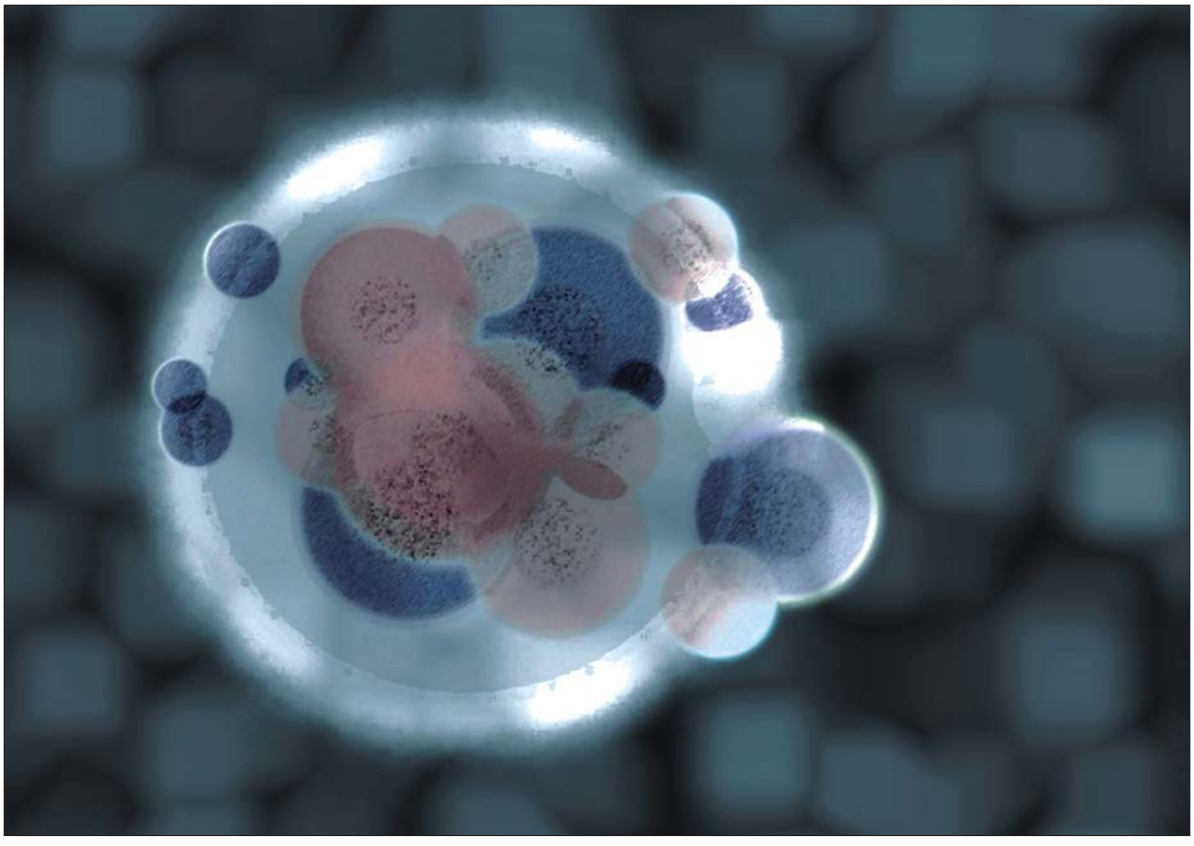

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Ameliorative Effects of Potassium and Calcium on the Salinity Stress in Embryo Culture of Cucumber (*Cucumis sativus* L.)

