} = \frac{\text{Root length of stressed plant}}{\text{Root length of control plant}} \times 100$$

Estimation of organic solutes:

At time of harvesting, shoots were taken randomly, weighed immediately and extracted in 15 cm³ of 80% (v/v) ethanol. Roots were dissected from these seedlings, washed quickly with deionized water, blotted, weighed immediately and extracted in 15cm³ of ethanol. The extraction was carried out at 60 °C for three hours, then filtered and the total volume was made up to 20 cm³ with 80% ethanol. The extraction was stored in a cold room at -4 °C, 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). About 2 cm³ of ethanol extract, was mixed with 2 cm³ of acid ninhydrin reagent in test tubes and incubated in a water bath at 95°C for 1 hour. The reagent was prepared by dissolving 1.25 g of ninhydrin in a mixture of 30 cm³ of glacial acetic acid and 20 cm³ of 6M orthophosphoric acid. The tubes were loosely sealed with marbles to prevent excessive

evaporation. After cooling, 4 cm³ of toluene was added to each test tube and the solution was mixed thoroughly, allowed layers to separate. The pink colored upper toluene layer was removed and the optical density was read at 518 nm.

Estimation of total amino acids:

Total amino acids excluding praline, were measured colorimetrically following the procedure outlined by Rosen (1957). 1cm³ of ninhydrin reagent (Sigma products) diluted (1:3) with 80% ethanol and 1 cm³ of ethanol extract of samples were mixed and heated in 20 cm³ test tubes covered with marbles at 90°C for 15 minutes in water bath. The samples were allowed to cool for half an hour and 10 cm³ of 50% (v/v). N-propanol was added and thoroughly mixed and allowed to stand for half an hour for color development. A range of standard solutions (0.01 to 1.0 mM) was prepared using glutamic acid. The optical density was read at 570 nm.

Estimation of total soluble sugars:

Total soluble carbohydrates were estimated colorimetrically using the procedure outlined by Yemm and Willis (1954). The reagent was prepared by dissolving 2 g of anthrone in a liter of concentrated sulphuric acid. The ethanol extract (0.2 cm³) was placed in 20 cm³ test tubes held in an ice bath below 0 °C. The samples were allowed to cool for 15 minutes. Antherone reagent (2 cm³) was added drop by drop to each test tube mixed by lightly shaking the test tubes in the ice bath. The samples were kept in the ice bath for about 15 minutes, on removal they were heated at 60 °C and cool for 15 minutes in a water bath with the test tubes loosely sealed with marbles. The dark green color was read at 620 nm.

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 HNO₃ 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 by reaction with Hg(SCN)₂ immobilized in an epoxy resin bead in a solid-phase reactor (SPR). The thiocyanate ions released were determined spectrophotometrically at 480 nm after completing reaction with Fe(III). The analytical curve for chloride was linear in the concentration range from 5.6×10^{-5} to 2.2×10^{-4} mol l⁻¹ with a detection limit of 1.4×10^{-5} mol l⁻¹. The relative standard deviation (R.S.D.) was 2.2% for a solution containing 2.2×10^{-4} mol l⁻¹ (n = 10). The simple manifold allows a routine analytical frequency of 100 determinations per hour. The main advantage of the developed method is the 400% reduction of the Hg waste solution generated when compared to conventional methods for chloride determination based on the same spectrophotometric reaction (Silva et al., 2005).

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:

Morphological parameters:

Shoots and roots length:

The highest shoots length (17.92 cm) was observed in control plants, followed by treated plants with NaCl at 50 and 100 mM with no significant differences between the two concentrations. In addition, no significant differences were observed when NaCl was added at 125 and 200 mM. However, the lowest shoot length (3.90 cm) was recorded when NaCl was added at the highest level (200 mM). Data in table 2 also shows that the reduction in roots length was due to the increase of salt level and the lowest length (1.60 cm) was observed when at salinity level of 200 mM. No significant difference was observed between plants treated with 50 mM NaCl and control plants which resulted in the highest roots length (9.39 cm) (Table 2).

