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Influence of Salinity on Growth and Physiology of *in Vitro* Grown Cucumber (*Cucumis Sativus L.*).

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ABSTRACT

Growth and physiological traits of cucumber (*Cucumis sativus L.*) were studied under salt stress *in vitro*. Microshoots were grown on Murashige and Skooge (MS) solid proliferation media containing 2. 89 μM GA_3. Salinity was induced by incorporating 0. 0, 50, 75, or 100 mM of NaCl to the culture media. Microshoots were exposed to direct and gradual shock of salinity. Gradual salt shock was induced by gradually transferring microshoots every week to different NaCl concentration from 0. 0 to 50 to 75 to 100 mM (starting from the control and ending with 100 mM NaCl). Growth parameters (shoot length, fresh shoot weight, dry shoot weight, root length, and root number) were generally reduced with elevated salt level in the direct and gradual salt shock. This reduction was less impaired in the gradual salt shock treatment. Root length was enhanced at 50 mM NaCl in both salt shock treatments. Leaf osmotic potential was significantly reduced (more negative) with increasing salinity. Na concentration was increased in salinized microshoots, K and Ca were reduced with elevated salinity and this reduction was less pronounced in the gradual salt shock. Increasing salt stress significantly reduced N to similar levels in the direct and gradual salt shock treatments. K/Na ratio decreased in salinized microshoot as compared with control and gradually microshoot showed higher K/Na ratio than directly salinized microshoot. Increased NaCl level slightly increased P and Mg concentration in the gradually salinized microshoots and significantly decreased P and Mg concentration in the directly salinized microshoots. Increasing salinity increased ash percent and reduced soluble protein percent, fat, and fiber in microshoots under both salt shocks. Moisture percent significantly decreased in directly salinized microshoots whereas it was maintained constant in gradually salinized microshoots. Carbohydrates content decreased at 50 and 75 mM NaCl, but increased at 100 mM NaCl. Elevated salinity significantly reduced microshoot crude protein content and increased proline and reducing sugars content. Microshoots accumulated higher moisture, reducing sugars, soluble protein, crude protein, proline and lower fat and carbohydrates when gradually salinized than when directly exposed to salt stress.

KEYWORDS: Cucumber, *in vitro*, mineral uptake, salinity.

INTRODUCTION

Salinity at present is one of the most serious environmental stresses influencing crop growth and productivity (Al-Karaki, 2000; Hilal *et al.* , 1998; Lopez *et al.* , 2002). It is affecting fully one-third of the irrigated land on the earth (Cano *et al.* , 1998; Satti and Lopez, 1994). Salinity is prevalent in both irrigated areas when farmers irrigate crops using poor quality water and practice poor irrigation management, and in dry areas when precipitation is not enough to remove excess soluble salt from the soil (Mohammad *et al.* , 1998; Shibli, 1993).

Cucumber is a high-yielding greenhouse crop. Under optimal cultivation condition, a cucumber plant can produce 19 Kg of marketable fruits after 19 weeks of growth (Adams and Winsor, 1992). Cucumber has been

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classified as moderately salt sensitive, since the best growth and fruit development occur at low salinity (Mass and Hoftman, 1977). The growth of greenhouse cucumber was reported to be influenced significantly using salinities above 1. 3 dS/m and the yield decreased by 15. 9% for each unit of increase of EC of irrigated water above 1. 3 dS/m (Chartzoulzkis, 1992).

Plant tissue culture provides useful information to elucidate plant response to salt stress (Shibli *et al*. , 1992). Such system offers greater control than *in vivo* growth conditions and has the advantage of small scale with clear visibility for monitoring shoot and root responses in the presence of imposed stress (Shibli *et al*. , 1992). The response of *in vitro* stressed cultures mimics the *in vivo* stressed plants (Swwan *et al*. , 2000). *In vitro* culture offers also a mean to focus exclusively on physiological and biochemical processes which contribute to salinity tolerance (Rus *et al*. , 1999; Shibli and Aljuboory, 2002).

This study was conducted to study the growth and physiological responses of *in vitro* grown cucumber under induced salinity.