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Abstract: The effect of varying potassium (0-control, 5, 10, 18 and 28 mM as potassium nitrate) and calcium (0-control, 5, 10, 18 and 28 mM as calcium nitrate) supply on growth of NaCl stressed (0-control, 100 and 150 mM) mature embryos of cucumber (*Cucumis sativus* L.) *in vitro* condition. NaCl caused decrease root (rooting percent, root number, root length and root fresh weight) and shoot (shoot length, shoot FW, leaf FW, whole plant FW) growth. Root/shoot length index and root/plant FW index was generally lower in the 100 and 150 mM NaCl concentrations than control (absence of NaCl). There was an increase in callus induction of NaCl treated cucumber embryos. Callus induction was decreased by $K^+$and Ca^{2+} applications at both NaCl concentrations In general, root and shoot growth of cucumber embryos was improved by supplemental potassium (10 mM potassium nitrate) and calcium (20 mM calcium nitrate).

Key words: Salt stress, Ca^{2+}, K^+, cucumber, embryo culture

INTRODUCTION

Salinity is at present one of the most serious environmental problems influencing crop growth around the world. The salinity of the soil and irrigated water is a problem that restricts yield on almost 40 million hectares of irrigated land, which is approximately one-third of the irrigated land on earth[1].

Salt causes stress and damage on the plant during the vegetation period from germination-emergence through growth-development and harvesting time[2]. It is generally accepted that three major hazards are associated with saline habitats. These may be described as: water stress, specific ion toxicity and nutrient ion imbalance[3]. Exposure to NaCl salinity affects transport processes in the plant, the result of which can be an alteration of the nutritional status and tissue ion balance. Salt stress inhibits the uptake and transport of potassium (K^+) and calcium (Ca^{2+}), nutrients that influence plant growth[4,5].

It is well known $Ca^{2+}$is involved in cell elongation, cell division, the structural stability and permeability of cell membranes. It plays a regulatory role in balance of cation-amion. It acts as an activator for only a few enzymes. Also, potassium is required for turgor build up in plants and maintains the osmotic potential of cells, which in guard cells governs the opening of stomata. It affects the cell extension and cell walls thick and stability. K^+plays role in enzyme activation, protein synthesis, photosynthesis[6,7].

Excess of salinity, nevertheless, causes a range of deleterious effects such as inhibition of photosynthesis, pigment synthesis, damage to plasma membrane permeability and other metabolic disturbances[8-10]. Different mechanisms leading to salinity tolerance in plant populations and species have been found[11-13]. One of this is amelioration of growth and metabolism of plants stressed with NaCl by supplemental $Ca^{2+}$and K^+[14,15].

However, in some cases, the mechanisms underlying the tolerance to salinity are not fully understood. Plant cell and tissue culture methods could be useful for understanding mechanisms of salinity in plants[16-18].

The present study on cucumber was designed to determination of amelioration effect of different potassium and calcium concentrations on salinity stress in cucumber embryo culture.

MATERIALS AND METHODS

Seeds of *Cucumis sativus* L. cvs. Beith Alpha F1 were put deionised water for imbibition and soften of seed coat. After seed coats were separated from seeds, seeds without coat were obtained and surface sterilized with 75% alcohol solution and 15% sodium hypochlorite solution (7% active chlorine) for 2-3 min. After seeds without coat were washed with sterile distilled water and prepared for sowing culture media.

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Table 1: Composition of basal MS media supplemented with concentrations of KNO_3 and $Ca(NO_3)_2$ for K^+ and Ca^{2+} experiments

K^+ experiment	NaCl	Ca^{2+} experiment
0 (mM)	0 (mM)	0 (mM)
5 (mM)	100 (mM)	5 (mM)
10 (mM)	150 (mM)	10 (mM)
18 (mM)		15 (mM)
28 (mM)		20 (mM)
		25 (mM)

In study, basal medium was MS supplemented with 100 mg L^{-1} myo-inositol, 0.5 mg L^{-1} nicotinic acid, 0.5 mg L^{-1} pyridoxine and 0.1 mg L^{-1} thiamine, 1.0 mg L^{-1} 2-4 D, 1.0 mg L^{-1} kinetin, 30 g sucrose and 8 g Difco Bacto agar. One seed was placed per test tube (10 cm length and 1.5 cm dia.) contained 10 ml of medium supplemented with different concentrations of NaCl, KNO_3 and $Ca(NO_3)_2$ that they were experiment treatments (Table 1). All culture media were adjusted to pH 5.7–5.8 and autoclaved for 15-20 min at 120°C and 1 atm. All cultures were incubated in a growth room at 25±2°C under a photoperiod at 16 h light provided by fluorescent tubes.