Table (2): Effect of different NaCl concentrations in culture medium on growth and fresh and dry weights of 14-day old wheat plants grown at 25 °C*.

NaCl concentrations (mM)	Fresh weight (gm)		Dry weight (gm)		Length (cm)		% of shoots and roots length to control	
	Shoots	roots	Shoots	Roots	shoots	Roots	Shoots	Roots
0	0.318a	0.626a	0.071a	0.030a	17.92a	9.39a	100a	100a
50	0.257b	0.578b	0.061b	0.022b	15.73b	8.71a	87.77b	92.76a
75	0.174c	0.399c	0.054c	0.019c	14.18b	5.72b	79.12b	60.92b

NaCl concentrations (mM)	Fresh weight (gm)		Dry weight (gm)		Length (cm)		% of shoots and roots length to control	
	Shoots	roots	Shoots	Roots	shoots	Roots	Shoots	Roots
100	0.166c	0.365c	0.051d	0.017c	11.79c	4.14c	65.79c	44.09c
125	0.128d	0.298d	0.046e	0.015d	9.43d	3.75cd	52.62d	39.93c
150	0.122d	0.264d	0.045e	0.014d	7.95d	2.80d	44.36d	29.82d
200	0.099e	0.065e	0.017f	0.007e	3.90e	1.60e	21.76e	17.03e
L.S.D.	0.0338	0.0468	0.0021	0.0021	2.012	1.145	10.639	9.599

*Each value is the mean of ten samples.

The labeled with the same letter are not significant as indicated by LSD ($P < 0.05$)

Shoots and roots length:

The effect of different NaCl treatments on percent of shoots and roots length to control was observed in table 2.

Increase in salt treatments resulted in gradual and significant decrease in the percentage of shoots and roots length to control. No significance difference in percentage of shoots length to control was observed when plants were treated with 125 mM and 150 mM of NaCl. Moreover, no significance difference in percent of roots to the control was observed when plants were treated with 100 mM and 125 mM of NaCl. The lowest values were observed when NaCl was added at 200 mM (21.76 and 17.03 for shoots and roots length to control respectively) (Table 2).

Fresh and dry weight:

Fresh weight:

The highest level of NaCl (200 mM) resulted in the lowest fresh weight for shoots (0.099 gm) and roots (0.065 gm) (Table 2). It was followed by treatment with 125 and 150 mM of NaCl with no significant differences. In addition, no significant difference was observed when NaCl was added at 75 and 100 mM, and the highest values (0.318 gm and 0.626 gm for shoots and roots fresh weight respectively) were recorded in non-stressed plants (Table 2).

Dry weight:

It was obvious clear that all studied treatments significantly decreased the dry weight for shoots and roots (Table 2). Control plants resulted in the highest dry weight for shoots (0.071 gm) and roots (0.030 gm). The gradual decrease in dry weights was due to the gradual increase in NaCl level. At 200 mM, the lowest values for shoots and roots dry weight were 0.017 gm and 0.007 gm, respectively (Table 2).

Organic compounds:

Proline:

The effect of different salt treatments on proline concentration of salt stressed-wheat plants has been shown in Table (3). The highest values ($4.337 \mu\text{g g}^{-1}$ D.W. and $3.511 \mu\text{g g}^{-1}$ D.W. for shoots and roots respectively) were recorded when NaCl was added at 200 mM. It was followed significantly by treating with 125 and 150 mM of NaCl with no significant concentration between the two concentrations. On the other side, the lowest values ($0.466 \mu\text{g g}^{-1}$ D.W. and $0.231 \mu\text{g g}^{-1}$ D.W. for shoots and roots respectively) were observed in control plants. No significant differences were observed between control plants and treatment with NaCl at low concentrations (50 and 75 mM) (Table 3).

Table (3): Effect of different NaCl concentrations in culture medium , on proline and total amino acids (expressed as $\mu\text{g/g}^{-1}$ D.W.) and total soluble sugars (expressed as mg g^{-1} D.W.) of 14-day old wheat plants grown at 25 °C*.

