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Interactive Effects of NaCl Salinity and Phosphorus on Growth and Mineral Contents of Cucumber Microcultured on Proliferation Medium.

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ABSTRACT

The goal of this work was to test the *in vitro* responses of cucumber microshoots to salt stress as affected by increased phosphorus (P) in the proliferation medium. Salinity was induced by incorporating NaCl at different levels (0.0, 25, 50, 75 and 100 mM) to the proliferation medium. Increased P in the proliferation medium was efficient to ameliorate the adverse effects of salinity. Vegetative and root growth of cucumber microshoots were negatively affected by salinity, whereas increasing P level improved the microshoot culture with elevated NaCl levels. The microshoots content of K, Ca and P were reduced by *in-vitro* induced salinity and this reduction was less pronounced as P level increased in the proliferation medium.

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INTRODUCTION

Agricultural crops are continuously exposed to a variety of biotic and abiotic stresses resulting in abnormal growth and development and low productivity [1, 2]. Salt stress is one of the most significant environmental stress factors that adversely affect crop growth and productivity world-wide [3]. Mitigating the adverse effects of salinity would have positive impacts on agricultural productivity. Therefore, choosing proper agricultural practices under salinity and osmotic stress conditions is becoming essential [4]. Previous studies suggested that mineral nutrient-status of crops greatly affect their adaptation and resistance to abiotic stresses. Phosphorus (P) is one of the essential mineral nutrients and is reported to be involved in mitigatingthe negative impact of both salinity and osmotic stresses on crops growth and development [5].

Recent developments in biotechnology have wide applications in plant sciences [6]. The application of plant tissue culture techniques in stress physiological studies, particularly salt and water stress, can be justified by the fact that *in vitro* system has the advantage of relatively little space needed to culture and rigorously control physical environment and nutrient status parameters, which are difficult to regulate with traditional experimental system [5,7].

Shoot apex culture has been widely used to evaluate plant physiological responses to salinity and osmotic stress in various species, including cherry [5], cucumber [5] and tomato [9]. With regards to the whole plant, a similar response to salt stress could be expected in plantlets grown through *in vitro* shoot apex culture [9], because such explants can be considered mini-replicas of a plant with anatomical organization and ability to root and grow into whole plant.

Although, various *in vitro* studies focused on aspects of plant regeneration in cucumber [10-12], little is known about physiological conditions affecting cucumber *in vitro* cultures. Recently, the responses of cucumber microshoots to salt and osmotic stresses were reported [3,5]. The objective of the present study was to investigate the effects of P with NaCl salinity on growth and mineral contents of cucumber microshoots grown on proliferation medium.

MATERIALS AND METHODS

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Seeds of cucumber (*Cucumissativus*L.) were decoatedand soaked in 70% ethanol for 1 minute, and then surface sterilized by immersing in 1% sodium hypochlorite for 20 minutes. Finally the seeds were rinsed three times with autoclaved distilled water. The sterilized seeds were germinated on modified MS [13]bioregulators-free medium. Cultures were maintained at 22 ºC for six days.

After six days of culture, the cotyledonary nodes (included both cotyledons, an intact apical bud and 0.5 cm length of intact hypocotyls) were dissected and inoculated on modified MS medium containing 2 $mg.l^{-1}$Kn for three weeks in order to obtain higher number of nodal explants. Thereafter, single node stem cuttings were inoculated on MS medium containing 1 $mg.l^{-1}$ Kn. Two weeks later, well developed plantlets (microshoots) were obtained.

Microshoots (about three cm long) were inoculated on proliferation medium containing 1 $mg.l^{-1}$Knand supplemented with a combination of NaCl, at 0.0, 25, 50, 75, 100 mM with 0.5, 1.0 or 2.0 mM P using KH_2PO_4 (making 15 combinations).

Data were recorded after one month for shoot length, root number and length and their fresh and dry weights. Dry weights were determined for shoots and roots after drying the samples to a constant weight at 78ºC.

For minerals content, shoots were dried to a constant weight. About 250 mg of ground tissue were digested with 10 ml of concentrated sulfuric acid in presence of selenium reagent mixture and heated to 300 ºC for 3 h. The solution was then diluted to 100 ml by addition of distilled water. Potassium was determined using flame photometer and calcium was determined using atomic absorption spectrophotometer. Phosphorus content was measured according to Watanabe and Olsen [14] and chloride was measured by potentiometric titration [15].

Treatments were arranged in a completely randomized design with 15 replicates. Data were subjected to the analysis of variance and means were separated according to the least significant difference (LSD) at 0.05 level of probability.

RESULTS AND DISCUSSION

Interaction of NaCl X P exerted significant effects on vegetative growth, except for shoot dry weight percentage, where the separate effects of both NaCl and P were only significant (Table 1). Therefore significant effects will be presented.NaCl X P interaction exerted significant effects on shoot growth (Table 1). Increased NaCl concentration reduced shoot growth, while P tended to alleviate significantly this reduction (Table 1).