Materials and Methods

Establishment of *In Vitro* Mother Stock Cultures

In vitro cultures of cucumber QS1034 (product of Holland, S&G Company, Pr; 758341/02-03) were established from seeds. Seeds were sterilized with 70% alcohol for 30 seconds, followed by 3. 5% sodium hypochlorite for 15 minutes and then rinsed with sterile distilled water for 3 times (each time for 15 minutes) under a laminar air-flow cabinet. Surface sterilized seeds were germinated on half strength MS medium (Murashige and Skoog, 1962). The pH of the medium was adjusted to 5. 8 using 0. 1 N NaOH and 0. 1 N HCl, solidified with 8. 0 g/L Bacto agar, dispensed in test tubes (8 ml/ test tube) and autoclaved at 121 °C and 1. 5 Kg/cm pressure. Cultures were maintained in the growth chamber (22- 2 °C), under 16 hr light (photosynthetic photon flux 40-60 mol m-□-1/ 8 hr dark). Shoot tips were excised aseptically and were inoculated on MS medium containing 30 g/L sucrose, 9. 30 μM kinetine for 3 weeks. Subcultures were performed for three times until a massive mother stock of microshoots had become available before initiating experiments.

Microshoot Physiological Responses to Salt Stress

NaCl levels of 0. 0 (control), 50, 75, and 100 mM were incorporated to 40 ml solid MS proliferation media containing 2. 89 μM GA_3 in 250 ml erlenmeyer flasks. Microshoots were directly subcultured to the salt levels. In another treatment, microshoots were gradually exposed to NaCl salinity stress by subculturing microshoots on solid proliferation media without NaCl (control). After one week, five flasks were retained and all other microshoots were subcultured on medium containing 50 mM NaCl. After one week, five flasks were retained at 50 mM and others were transferred to media containing 75 mM NaCl. Similarly, and after one week, five flasks were retained at this level while others were transferred to media containing 100 mM NaCl. After three weeks from the last subculture, cultures from the different salt levels were used for data collection. Shoot height, number of roots, root length, and number of roots (% of control) (Mercado *et al*., 2000), and fresh and dry shoot weight were recorded after six weeks. Treatments were arranged in a Completely Randomized Design (CRD) with three replicates (a flask in each replicate). Each flask contained four microshoot cultures.

Tolerance index was determined as fresh weight on salt medium/ fresh weight on control x100 (Cano *et al*. , 1998). Osmotic potential of leaf cell sap samples was measured using a Wescore-5500 Vapor Pressure Osmometer. Shoots were dried to a constant weight at 70 ˚C and then grounded using a small mortar to pass 1. 0 mm sieve size. Then sample of 0. 5g of well-mixed, dry, and ground plant material was weighed and ignited in Muffle Furnace at 550 ˚C and the ash was dissolved in five ml of 2N HCl for determination of Na, K, P, Ca, and Mg. Sodium and potassium concentrations were analyzed

using flame photometer (Corning, M410). Calcium and magnesium concentrations were determined using atomic absorption spectrophotometer (Elmer-Perkin, 2380). Phosphorus concentrations were analyzed using Vanadate-Molybdate Yellow method by Spectrophotometer (Spectronic 20 D) (Chapman and Pratt, 1961). Analysis of total nitrogen was performed using Micro-Kjeldahl digestion procedure and crude protein content was determined by multiplying the total nitrogen by a factor of 6. 25. (Bulman and Smith, 1993). Total water-soluble protein content was determined according to Lowery *et al*. (1951) by spectrophotometer at wavelength of 750 nm. Proline concentration was determined using a standard curve and calculated on fresh weight basis as described by Bates *et al*. (1973). Moisture, ash, crude fat, crude fiber, reducing sugars, and total carbohydrate were analyzed according to AOAC (1984).

Statistical Analysis

Statistical analysis was performed as two factor arrangement in a Completely Randomized Design (CRD), with three replications. Collected data were subjected to Analysis of Variance (ANOVA) using MSTATC program (Michigan State Univ. , East Lansing, MI). Probability of significance was used to indicate significant treatments and interaction effects and means were separated according to the Least Significant Difference (LSD) at 0. 05 levels of probability.