NaCl concentrations (mM)	Proline		Total amino acids		Total soluble sugars		Total organic solutes	
	Shoots	roots	Shoots	Roots	shoots	roots	shoots	Roots
0	0.466d	0.231e	4.739d	5.90e	35.73e	36.30a	40.69e	42.43d
50	0.499cd	0.437de	5.467cd	10.88d	37.66e	38.16cd	43.62e	49.48bc
75	0.599cd	0.868cde	6.533bc	13.03c	54.45d	39.45c	61.58d	53.35bc
100	0.659cd	1.102cd	6.712bc	19.68a	61.83cd	42.92b	69.30d	63.68a
125	1.252bc	1.312bc	6.911 b	20.18a	70.91 c	42.92b	79.07c	64.98a
150	1.744b	1.931b	9.381 a	15.38b	97.82 b	42.99b	108.95b	65.10a
200	4.337a	3.511a	9.924 a	3.90e	134.45a	46.94b	148.75a	54.35b
L.S.D.	0.70	0.66	1.32	2.09	9.14	2.65	7.94	6.62

*Each value is the mean of three samples .

The labeled with the same letter are not significant as indicated by LSD ($P < 0.05$)

Total amino acids (TAA):

In general, the gradual increase in salt level resulted in a gradual and significant increase in TAA level of shoots (Table 3). The highest concentration ($20.183 \mu\text{g g}^{-1}$ D.W.) of TAA in roots was observed when NaCl was added at 125 mM followed by the treatment with 100 mM NaCl (Table 3). Treatment with NaCl at 150 mM significantly increased TAA concentration ($15.379 \mu\text{g g}^{-1}$ D.W.) compared with treatment at 75 mM (13.03 mM). However, the lowest values were observed in both the control and 200 mM NaCl (Table 3).

Total soluble sugars (TSS):

The lowest concentration of total soluble sugars of shoots (35.73 mg g^{-1} D.W.) was observed in control plants, followed by the treatment with 50 mM NaCl (Table 3). Increase of NaCl level caused observable increase in TSS of shoots and the highest concentration (134.45 mg g^{-1} D.W.) was noticed when NaCl concentration was added at 200 mM. Concerning TSS of roots, data in Table (3) also show that the highest concentration (46.94 mg g^{-1} D.W.) was observed when

NaCl was added at 200 mM and the lowest concentration (36.30 mg g^{-1} D.W.) was observed in non-stressed treatment (control) followed by the treatment with 50 mM NaCl. Moreover, no significant difference was observed in TSS of roots when NaCl was added at 100, 125, 150 and 200 mM .

Total organic solutes (TOS):

Concerning total organic solutes in shoots, data tabulated in Table (3) clearly exhibit that the gradual increase in TOS (43.62, 61.58, 69.30, 79.07, 108.95 and 148.75 mg g^{-1} D.W.) was due to the gradual increase in the salt levels and the lowest concentration (40.69 mg g^{-1} D.W.) was recorded in the control plants. On the other side, the highest value of TOS in roots (65.10 mg g^{-1} D.W.) was noticed when NaCl was added at 150 mM, followed by the treatments with 150 and 100 mM of NaCl. In addition, no significant differences were observed at 50, 75 and 200 mM of NaCl (Table 3).

Inorganic compounds:

Sodium:

Concerning Na concentration It was clear that the

gradual increase in salt levels resulted in gradual and significant increase in sodium concentration (Table 4). The lowest values were observed in control wheat plants in both shoots (8.05 mM g⁻¹ D.W.) and roots (21.16 mM g⁻¹ D.W.). The highest values (91.15 mM g⁻¹ D.W. for

shoots and 197.28 mM g⁻¹ D.W. for roots) were recorded when NaCl was added at the highest level (200 mM). It was significantly followed by treatment with NaCl at 150 and 200 mM with no significant differences either for shoots or for roots.