Table 1: Interactive effects of phosphorus with *in vitro*-induced salinity on vegetative growth of cucumber microcultured on proliferation medium.

P (mM)	NaCl (mM)				
	0	25	50	75	100
Shoot Length (cm)					
0.5	9.50 b[(1)]	9.12 bc	8.68 cde	7.91 f	5.35 h
1.0	9.75 ab	9.28 bc	8.74 cde	8.10 ef	5.70 gh
2.0	10.18 a	9.57 ab	8.82 cd	8.21 def	6.20 g
Shoot Fresh Weight (g)					
0.5	2.35 bc	2.18 cde	1.95 fgh	1.75 hij	1.60 j
1.0	2.43 ab	2.26 bcd	2.00 efg	1.77 hij	1.64 ij
2.0	2.56 a	2.34 abc	2.10 def	1.83 ghi	1.71 ij
Shoot Dry Weight (g)					
0.5	0.193 bc	0.166 ef	0.140 g	0.116 hi	0.090 j
1.0	0.205 ab	0.175 de	0.145 fg	0.119 hi	0.097 ij
2.0	0.213 a	0.188 cd	0.163 de	0.124 h	0.104 ij

[1]For each parameter, means followed by different letters are significantly different according to LSD test ($P<0.05$).

In general increasing NaCl salinity, resulted in shorter shoots (Table 1) In absence of NaCl, increasing P concentration from 0.5 to 2 mM, increased shoot length from 9.5 to 10.18 cm (Table 1). In absence of NaCl, an increase of about 2.6 and 7.3% was noticed in shoot length at 1 and 2 mM P, respectively (Table 1). In presence of NaCl, P resulted in less shoot length reduction percentages, and in some cases (25 mMNaCl X 2 mM P) an increase rather than a reduction was observed (Table 1). The 100 mMNaCl X 0.5 mM P produced the shortest shoots (Table 1) and the most severe reduction percentages (Table 1); at the same concentration of NaCl, the 2 mM P gave significantly longer shoots than the 0.5 mM P.

Microshoot fresh and dry weights decreased concomitantly with salinity concentration up to 50 mMNaCl (Table 1). Heaviest and lightest shoot fresh and dry weights were obtained at 0 mMNaCl X 1 or 2 mM P and 100 mMNaCl X0.5 mM P, respectively. P counteracted the unfavorable effects of salinity on dry weight and corresponding reduction percentages (Table 1).

Adventitious root growth was significantly affected by the NaCl X P interaction, except for adventitious root dry weight percentages (Table 2) where the separate effect of NaCl was significant. Therefore significant effects will be presented.

Table 2: Interactive effects of phosphorus with *in vitro*-induced salinity on adventitious root growth of cucumber microcultured on proliferation medium.

P (mM)	NaCl (mM)				
	0	25	50	75	100
Root Number					
0.5	6.22 de[(1)]	5.42 ef	4.72 fg	4.28 gh	3.82 h
1.0	7.85 ab	7.10 bc	6.77 cd	4.43 gh	3.92 gh
2.0	8.48 a	7.56 bc	6.93 cd	4.63 fgh	3.97 gh
Root Length (cm)					
0.5	7.64 cd	7.13 d	6.27 ef	5.85 fg	4.93 h
1.0	8.65 ab	8.08 bc	7.11 de	6.24 f	5.14 gh
2.0	8.97 a	8.23 abc	7.46 cd	6.30 ef	5.16 gh
Root Fresh Weight (g)					
0.5	0.220 c	0.197 d	0.129 f	0.110 g	0.081 h
1.0	0.237 b	0.215 cd	0.140 e	0.125 fg	0.087 h
2.0	0.244 a	0.219 c	0.148 e	0.126 f	0.089 h
Root Dry Weight (g)					
0.5	0.0138 d	0.0121 e	0.0077 h	0.0061 i	0.0041 k
1.0	0.0155 b	0.0135 d	0.0087 g	0.0074 h	0.0045 j
2.0	0.0160 a	0.0140 c	0.0093 f	0.0076 h	0.0048 j

[1]For each parameter, means followed by different letters are significantly different according to LSD test (P<0.05).

At NaCl concentrations up to 75 mM, reduction in adventitious root number was observed (Table 2). Within the same concentration of NaCl less than or equal to 50 mM, P significantly increased adventitious root number (Table 2,); at 75 and 100 mMNaCl, P had no significant effects on adventitious root number. At the 0.5 mM P, percent reduction was -38.5 at 100 mMNaCl. In absence of NaCl, P at 1 and 2 mM gave about 26 and 36% increase in adventitious root number (Table 2), The respective percent increase in adventitious roots was reduced to about 9 and 12 at 50 mM of NaCl. Further increase in salinity to 100 mM even in presence of P aggregated the percent reduction in adventitious root number to almost –36 % (Table 2).Phosphorus was reported to enhance root growth through improving root length and surface area of salt stressed tomato plants [16]. The results of the present study indicated that at any imposed NaCl concentration, vegetative and adventitious root growth were enhanced with P. Plants have developed several cellular and molecular mechanisms to cope with abiotic stresses [17].