Results and Discussion

Growth and Osmotic Potential

Increasing salinity level decreased most growth parameters (shoot fresh weight, shoot dry weight, and shoot height). The reduction in shoot fresh weight was less aggravated in gradual shock salinity treatment than direct shock treatment and was more pronounced at 75 and 100 mM (Table 1). Microshoot height and dry weight (Table 2) did not differ when microshoots were exposed either directly or gradually to salt stress; the differences were only noticed among salt levels. The changes in fresh and dry weights of cucumber microshoot accounted by salinity were similar and in agreement with those described previously (Al-Harbi, 1995; Lecheno et al. , 1997). However, Shibli et al. (2000) reported that microshoot fresh weight significantly increased in Galla and M 26 when salinized at 50 mM NaCl, and a slight decrease in fresh weight was obtained in all microshoots when gradually salinized at 100 mM NaCl. Chartzoulakis (1992) reported that cucumber shoot height showed an average reduction of 20, 54, and 80% with salinity levels of 2. 7, 5. 0, and 10. 7 dS/m, respectively. Reduction in growth with increasing salinity could be attributed to salinity induced water deficit (Satti and Lopez, 1994), ion toxicity associated with excessive uptake particularly of Na^+ and Cl^- (Satti and Lopez, 1994), and nutritional imbalance as a result of depressed uptake, shoot transport and impaired internal distribution of minerals especially K^+ and Ca^+2 (Munns, 1993).

Root length at 50 mM NaCl was increased significantly as compared with the control, while further increase in salinity level resulted in significant reduction in root length in both salt shocks (Table 1). Microshoot showed higher root length when gradually salinized than when directly salinized. Unlike the present result, significant decrease in root length with increased salinity level was detected in cucumber seedling by Chartzoulakis (1992) and Huan Wen *et al*. (1999). Shibli and Al-Juboory (2002) reported that increasing salinity more than 75 mM NaCl significantly decreased root length of Nabali-olive microshoot. Cucumber microshoot showed significantly lower root number (Table 2) and rooting percent (Table 3) as salinity level increased; gradually salinized microshoots showed significantly higher rooting than directly salinized microshoots. Reduction in rooting percent with increased salinity was reported in other crops (Cano *et al*. , 1998; Shibli *et al*. , 1998; Smith *et al*. , 1992).

Salt tolerance, as depicted by tolerance index was decreased significantly with increased salinity level in

both salt shock treatments (Table 1). Microshoots showed significantly higher tolerance index when gradually salinized at 75 and 100 mM NaCl than when directly exposed to salt stress. According to Cruz *et al*. (1990), the effect of salinity on plant was expressed as reduced shoot biomass; therefore vegetative growth is the most widely used index in studies on salt tolerance. On the other hand, Cano *et al*. (1998) reported that the different degree of salt tolerance between *Lycopersicon esculentum* and *L. pennellii* was not clearly shown on the basis of shoot growth of plantlets.

Cucumber microshoots showed a significant decrease (more negative) in leaf osmotic potential as salinity level increased in both salt shock treatments; reduction was more pronounced for the gradually salinized microshoots (Table 3). Leaf osmotic potential was reported to decrease (more negative) with increased salinity stress in tomato (Mohammad *et al*. , 1998; Smith *et al*. , 1992). Reduction in medium osmotic potential could be a major factor for the reduction of growth and mineral content of the plant tissues (Shibli *et al*. , 1992; Smith *et al*. , 1992). Halophytic as well as glycophytic plants species adjust to high salt concentration by lowering tissue osmotic potential with increased uptake of solutes (Greenway and Munns, 1980). The decrease in the osmotic potential of plants grown in salt stress may result from water stress or an increase in dissolved solutes or combination of both (Ashraf, 1989).

Inorganic Component

Microshoot Na concentration was increased significantly with increasing salinity level. However, there was significantly lower Na accumulation in microshoots treated insubjected to gradual salinity shock under all salt levels compared with those of the direct shock (Table 4). Accumulation patterns of Na observed in this study was similar to those reported previously in cucumber shoots (Al-baker, 2001; Al-Harbi *et al*. , 1993; Al-Harbi, 1995; Chartzoulakis, 1992) and in other plant species (Ali *et al*. , 1993; Al-Karaki, 2000; Shibli *et al*. , 1998). It has been reported that Na is not considered an essential element for plant growth, and plants accumulate Na at the expense of K^+ and Ca^{+2} in saline conditions (Cramer *et al*. , 1985). Increasing the Na content disturbs the nutrient balance, osmotic regulation, and causes specific ion toxicity (Gunes *et al*. , 1996).

The increase in salinity level significantly decreased microshoot K content when directly salinized at 75 and 100 mM NaCl, whereas it had no effect in gradual shock treated microshoots (Table 4). However, when salt levels increased, the microshoots treated in gradual shock salinity accumulated more K than in the direct shock salinity. This agrees with Al-Harbi (1995) findings where increasing salinity level significantly reduced K content in cucumber shoots. Also, the increase of NaCl level was reported to be accompanied by corresponding reduction in K concentration (Al-Karaki, 2000; Essa, 2002; Knight *et al*. , 1992; Shibli *et al*. , 2001). The reduction in K content with increasing salinity could have resulted from competition between Na and K on absorptive sites of the plant roots (Ali *et al*. , 1993; Cordovilla *et al*. , 1995; Essa, 2002; Shibli, 1993). According to Greenway and Munns (1980), the reduction in K concentration could inhibit growth by reducing capacity for osmotic adjustment and turgor maintenance or by adversely affecting metabolic functions.