Table (4): Effect of different NaCl concentrations in culture medium , on the ionic contents of 14- day old wheat plants grown at 25 °C*.

NaCl concentrations (mM)	Sodium		Chloride		Potassium		Total inorganic solutes	
	Shoots	Roots	shoots	Roots	shoots	Roots	Shoots	Roots
0	8.05f	21.16f	10.25e	14.34f	75.35a	70.96a	95.33f	93.65e
50	56.84e	104.88e	14.91e	33.71e	68.75a	65.67a	178.51e	206.50d
75	68.03d	149.50d	25.87d	46.77d	56.18b	37.01b	220.80d	235.71c
100	73.51c	159.77c	29.19d	50.89cd	53.59b	32.12bc	240.28d	244.35c
125	83.51b	181.44b	40.28c	56.79c	51.08bc	31.21bc	270.71c	269.11b
150	87.67ab	188.49ab	49.84b	66.14b	46.59cd	29.48c	309.11b	272.31b
200	91.15a	197.28a	90.19a	155.90a	43.50d	17.61d	366.39a	378.90a
L.S.D.	4.63	9.92	6.626	5.957	6.619	6.61	26.47	15.63

*Each value is the mean of three samples calculated as mM g⁻¹ dry weight.

The labeled with the same letter are not significant as indicated by LSD (P<0.05)

Chloride:

The significant increase in chloride concentration for shoots (14.91, 25.87, 29.19, 40.28, 49.84, 90.19 mM g⁻¹ D.W.) and for roots (33.71, 46.77, 50.89, 56.79, 66.14, 155.90 mM g⁻¹ D.W.) was due to the gradual increase in salt levels, on the other hand, the lowest Cl concentrations (10.25 and 14.34 mM g⁻¹ D.W. for shoots and roots respectively) were recorded in non-stressed control plants (Table 4) .

Potassium:

The gradual decrease in potassium concentration was due to the gradual increase in salt treatments and the highest potassium concentrations (75.35 and 70.96 mM g⁻¹ D.W. for shoots and roots respectively) were recorded in the control plants (Table 4). It was followed

by treatment with the lowest salt level (50 mM) with no significant differences. In additions, no significant differences were observed when NaCl was added at 75 and 100 mM either for shoots or for roots, and the lowest K concentration (43.50 and 17.61 mM g⁻¹ D.W.) was recorded when NaCl was added at the highest level (200 mM) .

Total inorganic solutes (TIS):

As presented in Table (4), salt treatments increased the total of inorganic solutes (TIS) and the highest values (366.39 and 378.90 mM g⁻¹ D.W. for shoots and roots respectively) were noticed when the highest level of NaCl (200 mM) was added. However, the lowest values (95.33 and 93.65 mM g⁻¹ D.W. for shoots and roots respectively) were observed in control plants. On

the other side, no significant differences were observed when NaCl was added at 75 and 100 mM. Moreover, no significant difference was recorded in total inorganic solutes of roots when NaCl was added at 125 and 150 mM. The same levels resulted in significant differences in total inorganic solutes for shoots (270.71 and 309.11 mM g⁻¹ D.W. for salt treatments at 125 and 150 mM respectively).

Discussion

In general, the increase of salt in soil or irrigation water will result in an observable reduction in growth and productivity of stressed plants (Israeli et al., 1986). The elongation of the main shoot of two cherry varieties (cv. 'Bigarreau Burlat' [BB] and 'Tragana Edessis' [TE]) grown under 25 or 50 mmol L⁻¹ NaCl was significantly reduced relative to the control under NaCl-induced salinity stress (Papadakis et al., 2007). This is a common response of plants cultivated under saline conditions, leading to a reduction of growth (Greenway and Munns, 1980). Roots appear more resistant than shoots (Munns, 2002). Schulze (1986) pointed that there are two factors cause growth inhibition; the salt stress (first) and, the mechanism of carbon distribution during stress condition (second).

