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GROWTH, NUTRIENT STATUS AND SOME OXIDASES ENZYME ACTIVITY OF CUCUMBER PLANTS AS AFFECTED BY SODIUM CHLORIDE SALINTY

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ABSTRACT

Growth and nutritional status as well as ascorbic acid oxidase and peroxidase activities in cucumber (*Cucumus sativus* L. var. Salprita) plants grown under NaCl salinity were studied. The growth was decreased with increase of NaCl level. Highly significant negative correlations were found between salinity level and the uptake of all determined elements except that of manganese (Mn) in the shoots. Under salinity stress conditions, potassium (K) and calcium (Ca) were found to migrate from roots to shoots, while iron (Fe) tends to accumulate in the roots and uptake of zinc (Zn) and copper (Cu) was impaired. Potassium and phosphorus (P) concentrations were dramatically decreased as the level of NaCl was increased, meanwhile magnesium (Mg) showed a slight decrease and Ca concentration even increased in the shoots. Micronutrient concentrations showed different trends as the salt concentration increased. Iron (Fe) was severely decreased in the shoots, zinc (Zn) was decreased in the roots, Copper (Cu) was decreased in both shoots and roots while manganese (Mn) was increased in the shoots. Nutrient concentration ratios were disturbed. P/K ratio was deviated negatively in the shoots and positively in the roots of salt treated plants as compared to control. However, K/Mg was deviated positively in the shoot and negatively in the root. K/Zn was negatively deviated in the root at high salt level and positively in the shoot under the low level. Fe/Zn was negatively deviated from control plants in the shoots and roots under low salt level and positively in the roots of the plants grown under the high level. Ascorbic acid oxidase and peroxidase activities in cucumber leaves were severely inhibited as the salt level increased in the root medium. Thus, activities of these enzymes can be used as indicators for salt stress conditions.

INTRODUCTION

Salinity is a wide spread problem in the arid and semi-arid areas. Presence of soluble salts in the plant growth medium affects the growth as inducing many physiological disorders within the plant tissues (Mengel and Kirkby, 1987, Munns, 1993; Cordovilla *et al*, 1995). Protein synthesis, carbon dioxide assimilation, respiration and enzyme activity were reported to affect by salinity levels of the growth medium (Helal and Mengel, 1979; El-Fouly and Jung, 1972; Mizrahi *et al*, 1970; Meiri and Shalhevet, 1973). Salinity may also affects nutrient uptake and translocation (Yahya, 1998).

Cucumber is one of the important vegetable crops. It is classified as sensitive to salinity, specially in the seedling stage where the plants are more sensitive (Lehman *et al*., 1984).

Available data revealed that balances of macro- and micronutrients within the plant tissues grown under saline stress conditions still in need for further investigations. In addition, rare studies on oxidases enzyme activity in plants grown under salinity stress were conducted.

The present paper aimed to study the effect of NaCl salinity on growth and nutrient status in cucumber plant tissues. Some oxidases enzyme activity in the leaves was also studied.

MATERIALS AND METHODS

A water culture experiment was conducted in the laboratory cultivation unit of the programme "Micronutrients and Other Plant Nutrition Problems in Egypt", National Research Centre, Dokki, Cairo, Egypt with cucumber plants (*Cucumus sativus* L. var. Salprita).

Germination and growth medium

Seeds were germinated for 4 days in dark at 20° C on filter papers moistened with 0.1 mM calcium chloride. Healthy and vigorous seedlings were transplanted and grown in Hoagland and Arnon (1950) nutrient solution using 1.0 L plastic vessels. Chemical composition of the nutrient medium is shown in Table 1.

Growth conditions

Plants were exposed to a light/dark cycle of 16 hours fluorescent light at 300-350 $\mu M.m^{-2}.sec^{-1}$. and 8 hours dark. Relative humidity in the growth chamber was adjusted at 50-60 % and temperature at 28-30° C. Media were continuously aerated using air pump.