Reduction in microshoot P concentration when directly salinized was significant only at 100 mM NaCl, whereas the increase in microshoot P content when gradually salinized was significant at 50 mM NaCl (Table 4). Generally, microshoot accumulates more P when gradually salinized than when exposed directly to salt stress. This agrees with earlier findings (Shyab *et al*. , 2003) where the percentage of P of sour orange microshoot was reduced with elevated salinity with reduction less aggravated in the gradual shock experiment. Data on reduced shoot P content with increased NaCl were in agreement with results of others who reported that NaCl reduced P transport and uptake in many plants (Al-Karaki, 1997, Shibli et al. , 1998; Shibli

et al. , 2001). The decrease in shoot P associated with increased NaCl level might be attributed to low $H_2PO_4^{-2}$ activity; the preferred phosphate form for plant uptake (Al-Karaki, 1997).

The increase in salinity level significantly decreased Ca concentration at 75 and 100 mM NaCl in direct shock treated microshoot, whereas the decrease in microshoot Ca concentration was not significant when gradually salinized (Table 4). However, when salt levels increased, the microshoot accumulated more Ca in the gradual salinity shock than in the direct shock. It has been previously reported that the decrease in Ca concentration in salinized cucumber shoots was not significant (Al-Harbi, 1995). Knight *et al.* (1992) reported an intermediate proportional increase in leaf Na and decrease in Ca content to occur with elevated NaCl levels. Also, reduction in Ca concentration was observed in leaves of celery (Paradossi *et al.* , 1999), guava (Ebert *et al.* , 2002), and soybean (Essa *et al.* , 2002) under salinity treatments. It has been shown that increasing concentration of NaCl causes progressively greater displacement of Ca from cell membrane and disturbs intracellular Ca concentration (Cramer *et al.* , 1985).

The increase in salinity level significantly decreased microshoot Mg contents when directly salinized, whereas Mg concentration increased in the gradually salinized microshoots (Table 4). Cucumber microshoots showed significantly higher Mg concentration when salinized gradually than when exposed directly to salinity. An increase in leaf Mg concentration under salt stress was reported in soybean (Essa *et al.* , 2002) and in tomato (Perez-Alfocea *et al.* , 1996). Shibli *et al.* (1998) reported reductions in shoot Mg content with increased NaCl salinity in hydroponic grown tomato. The decrease in Mg absorption is responsible for decreased chlorophyll content (Leidi *et al.* , 1991). However, the reduction in the content of this macronutrient induced by salinity does not appear to be responsible for growth inhibition in faba bean plants (Cordovilla *et al.* , 1995).

Salinized cucumber microshoot showed significantly lower K/Na ratio as compared to the control in both salt shock treatments (Table 5). Higher K/Na ratio was obtained in the gradual salt shock treatment. Similar result was reported in cucumber shoot (34). Reduced K/Na was obtained in tomato (Al-Baker, 2001). Reduced K/Na was obtained in tomato (Al-Karaki, 2000), soybean (Essa *et al.* , 2002), and celery (Paradossi *et al.* , 1999) under salt stress. Increasing Na content and decreasing K/Na ratio in plant leaves could be attributed to competition between Na^+ and K^+ ions on the absorptive sites of the plant roots (Essa *et al.* , 2002). Salt tolerance in some plant species has been related to exclude Na from leaves and maintain higher K/Na ratio in plant tissues (Creda *et al.* , 1995; Perez-Alfocea *et al.* , 1993).

Regardless of the salt shock method, a significant decrease in cucumber microshoot N content was obtained when stressed with NaCl compared to the control (Table 5). This decrease was more aggravated at the 100 mM NaCl compared with the 50 and 75 mM levels, which were similar. In contrast, Al-Harbi (1995) found that there was no consistent salinity effect on N concentration in cucumber shoot. Pardossi *et al.* (1999) reported that salt stress hardly affects the content of N, which decreased significantly in celery leaves at 300 mM NaCl. Similar reduction in shoot N content was observed in hydroponic and *in vitro* grown tomato (Flores *et al.*, 2001; Shibli *et al.*, 1998; Al Rawahy *et al.* , 1992; Ali *et al.* , 1993). The decreased N content of plants by salinity could be explained by the ionic interference with NO_3^- translocation across the root plasma membrane (antagonism between Cl^- and NO_3^-) (Ali *et al.* , 1993; Aslam *et al.* , 1984; Gunes *et al.* , 1996). Ali *et al.* (1993) attributed the reduced tomato growth to the decreased N uptake under saline conditions.