Nieman et al. (1988) clarified that the response to salt stress is proved by losing of water and turgor pressure which result in a remarkable reduction in growth rate. This reduction in growth might be associated with the disorders in the metabolic processes (enzymes activities, proteins biosynthesis, mitochondria and chloroplast activities) (Mengel and Kirkby, 1987). Gunes et al. (1996) emphasized that the reduction of plant growth under salt stress conditions might be due to the accumulation of some ions in plant tissues specially sodium and chloride, another suggestion is the imbalance between organic and inorganic compounds

under salinity conditions. Greenway (1963) attributed the reduction in shoots and roots length under salt stress conditions to the accumulation of soluble ions and/ or to the changes occur on cell ions. In salt stressed pepper (*Capsicum annuum* L.) plants, the reduction of shoots length was due to the reduction in RNA, DNA and protein synthesis (Streb and Feierabend, 1996). Moreover, Dunlap and Binzel (1996) confirmed that the increment of NaCl levels cause a decrease in IAA level in salt stressed tomato (*Solanum lycopersicum* L.) plants and cause changes in IAA metabolism consequently affect the shoots and roots length. Hormonal signals originating from the roots probably regulate this decline in growth (Munns, 2002). Furthermore, Nassar et al. (1999) attributed the reduction in shoots and roots length in salinity stressed plants to the obstacles in water absorption even in muddy soils.

Salinity has a direct effect on most of the metabolic processes and growth; it causes reduction in dry weight via, decrement of water potential in the culture soil and prevention of water absorption by plants roots. The high rate of some specific ions which might have toxic effects and cause many physiological disorders (Simpson, 1981).

The reduction of fresh weight in salt stresses plants might be due to the reduction of Adenosine triphosphate (ATP), Uridine diphosphate glucose (UDPG) and Uridine triphosphate (UTP) (Nieman et al., 1988).

Gill et al. (2001) noticed significant increase in total soluble sugar, reducing sugar content in response to various abiotic stresses in different parts of sorghum (*Sorghum* L.) seedlings. The accumulation of total soluble sugars under salt condition could be attributed to the fact that seed carbohydrate metabolism under stress conditions can be considered as a dynamic process involving concomitantly occurring processes of

polysaccharide degradation and synthesis of new compounds and nevertheless, some researchers agree that salinity and water stress induce soluble sugar accumulation (Wang and Stutte, 1992; Kameli and Losel, 1995).

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

Aledesuquy (2000) noticed that the chemical and biological changes of NaCl accumulation in salt stressed tobacco (*Nicotiana tabacum* L.) tolerant lines are due to the accumulation of sugars and other solutes. Such accumulation increases the osmotic potential, preserve the turgor pressure, and consequently, increase plant tolerance for salt stress. It is useful physiologically to have an increase in carbohydrate content, as this would indicate that growth is still continuing and the cultures have not reached stationary phase yet, on the other hand, decrease in carbohydrate content in stressed cells might indicate a decrease in growth rate (Potluri and Prasad, 1993). In addition, Aledesuquy et al. (1998) suggested that reducing sugars and other solutes contributed more to the solute potential and therefore maintained turgor leading to tolerance. Proline concentration in mango (*Mangifera indica*) leaves increased by increasing chloride salts of Na, Ca and Mg at a ratio of 3:1:1,