Table 1: Chemical composition of Hoagland and Arnon (1950) nutrient solution

Salt	Full-strength nutrient solution	
	Cations (mg /L)	Anions (mg /L)
KNO_3	K = 391	NO_3-N = 140.08
$Ca(NO_3)_2.4H_2O$	Ca = 120.24	NO_3-N = 84.048
$Mg.SO_4.7H_2O$	Mg = 48.64	SO_4-S = 64.123
$NH_4H_2PO_4$	NH_4-N = 14.008	H_2PO_4-P = 30.975
Na-Fe-EDTA	Fe = 2.34	
H_3BO_4		B = 1.0
$MnCl_2.4H_2O$	Mn = 2.008	Cl = 2.60
$ZnSO_4.7H_2O$	Zn = 0.2	SO_4-S = 0.1
$CuSO_4.5H_2O$	Cu = 0.082	SO_4-S = 0.041
$Na_2MO_4.2H_2O$	Na = 0.02	MO = 0.043

Treatments

The treatments were carried out in 3 replicates as follows:

- Control: Hoagland and Arnon's nutrient solution
- Hoagland and Arnon's nutrient solution + 100 mM NaCl
- Hoagland and Arnon's nutrient solution + 200 mM NaCl

Chemical analysis

On the 2nd week, plants were collected. The 3rd mature leaf was used to determine ascorbic acid oxidase (Tono and Fujita, 1982) and peroxidase (Chance and Maehly, 1955) as specific activity (EU/mg protein). The plants were washed with tap water, 0.001 N HCl and bidistilled water. Shoots and roots were separated, oven dried at 70° C for 24 hours, and then weighed.

Plant materials were ground and dry-ashed in a muffle furnace at 550°C for 6 hours using 3.0 N HNO_3. The residue was then suspended in 0.3 N HCl.
Phosphorus was photometrically determined using the molybdate-vanadate method according to Jackson (1973). Sodium, K and Ca were measured using Dr. Lang M8D-Flamephotometer. Mg, Fe, Mn, Zn and Cu were determined using Atomic Absorption Spectrophotometer (Chapman and Pratt, 1978).
Data were statistically analyzed using Costate Statistical Package (Anonymous, 1989).

RESULTS AND DISCUSSION

Plant growth:

Fig. 1 shows that dry weight of the different plant organs was reduced as affected by NaCl salinity. With the treatment 100 mM, the plants may slightly coped with salt stress, however with 200 mM treatment, both shoot and root growth were significantly reduced. Under such conditions, CO_2 assimilation dropped down (Mengel and Kirkby, 1987). Because of the high respiration rate, plants grown under salt stress conditions were energy poor (Helal and Mengel, 1981). On the other hand, they depleted storage carbohydrate in a greater extent (Luettge *et al*., 1971) rendering low plant biomass content. Reduction of dry biomass may be also due to the inhibition of leaf water potential (Izzo *et al*., 1991).

Fig. 1: Dry weight of cucumber plants as affected by NaCl salinity levels in the growth medium

Nutrient status

- **Uptake**

(Table 2 shows that the uptakes of P, K, Mg, Ca, Fe, Zn and Cu were negatively correlated with the increase of NaCl level in the growth medium, whereas Mn uptake by the shoot was increased. This may be attributed to the plant osmoregulation to fulfil photosynthesis Mn-minimum requirements. Uptake of all nutrients other than Na with the treatment 100 mM was higher than those with the treatment 200 mM. Similar results were reported by Durand and Lacan (1994). Significant reduction in ratios over control was calculated for most of the studied elements (Fig. 2). Calcium uptake reduction

Fig. 2 : Nutrient uptake (increase/decrease % above and down control) by cucumber plants as affected by NaCl salinity in the growth medium

was higher in shoots which, suggest that most of Ca migrate to the shoot -as an osmoregulation- to resist salt detrimental effects on shoot cells. On the contrary, reduction of iron uptake was higher in shoots compared to roots, which suggest Fe accumulation in the roots because of the lake of the processes energize translocation (Helal and Mengel, 1981). Similar trend was found with Zn and Cu. High reduction in the nutrients uptake under high salinity levels can be because of the antagonism between Na and K, Ca, Mg and phosphate (Yahya, 1998). Depress of water potential cause by salt stress can also restrict passive uptake of nutrients (Mengel and Kirkby, 1987). Lack of energy in the plants suffering from salinity stress can also impair the active uptake process of the elements (Helal and Mengel, 1979).

Table 2: Nutrients uptake by cucumber plants as affected by NaCl salinity in the growth medium