In potassium and calcium experiments were used completely randomized block experimental design with three replications and each treatment were contained 20 tubes (seeds). Results were expressed as means of three replications. Differences among the means were compared by Fischer's Least Significant Difference Test.

RESULTS

Effects of NaCl: All characters were negatively affected by increasing salinity non-supplement by both potassium and calcium in this study. On increasing the concentration of NaCl; rooting ratio, root number, root length, root weight, plant length, shoot weight, leaf weight, whole plant weight, root/plant length index, root/plant weight index decreased except callus induction rate (Table 2-7).

Restrictive effects of salinity almost all growth characteristics were confirmed by previous investigations in cucumber[19], in melon[20] and pumpkin[21]. On the other hand, adversely effects of salt stress was observed *in vitro* conditions of tomato[17,22,23], pepper[24], cabbage [25] and chinese cabbage[26].

The burden of high salt concentrations for plants is due to the osmotic retention of water and specific ionic effects on the protoplasm. Water is osmotically held in salt solutions so that as the concentration of salt increases water becomes less and less accessible to the plant. An excess of $Na^+$and, to an even greater extent, an Cl^- in the protoplasm leads to disturbances in the ionic balance ($K^+$and Ca^{2+} to Na^+) as well as ion specific effects on enzyme proteins and membranes[2]. Therefore,

Table 2: Effect of K^+ (KNO_3) on root characters in cucumber embryos stressed with NaCl (Values comparisons between means were made with Fischer's least significant test within each columns. Values followed by a common letter are not significantly different, P<0.05)

NaCl (mM)	K^+ (mM)	Rooting (%)	Root number	Root length (mm)	Root FW (g)
0	0	87.27bc	7.46c	8.46cd	0.53cd
	5	90.90ab	8.79c	9.43c	0.58bc
	10	91.66ab	10.59b	10.06bc	0.60bc
	18	97.43a	14.38a	13.43a	0.77a
	28	94.44a	10.94b	11.96ab	0.63b
100	0	46.66d	0.01e	4.09fgh	0.29fgh
	5	55.55d	0.82d	4.62efg	0.31efg
	10	67.22a-d	1.59d	6.49de	0.33ef
	18	63.16bd	1.20d	5.79ef	0.46d
	28	59.25cd	0.87d	5.03efg	0.36e
150	0	38.88d	0.01e	2.37h	0.13j
	5	44.44d	0.12e	2.78gh	0.16ij
	10	57.22d	0.97d	5.19ef	0.22hı
	18	45.07d	0.33d	4.80efg	0.24gh
	28	42.85d	0.20d	3.72fgh	0.28fgh
	F	0.01	0.01	0.01	0.01

Table 3: Effect of K^+ (KNO_3) on top tissues characters in cucumber embryos stressed with NaCl (Values comparisons between means were made with Fischer's least significant test within each columns. Values followed by a common letter are not significantly different, P<0.05)

NaCl (mM)	K^+ (mM)	Shoot length (mm)	Shoot FW (g)	Leaf FW (g)	Whole plan FW(g)
0	0	20.30bc	0.92def	0.46def	1.45de
	5	22.29bc	1.28c	0.64c	1.86c
	10	26.48ab	1.69b	0.84b	2.29b
	18	31.81a	2.27a	1.13a	3.04a
	28	27.85ab	2.19a	1.09a	2.83a
100	0	4.56e	0.80efg	0.40efg	0.94gh
	5	6.34de	0.87def	0.43def	1.04fgh
	10	14.02cd	1.12cd	0.56cd	1.59cd
	18	11.39de	0.95de	0.47de	1.29ef
	28	7.84de	0.95de	0.47de	1.25efg
150	0	2.30e	0.54g	0.27g	0.77h
	5	2.91e	0.62fe	0.31fe	0.87h
	10	4.26e	0.64efg	0.32efg	0.90h
	18	3.48e	0.64efg	0.32efg	0.95gh
	28	3.13e	0.64efg	0.32efg	0.93gh
	F	0.05	0.01	0.01	0.01