Chemical Analysis

Moisture

Percent moisture was significantly decreased in microshoots when exposed directly or gradually to salt stress (Table 6). However, the gradually salinized microshoot had significantly higher moisture content than

the directly salinized microshoot. This agrees with previous results of Al-Harbi (1993) where cucumber water uptake was significantly reduced under the condition of high salt concentration (8. 5 mS/cm) in the nutrient solution. Huan-Wen *et al*. (1999) reported that water content in tissues of cucumber seedlings increased slightly under low salt concentration (100 mmol/l) and decreased at higher concentrations. According to Khan *et al*. (1999), when succulence at low salt concentration was increased, the plant *(Halopyrum mucronatum L*. Stapf) achieved more positive turgor and had a substantial promotion of growth; with further increase in salinity, the water content of plant decreased leading to turgor loss with consequent inhibition of growth. Increased uptake of Na^+ and Cl^- resulted in a decrease of cell water potential, thus promoting water uptake (Khan *et al*. , 1999).

Protein Content

NaCl significantly reduced microshoot content of crude protein at 75 and 100 mM NaCl in both salt shock treatments (Table 6). The reduction in salinized microshoot crude protein content was less aggravated in the gradual salt shock than the direct salt shock treatment. Reduction in crude protein content was reported in rice leaf (Lutts *et al*. , 1996), rice grain (Sultana *et al*. , 1999) and in *Coleus blume* tissues (Gilbert *et al*. , 1998) in response to salinity. Lower level of protein in salt stressed plant parts is a result of the decreased synthesis of protein as well as increased activities of protein hydrolyzing enzymes (Pessarakli and Tucker, 1994).

Each increase in salinity level resulted in a significant decrease in leaf soluble protein. The gradually salinized microshoots showed significantly higher soluble protein content than the directly salinized microshoots (Table 7). This agrees with other results obtained in cucumber seedling (Hun-Wen *et al*. , 1999), rice leaf (Luttus *et al*. , 1996), and cowpea leaf (Silveira *et al*. , 2001) under salt stress. Similarly, Abu-Khadijeh (2002) reported that the leaf soluble protein content was significantly reduced as salinity level increased in hydroponic and *in vitro* grown tomato. The progressively lower leaf content of soluble protein in salt stressed cowpea was correlated with low Nitrate Reductase activity (NR) and could account for a decline in plant growth (Silveira *et al*. , 2001).

Fat Content

Regardless of the salt shock method, fat percent of microshoot decreased significantly for each increase of salt level up to 75 mM (Table 7). Significantly, lower fat content was found in the gradual salinized microshoot. Phospholipid content of bean roots was found to decrease or to remain unchanged as a consequence of saline treatment (80 mM NaCl) (Cachorro *et al*. , 1993). On the other hand, roots of salt-treated *Chloris gayanakunth* accumulated more concentration of reserve lipids (Cordoba *et al*. , 2001). Several reports have suggested that lipids might be involved in the protection against salt stress (Huflejt *et al*. , 1990; Ritter and Yopp, 1993). It has been reported (Luttus *et al*. , 1996) that membrane alteration recorded after short-term exposure to salt stress could be ascribed to peroxidation of unsaturated fatty acids rather than to membrane protein modification. Also, it is possible that the first stage of anion uptake is its penetration to the lipid fraction of the root membrane (Al-Rawahy *et al*. , 1992). Therefore, different degrees of resistance of various plants to salinity might be due to the specific lipid composition of their roots (Kafkafi, 1987).

Fiber Content

Fiber percent of microshoot decreased significantly with increasing salt stress as compared with the control. No significant difference was found between the gradual and the direct salt shock (Table 7). This is in agreement with Fowler *et al*. (1992) who reported that salinity stress decreased fiber concentration in Russian thistle, thereby, digestibility and quality were improved.