Nutrients / Treatments	Macronutrients uptake (mg/g dry weight)					Micronutrients uptake (µg/g dry weight)			
	P	K	Mg	Na	Ca	Fe	Mn	Zn	Cu
Shoot uptake									
Control	5.11	30.4	1.68	1.25	6.37	78.5	10.7	39.3	8.22
100 mM	3.60	23.5	1.29	25.1	5.67	35.2	12.2	27.6	3.95
200 mM	2.60	19.67	1.04	34.8	5.82	25.1	12.9	27.2	2.23
Mean	3.77	24.45	1.33	20.36	5.62	46.25	11.95	31.37	5.13
± SD	1.26	5.42	0.32	17.25	0.23	28.37	1.15	6.89	3.89
R	-0.99	-0.97	-0.99	0.97	-0.88	-0.94	0.98	-0.87	-0.92
Root uptake									
Control	0.88	10.1	0.10	0.15	0.52	73.4	0.92	28.9	3.37
100 mM	0.75	3.46	0.06	0.74	0.29	62.4	0.72	26.8	1.88
200 mM	0.23	0.41	0.04	0.47	0.31	30.7	0.46	7.99	0.82
Mean	0.62	4.65	0.066	0.45	0.37	55.5	0.68	21.2	2.02
± SD	0.34	4.95	0.030	0.24	0.12	22.2	0.25	11.5	1.28
R	-0.96	-0.99	-0.98	0.539	-0.822	-0.96	-0.99	-0.91	-0.99
Total uptake									
Control	5.99	40.5	1.78	1.40	6.89	151.9	11.62	68.2	11.6
100 mM	4.35	27.0	1.35	25.8	5.96	97.6	12.93	54.4	5.83
200 mM	2.83	20.2	1.08	35.3	6.13	55.8	13.37	35.9	4.05
Mean	4.39	29.23	1.40	20.82	6.32	101.75	12.47	52.84	7.15
± SD	1.58	10.38	0.35	17.47	0.49	48.18	0.87	16.22	3.94
R	-0.99	-0.98	-0.99	0.968	-0.76	-0.99	0.99	-0.99	-0.95

***Concentrations**

As a result of uptake reduction, macronutrient concentrations were decreased (Fig.3). Phosphorus concentration was decreased in both shoots and roots, while potassium was dramatically decreased in the roots. Mg concentrations were less affected, while calcium concentrations were even increased. Potassium migration to the shoots can be explained as a plant adaptation to balance sodium in the cell vacuole, while Ca concentration increased to withstand detrimental effects of Na (Mengel and Kirkby, 1987). Shoot iron concentration was greatly decreased, while zinc was mostly decreased in the roots with the treatment 200 mM (Fig. 4). Copper concentration showed decreases in both shoot and root, however Mn concentration in the shoots was increased. As previously mentioned, under

salt stress, iron appeared to accumulate in the root while zinc and copper uptake processes are impaired.

Fig. 3: Sodium, P, K, Mg and Ca concentrations in shoots and roots of cucumber plants as affected by NaCl salinity.

- **Nutrient balance**

Nutrient balance in the plant tissues was found to also affected by salt stress in the growth medium (Mengel and Kirkby, 1987). As most of potassium migrated to the shoot tissues, P/K ratio was decreased in shoots and increased in roots while K/Mg was increased in shoots and decreased in roots (Table 3). Lake of Zn translocation (Yahya, 1998) led to a negative deviation of K/Zn ratio in the roots grown under high salt concentration (200 mM). For the same reason, negative deviations were calculated for Fe/Zn in the shoots. Because of the high accumulation of Fe in the roots with high salt concentration, a positive deviation was found in Fe/Zn in the roots grown under high salt stress. Foliar application of some nutrients can be of great benefit to correct nutrient concentrations in the shoots and consequently nutrient balance (Salama *et al*., 1996)

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Table 3: Nutrient concentration ratios in cucumber plant tissues as affected by NaCl salinity

Ratios / Treatments	P/K	Deviation % from control	K/Mg	Deviation % from control	K/Zn	Deviation % from control	Fe/Zn	Deviation % from control
Shoot nutrient ratios								
Control	0.168	--	18.07	--	770.2	--	1.99	--
100 mM	0.135	-19.6	18.1	+0.16	850.9	+10.5	1.27	-36.1
200 mM	0.131	-22.0	19.0	+5.14	725.9	-5.75	0.92	-53.7
Root nutrient ratios								
Control	0.087	--	96.7	--	351.0	--	2.54	--
100 mM	0.218	+150.5	62.4	-34.4	129.2	-63.2	2.32	-8.7
200 mM	0.558	+541.3	10.75	-88.9	50.0	-85.7	3.83	+50.8

Fig. 4: Iron, Mn, Zn and Cu concentrations in shoots and roots of cucumber plants as affected by NaCl salinity.