Significant effects of NaCl X P on adventitious root length was clearly demonstrated (Table 2), though adventitious root length was reduced with salinity, it increased by P treatment within concentrations of NaCl less than or equal to 50 mM only (Table 2). Each increase in NaCl concentration was associated with an increase in percent reduction in adventitious root length (-36% at 0.5 mM P X 100 mMNaCl) (Table 2). Increasing P to 1 and 2 mM in absence of NaCl increased the respective adventitious root length value by about 10 and 16%. At NaCl greater than 50 mM, P was unable to sustain increase in adventitious root length which was reduced by about 33% even in presence of 1 and 2 mM of P (Table 2). Adventitious root fresh and dry weights were reduced as salinity increased at all concentrations of P (Table 2). In contrast, adventitious root fresh and dry weights were enhanced at every concentration of NaCl by P at 1 and 2 mM. Heaviest and lightest adventitious root fresh and dry weights were recorded for the 2 mM P in absence of NaCl and for the 0.5 mM P in presence of 100 mMNaCl, respectively (Table 2). Percent reductions in adventitious root fresh and dry weights were progressively severe at NaCl concentrations greater than or equal to 50 mM (Table 2). P at 2 mM in absence of NaCl increased adventitious root fresh and dry weights by about 12 and 13%, respectively (Table 2).

NaCl, P and their interaction exerted significant effects on microshoots mineral composition (Table 3). In general, uptake of Na was increased by NaCl-induced salinity (Table 3). In absence of NaCl, Na content was not significantly affected by P concentrations. In contrast, and in presence of NaCl, P tended to play a significant role in decreasing Na content. At each of NaCl concentrations equal or higher than 25 mM, the 1 and 2 mM P gave almost similar Na contents. The highest Na uptake was observed at the 100 mMNaCl X 0.5 mM P, while at the same concentration of NaCl, the 1 and 2 mM P reduced Na uptake significantly (Table 3).

At 0 mMNaCl, Cl content followed a pattern , more or less similar to that of Na. Although Cl content was increased with NaCl-induced salinity, its uptake decreased as concentration of applied P increased; in general; the 1 and 2 mM P gave almost similar Cl contents (Table 3).Shibli*etal.*[18] reported that Na and Cl increased with imposed salinity, but their uptake was reduced with increasing P concentration The highest K content was obtained by 2 mM P in absence of NaCl. At all NaCl concentrations, 2 mM P resulted in a significant increase in

K uptake compared to other P concentrations (Table 3). The increased uptake of Na and decreased shoots content of K could be explained by the antagonistic relation between K and Na [19].

Table 3: Interactive effects of phosphorus with *in vitro*-inducedsalinity on mineral composition of cucumber microcultured on proliferation medium.

P (mM)	NaCl (mM)				
	0	25	50	75	100
Concentration (mg.g^{-1} DW)					
Na					
0.5	2.50 i [1]	22.51 g	33.28 cd	39.65 ab	42.56 a
1.0	2.44 i	17.75 h	27.59 ef	33.36 cd	37.26 bc
2.0	2.41 i	17.32 h	25.83 fg	30.31 de	35.37 c
Cl					
0.5	5.48 j	23.10 h	34.48 de	42.40 b	50.21 a
1.0	5.26 j	18.41 i	28.31 fg	35.51 de	40.51 ab
2.0	5.12 j	17.65 i	26.07 gh	31.18 ef	37.41 cd
K					
0.5	38.31 b	32.15 cd	29.52 def	25.22 f	18.41 g
1.0	41.22 b	37.31 b	31.58 de	27.19 ef	20.39 g
2.0	46.47 a	40.60 b	36.69 bc	30.50 de	2 5.62 f
Ca					
0.5	7.81 ab	6.50 d	5.44 f	4.82 fg	4.25 g
1.0	8.22 a	8.03 ab	7.25 bcd	6.43 de	5.33 f
2.0	8.35 a	8.11 ab	7.56 abc	6.75 cd	5.55 ef
P					
0.5	2.48 de	1.78 fg	1.51 gh	1.17 I	1.03 i
1.0	3.56 b	2.68 cd	2.10 ef	1.82 fg	1.64 fgh
2.0	4.48 a	3.14 bc	2.34 de	2.01 ef	1.76 fg

[1]For each parameter, means followed by different letters are significantly different according to LSD test (P<0.05).

Ca content decreased significantly with NaCl. This reduction was slowed down by increasing P concentration. The 1 and 2 mM P resulted in higher Ca content compared to the 0.5 mM P. However, in absence of NaCl, no significant changes in Ca uptake were detected among P concentrations (Table 3).In absence of NaCl, P content increased significantly with each increase in P concentration applied. NaCl concentrations greater than or equal to 25 mM resulted in a significant reduction in P content but increasing P concentration above 0.5 mM significantly increased P uptake (Table 3). Comparable to responses obtained in cucumber microshoots, Shibli*et al.*[18] using African violet noticed that reduction in P, Ca and K contents with elevated salinity was less as P concentration in the medium increased.

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