Ash Content

Irrespective of salt shock method, percent ash content of microshoots increased significantly for each salt level

up to 75 mM (Table 7). Ash content was similar for the gradual or the direct salt shock. This agrees with the result obtained by Shibli *et al*. (1998) who reported a significant increase in ash percent with the increased salinity level in hydroponic grown tomato. Also, ash content was increased with imposed salinity on *in vitro* grown African violet (Shibli *et al*. , 2001).

Total Carbohydrate Content

Increased NaCl significantly reduced microshoot carbohydrates content at 50 and 75 mM level, whereas at 100 mM carbohydrate content was significantly increased in both salt shock treatments (Table 8). Microshoot accumulated significantly less carbohydrate when gradually salinized at 100 mM NaCl than when directly salinized. This agrees with Dell-Amico *et al*. (1998) who reported that the total carbohydrate content was the highest in roots and leaves of tomato seedling treated with 100 mM NaCl which showed the poorest growth. The increase in total carbohydrate was reported recently in salt stressed wheat seedling grown in hydroponic culture (Kerepesi and Galiba, 2002). Carbohydrates are the source of energy and carbons needed for adaptive and /or defensive responses to stresses (Todaka *et al*. , 2000). Noiraud *et al*. (2000) reported that carbohydrate accumulation under salt stress is the result of an increase of carbohydrate synthesis and a decrease in carbohydrate catabolism. The general accumulation of carbohydrate could be the result of a decreased demand for energy as growth of celery plant was reduced under salt stress (Noiraud *et al*. , 2000).

Reducing Sugars

Reducing sugar content was significantly increased in the gradually and directly salinized microshoot with elevated salt level. Significantly higher accumulation was noticed in the gradually salinized microshoot (Table 8). This result is in agreement with the results reported by Kerepsi and Galabia (2002) and slightly different from those reported by Dell-Amico *et al.* (1998) who found that leaf content of reducing sugars was the highest in tomato seedlings treated with 50 mM NaCl solution. The increase of reducing sugar levels in the leaves of the hydroponic grown tomato plant by salinity could correspond to a general response to salinity (Flores *et al*. , 2001), whereby osmotic potential of the leaves is increased in order to achieve the necessary osmotic adjustment (Carvajal *et al*. , 1998).

Proline Content

Leaf proline content was significantly increased in the gradually and directly salinized microshoot with elevated salt level, where higher accumulation noticed in the gradually salinized microshoot (Table 8). A similar result was previously reported in leaves of cucumber seedlings (Huan-Weng *et al*. , 1999). Dell-Amico *et al*. (1998) reported that the highest proline accumulation was in the leaves of tomato seedlings treated with 100 mM NaCl. Accumulation of proline in plant tissues as a response to salt stress has been attributed to enhanced activities of the enzyme involved in proline biosynthesis (Charest and Pan, 1990) and to the inhibition of proline oxidase a proline catabolizing enzyme (Yoshiba *et al*. , 1997). Proline could be an adaptive response through decreasing osmotic potential in the cytoplasm avoiding water loss and /or enzyme stabilization (Gunes *et al*. , 1996; Pereze-Alfocea *et al*. , 1996).

CONCLUSIONS

Results indicate that growth of cucumber microshoots was adversely affected by gradual and direct salinity shock. Salt stress significantly reduced shoot growth (shoot length, fresh weight, and dry weight) and root growth (root length and root number). This reduction was less aggravated in the gradual shock treatment. Gradually salinized microshoots maintained constant K and Ca concentration and increased P and Mg concentration, while directly salinized microshoots showed a decrease in K, Ca, Mg and P concentration. Greater Na exclusion and higher ion accumulation of K, P, Ca, and Mg are suggested as an osmoregulation mechanism required for

salt tolerance. Increases in content of carbohydrates, reducing sugar and proline could correspond to a general response to salinity that is: increasing the osmotic potential of leaves in order to achieve the necessary osmotic adjustment. Evaluating salt stress tolerance on the basis of fresh matter accumulation, gradually salinized microshoots seem to be more salt tolerant than directly salinized microshoots. Salt stress tolerance was achieved by physiological and biochemical mechanisms as evidence by accumulation of more inorganic and organic matter which all contribute to more effective osmotic adjustment. Cucumber cultivar Q. S. 1034 could tolerate salinity up to 75 mM NaCl, therefore it can be planted in slightly saline soils.

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Table 1. Interactive effects of salinity level and salt shock method on shoot fresh weight, root length and tolerance index of *in vitro* grown cucumber (*Cucumis sativus* L.).