Enzyme activity

Ascorbic acid oxidase and peroxidase activities in cucumber leaves were inhibited as a result of salt stress in the plant growth medium (Fig. 5). Severe decreases in the activity of both enzymes were determined at the high salt level (200 mM). This may be due to the inhibition of their synthesis because of the lack and disturbance in nutrients uptake and distribution (Yahia, 1998), especially phosphate, Mn and Zn. Inhibition of CO_2 assimilation (El-Fouly and Yung, 1972) and protein synthesis (Mengel and Kirkby, 1987) and lack of energy (Helal and Mengel, 1981) may also be direct reasons for enzyme activity inhibition. This may support the findings of Porath and Poljakoff-Mayber, 1964, 1968) with pea plants. In contrast, the activity of plasma membrane N^+-ATPase was reported to increase under salinity stress

(Reuveni *et al*., 1993). On the other hand, Greenway and Osmond (1972) found that dehydrogenases and aspartic acid transaminase in *Phaseolus vulgaris* showed no important responses with NaCl concentrations. Thus, inhibition of ascorbic acid oxidase and peroxidase can be used as indicators for salinity stress in the plant growth medium.

Fig. 5: Ascorbic acid oxidase and peroxidase activity in cucumber leaves as affected by NaCl salinity in the growth medium

CONCLUSIONS

From the present work, it can be concluded that, lack of nutrients uptake and disturbance of their distribution in the plant tissues due to NaCl salinity in the root medium led to a reduction in cucumber plant growth. The plants, to some extent, can cope with the low levels of salt stress, however, with the high levels, dramatic disturbance in nutrient balance and reduction in growth, nutrient uptake and oxidases enzyme activity take place. According to the previous studies, foliar application of some nutrients can be useful to correct the nutrient concentrations in the shoot and consequently the nutrient balance. Ascorbic acid oxidase and peroxidase activities can be used as indicators for salinity stress in the plant growth medium

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تأثير ملوحة كلوريد الصوديوم فى بيئة الجذور على النمو والحالة الغذائية وبعض انزيمات الأكسدة فى نبات الخيار

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نميت نباتات الخيار من صنف سالبريتا فى مزارع مائية فى مستويين من ملوحة كلوريد الصوديوم لدراسة أثر التركيزات الملحية على النمو، امتصاص العناصر الكبرى والصغرى وتوزيعها داخل أجزاء النبات وكذلك التوازن العنصرى داخل الأنسجة، بالاضافة الى نشاط انزيمى الأسكوربيك أسيد أكسيديز والبيروكسيديز. وجد أن النمو قد يقل بزيادة التركيز الملحى فى بيئة الجذور. كما وجد أن هناك ارتباطا سالبا معنويا بين امتصاص جميع العناصر التى تم تقديرها و زيادة المستوى الملحى فى بيئة الجذور ما عدا عنصر المنجنيز فى المجموع الخضرى للنبات. ولقد اتضح من الدراسة أنه تحت ظروف الاجهاد الملحى فان عناصر البوتاسيوم والكالسيوم تهاجر من الجذور الى المجموع الخضرى بينما عنصر الحديد يتراكم فى الجذور كما يثبط امتصاص عنصرى الزنك والنحاس . كما وجد أن تركيز كل من البوتاسيوم والفوسفور ينخفض بشدة مع زيادة التركيز الملحى، وكان الانخفاض فى تركيز عنصر الكالسيوم بسيطا بينما زاد تركيز عنصر المنجنيز فى المجموع الخضرى عن نباتات الدليل. هذا ولم تبين العناصر الصغرى اتجاها محددا حيث اختلفت تركيزاتها بين الزيادة والنقصان فى كل من الجذور والمجموع الخضرى عن الدليل، فبينما انخفض تركيز الحديد بشدة فى المجموع الخضرى، فقد انخفض تركيز الزنك فى الجذور وقل تركيز النحاس فى كلا من المجموع الخضرى والجذور فيما زاد تركيز المنجنيز فى المجموع الخضرى للنباتات. ولقد أوضحت النتائج أيضا أن التوازن العنصرى قد اختل داخل أعضاء النبات بسبب تواجد ملح كلوريد الصوديوم فى البيئة ، فبينما قلت نسبة فو/بو فى المجموع الخضرى فقد زادت فى الجذور عن الدليل. ولقد حدث العكس فى نسبة بو/مغ، بينما نقصت نسبة بو/خ فى الجذور ومع التركيز الملحى الأقل فى المجموع الخضرى و زادت فى المجموع الخضرى فى النباتات النامية على التركيز الملحى الأعلى، أما نسبة ح/خ فقد انخفضت فى المجموع الخضرى للنبات ومع التركيزالملحى الأقل فى الجذور وزادت فى الجذور مع التركيز الملحى الأعلى عن نباتات الدليل. أما عن انزيمات الأسكوربيك أسيد أكسيديز والبيروكسيديز فقد وجد أن نشاطها يقل مع زيادة التركيز الملحى فى بيئة الجذور مما يقترح معه أن قياساتها تصلح كدليل على الاجهاد الملحى للنبات.