respectively from 1000-4000 ppm in the irrigation water (Abd El-Karim, 1991). 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 (Hasegawa et al., 2000; 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). Zlatev and Stotanov (2005), suggested that proline accumulation of plants could be only useful as a possible drought injury sensor instead of its role in stress tolerance mechanism. However, Vendruscolo et al. (2007) found that proline is involved in tolerance mechanisms against oxidative stress and this was the main strategy of plants to avoid detrimental effects of water stress. Higher proline content in wheat (*Triticum* spp) plants after water stress has been reported by Vendruscolo et al. (2007) and Poustini et al. (2007). Rao and Rao (1979) observed increase of some acids (glutamic, aspartic, alanine and proline) in salt stressed *Arachis hypogaya* L. such result might be attributed to the slight increment of total amino acids in salt tolerant and salt sensitive lines and the significant increase of each amino acid (specially proline). Sensitive lines however, show considerable changes in amino acids content. In this concern, Priebe and Jager (1978) observed remarkable decrease in glutamic and aspartic acids. Moreover, free proline was increased in tolerant salt lines of wheat (*Triticum* spp.) and barley (*Hordeum vulgare* L.) plants and decrease in sensitive salt lines of both species (wheat and barley). In contrast, Willadino et al. (1996) observed that total amino acids in salt stressed maize (*Zea mays* 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. Proline content under salt stress conditions might associate with the series of proline analysis (Das et al., 1990). Proline accumulation under salt stress conditions might be attributed to the direct induction of proline bio-synthesis via activation of proline – 5 – carboxylate reductase enzyme and reduction of proline dehydrogenase enzyme during salt stress conditions (Delauney and Verma, 1993). High content of proline in salt stressed wheat plants did not negatively affect the enzymes activities, but it protects the enzymes set and the cell walls from the saline factors (Hanafy et al., 2002).

Concerning the reduction of K content under salt stress conditions, Nitsos and Evans (1969) suggested that potassium acts as activator element necessary for starch biosynthesis or implicates in many steps of proteins biosynthesis series (Evans and Wildes, 1971). Another explanation is the direct competition between sodium and potassium ions followed by the antagonism between both ions in the plasma membrane at the ion absorption site (Epstein and Rains, 1987). The osmotic adjustment can usually be accounted for by an increase in concentration of a variety of common solutes, including sugars, organic acids and ions especially K^+ (Taiz and Zeiger, 1991). The adverse correlation between K and Na ions refer to the antagonism between these two ions. Such antagonism might lead to a reduction in K concentration under saline conditions (Hu and Cramer, 1993). Moreover, Hanafy et al. (2002) supposed that the increase of sodium decrease the potassium concentration in salt stressed wheat (*Triticum* spp.) plants. This reduction might be due to that presence of sodium ions diminish the absorption of potassium ion and inhibit enzymes activities. The increment of sodium ion concentration in salt stressed

plants might considered as a result for plant ability to utilize Na ion and adjust the osmotic potential between plant tissue and outside atmosphere (Glenn, 1987). This osmotic adjustment is necessary for plant maintenance under salt stress condition (Flowers et al., 1977). In addition, Aly (1987) clarified that sodium is a prevailed ion in case of growing plants in a salty soils. 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, osmotic adjustment (Chaven and Karadge, 1980). Barakat et al. (1982) studied the effect of NaCl, Na_2CO_3 , Na_2SO_4 and their combinations and concentrations of 40 and 80 meq/l on minerals content of guava (*Psidium guajava*) seedlings. Leaves content of Na and Cl concentrations increased gradually with increasing of salinity levels. The increase in chloride concentration might cause increment in osmotic potential and / or toxicity, followed by reduction in water absorption and growth inhibition (Smith et al., 1983). Such retardants in growth might be attributed not only to direct effect of chloride in decrement the absorption rate of phosphor ion but also to the reduction of P transportation rate to the upper parts of plant (Ashour et al., 1970). In addition, chloride causes a reduction in nitrates content in salt stressed wheat (*Triticum* spp.) plants via inhibition of nitrates absorption rate (Botella et al., 1993). 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). Sodium accumulation in the vacuoles rather than in the cytoplasm and apoplasmic space in the root cells of the soybean (*Glycine max*) plants, leading to the difficulty in the transport of Na to leaves in soybean. Salt injury of soybean (*Glycine max*) is considered to be caused by the accumulation of Cl at high concentrations in all the compartments of root and leaf cells. In contrast,

the accumulation of Na in the cytoplasm of the root and leaf cells might disturb the metabolism and lead to the occurrence of salt toxicity in cucumber plants, which are

tolerant to Cl due to the stimulation of Cl accumulation in vacuoles when the Ca concentration was high in nutrient media (Dabuxilatu and Ikeda, 2005).

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