Salt Shock Methods	Salinity Level (mM)	Shoot Fresh Weight (gm)	Root Length (cm)	Tolerance Index
Control	0	11. 26 a*	10. 6 c	100 a
Direct Shock	50	7. 27 b	11. 63 b	64. 54 b
	75	4. 58 de	6. 83 e	40. 70 de
	100	3. 94 e	4. 21 g	34. 96 e
Gradual Shock	50	7. 60 b	12. 74 a	67. 46 b
	75	6. 01 c	8. 40 d	53. 41 c
	100	4. 80 d	5. 77 f	42. 66 d

* Means within columns having different letters are significantly different according to LSD at 0. 05 level.

Table 2. Separate effect of salinity level on dry weight, shoot height and root number of *in vitro* grown cucumber (*Cucumis sativus* L.).

Salinity Level (mM)	Shoot Dry Weight (gm)	Shoot Height (cm)	Root Number
0	0. 61 a*	13. 0 a	8. 33 a
50	0. 48 b	8. 19 b	7. 17 a
75	0. 41 c	5. 67 c	5. 50 b
100	0. 30 d	4. 16 d	3. 67 c

* Means within columns having different letters are significantly different according to LSD at 0. 05 level.

Table 3. Separate effects of salinity level and salt shock method on rooting % and leaf osmolarity of *in vitro* grown cucumber (*Cucumis sativus* L.).

Main effect	Rooting (%)	Osmotic Potential (Bar)
Salt shock methods		
Direct salt shock	69. 02 b*	- 8. 28 b
Gradual salt shock	79. 02 a	- 8. 58 a
Salt Level (mM)		
0	100 a	- 7. 22 d
50	86. 03 b	- 8. 21 c
75	66. 03 c	- 8. 72 b
100	44. 02 d	- 9. 59 a

*For each separate effect, means within columns having different letters are significantly different according to LSD at 0. 05 level.

Table 4. Interactive effects of salinity level and salt shock method on microshoot content of Na, K, P, Mg, and Ca of *in vitro* grown cucumber (*Cucumis sativus* L.)

Salt Shock	Salt Level	Na	K	P	Ca	Mg
Control	0	0. 41 f*	4. 83 a	0. 53 bc	0. 15 ab	0. 13 c
Direct Shock	50	3. 73 c	4. 35 ab	0. 57 ab	0. 13 ab	0. 1235d
	75	4. 19 b	3. 83 b	0. 49 c	0. 07 cd	0. 1212e
	100	4. 87 a	3. 16 c	0. 43 d	0. 05 d	0. 1180f
Gradual Shock	50	2. 60 e	4. 69 a	0. 58 a	0. 16 a	0. 1452 a
	75	3. 25 d	4. 53 a	0. 56 ab	0. 13 ab	0. 145 a
	100	3. 61 c	4. 47 a	0. 56 ab	0. 12 bc	0. 1293b

* Means within columns having different letters are significantly different according to LSD at 0. 05 level.

Table 5. Separate effects of t salinity level and salt shock method on microshoot content of N and K/Na ratio of *in vitro* grown cucumber (*Cucumis sativus* L.).

Main effect	Trait	
	K/Na ratio	N (%)
Salt shock method		
Direct salt shock	3. 59 b*	6. 63 a
Gradual salt shock	4. 02 a	6. 87 a
Salt level (mM)		
0	11. 62 a	7. 18 a
50	1. 49 b	7. 15 b
75	1. 15 b	6. 77 b
100	0. 95 b	5. 92 c

* For each separate effect, means within columns having different letters are significantly different according to LSD at 0. 05 level.

Table 6. Interactive effects of salinity level and salt shock method on microshoot contents of moisture and crude protein of *in vitro* grown cucumber (*Cucumis sativus* L.).

Salt Shock Method	Salt Level(mM)	Moisture (%)	Crude Protein (%)
Control	0	94. 58 a*	46. 13 a
Direct Shock	50	93. 62 a	45. 12 a
	75	92. 98 b	42. 43 b
	100	91. 04 c	36. 44 d
Gradual Shock	50	93. 40 b	45. 77 a
	75	93. 16 b	45. 10 a
	100	93. 12 b	40. 10 c

* Means within columns having different letters are significantly different according to LSD at 0. 05 level.

Table 7. Separate effects of salinity level and salt shock method on microshoot contents of soluble protein, fiber, fat, and ash of *in vitro* grown cucumber (*Cucumis sativus* L.).

Main Effect	Trait			
	Soluble Protein (mg/g FW)	Fat (%)	Fiber (%)	Ash(%)
Salt Shock Method				
Direct salt shock	12. 47 b*	1. 18 a	10. 67 a	17. 09 a
Gradual salt shock	13. 21 a	0. 89 b	9. 88 a	17. 39 a
Salinity Level (mM)				
0	14. 14 a	1. 69 a	11. 86 a	11. 87 c
50	13. 30 b	1. 31 b	10. 93 ab	18. 24 b
75	12. 60 c	0. 62 c	9. 77 bc	19. 22 a
100	11. 46 d	0. 47 c	9. 44 c	19. 64 a

* For each separate effect, means within columns having different letters are significantly different according to LSD at 0. 05 level.

Table 8. Interactive effects of salinity level and salt shock method on microshoot contents of carbohydrate, reducing sugar, and proline of *in vitro* grown cucumber (*Cucumis sativus* L.).

Salt Shock Method	Salt Level (mM)	Total Carbohydrate(%)	Reducing Sugar (%)	Proline (Mol / g FW)
Control	0	23. 93 bc*	3. 31 d	0. 82 e
Direct Shock	50	19. 34 e	7. 33 c	1. 62 d
	75	23. 05 cd	10. 11 b	2. 25 c
	100	31. 41 a	11. 04 b	3. 18 b
Gradual Shock	50	19. 42 e	9. 57 b	2. 08 c
	75	21. 17 de	15. 11 a	3. 20 b
	100	26. 26 b	16. 41 a	4. 18 a

* Means within columns having different letters are significantly different according to LSD at 0. 05 level.

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تأثير الملوحة على نمو وفسيولوجيا الخيار (*Cucumis Sativus* L.) المزروع داخل الأنابيب

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ملخـص

تمت دراسة تأثير الإجهاد الملحي على النمو و الصفات الفسيولوجية لنبات الخيار(*Cucumis sativus* L.) المزروع داخل الأنابيب، حيث تمت زراعة النباتات على وسط غذائي لموراشيج و سكوج يحتوي على 1 مليغرام/لتر من حامض الجبرلين ومزود بالتراكيز الملحية صفر ، 50 ، 75 و100 mM من كلوريد الصوديوم. تم تعريض النباتات إلى الإجهاد الملحي التدريجي والمباشر. تمت دراسة الإجهاد الملحي التدريجي بواسطة نقل النباتات بعد كل أسبوع من الزراعة على مستوى ملوحة إلى المستوى الذي يليه. زيادة تركيز الملوحة أدت إلى نقصان مقاييس النمو(طول الساق، الوزن الرطب، الوزن الجاف ،طول الجذر، وعدد الجذور) وكان هذا النقصان اقل باستخدام تأثير الإجهاد الملحي التدريجي. زاد طول الجذر معنويا على مستوى الملوحةmM 50 تحت تأثير الإجهاد الملحي التدريجي والمباشر. الضغط الاسموزي في الأوراق النباتية نقص معنويا (زاد بالسالب) مع زيادة الملوحة. أثرت الملوحة على تركيز العناصر الغذائية داخل النبات، فقد زاد محتوى الصوديوم و نقص محتوى البوتاسيوم و الكالسيوم وكان هذا النقصان اقل باستخدام تأثير الإجهاد الملحي التدريجي. أدت زيادة تركيز الملوحة إلى نقص معنوي في محتوى النيتروجين ونسبة البوتاسيوم إلى الصوديوم. وزاد تركيز الفسفور و المغنيسيوم في النباتات المعرضة تدريجيا إلى الإجهاد الملحي ولكنه نقص في النباتات عندما تعرضت مباشرة إلى الملوحة. النسبة المئوية للرماد زادت بزيادة الملوحة ولكن النسبة المئوية للدهن والألياف والبروتين الذائب تناقصت. وزادت النسبة المئوية للرطوبة عند مستوى الملوحة 50 ,75 mM وقلت على مستوى الملوحة الأعلى. وقل محتوى الكربوهيدرات معنويا على مستوى الملوحة 50 ,75 mM وزاد على مستوى الملوحة 100 mM. و بزيادة الملوحة قل محتوى البروتين الخام في النباتات وزاد محتوى البرولين و السكريات المختزلة. احتوت النباتات على نسب أعلى من الرطوبة والبروتين الخام و البروتين الذائب والسكريات المختزلة والبرولين وعلى نسب اقل من الكربوهيدرات والدهن عندما عرضت تدريجيا للملوحة.

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