Table 4: Effect of K^+ (KNO_3) on callus induction and root/plant indices characters of cucumber embryos stressed with NaCl (Values comparisons between means were made with Fischer's least significant test within each columns. Values followed by a common letter are not significantly different, P<0.05)

NaCl (mM)	K^+ (mM)	Callus induction (%)	Root/Shoot length index	Root/plant FW index
0	0	12.73bd	1.036abc	0.580a
	5	9.09cd	1.061abc	0.457bc
	10	8.33cd	1.347ab	0.575ab
	18	3.23d	1.441a	0.458bc
	28	5.88d	1.192abc	0.481b
100	0	53.33a	0.464c	0.325de
	5	44.44a	0.515bcd	0.343de
	10	32.78ad	0.864bc	0.358d
	18	36.84ac	0.720bcd	0.396d
	28	40.74ab	0.741bcd	0.417bcd
150	0	61.11a	0.417c	0.254g
	5	55.56a	0.425c	0.276def
	10	42.78a	0.465c	0.296def
	18	54.92a	0.429c	0.310def
	28	57.14a	0.442c	0.302def
	F	0.01	0.01	0.01

Table 5: Effect of Ca^{2+} ($Ca(NO_3)_2$) on root characters in cucumber embryos stressed with NaCl (Values comparisons between means were made with Fischer's least significant test within each columns. Values followed by a common letter are not significantly different, P<0.05)

NaCl (mM)	Ca^{2+} (mM)	Rooting (%)	Root number	Root length (mm)	Root FW (g)
0	0	87.57a	7.46d	8.35d	0.54b
	5	90.90a	8.79d	9.25d	0.58b
	10	100.00a	17.87a	14.26a	0.86a
	15	97.43a	16.46ab	13.66ab	0.78a
	20	94.87a	15.64b	12.10bc	0.63b
	25	91.41a	11.25c	11.52c	0.61b
100	0	25.55fg	0.01g	4.06fghij	0.17f
	5	38.88def	1.89ef	4.57efghi	0.23ef
	10	56.01bcd	1.94ef	5.02efgh	0.33cde
	15	61.85bc	2.10ef	5.24efg	0.36cd
	20	64.10bc	2.53ef	5.79ef	0.53b
	25	67.92b	2.82e	6.19e	0.57b
150	0	11.11g	0.01g	2.38j	0.23ef
	5	25.00fg	0.66fg	2.65ij	0.28de
	10	32.77efg	0.97efg	2.80ij	0.29de
	15	45.07cdef	1.05efg	3.13hij	0.31cde
	20	50.00bde	1.43efg	3.85f-j	0.41c
	25	47.61bde	1.28efg	3.65g-j	0.34cd
	F	0.05	0.01	0.01	0.01

Table 6: Effect of Ca^{2+} ($Ca(NO_3)_2$) on top tissues characters in cucumber embryos stressed with NaCl (Values comparisons between means were made with Fischer's least significant test within each columns. Values followed by a common letter are not significantly different, P<0.05)

NaCl (mM)	Ca^{2+} (mM)	Shoot length (mm)	Shoot FW (g)	Leaf FW (g)	Whole plant FW (g)
0	0	20.53b	0.90fg	0.45fg	1.45e
	5	22.29b	1.31cde	0.65cde	1.89cd
	10	35.14a	1.84a	0.92a	2.70a
	15	33.24a	1.69a	0.84a	2.47b
	20	25.06b	1.62ab	0.81ab	2.25b
	25	23.72b	1.41bc	0.70bc	2.02c
100	0	4.64ef	0.81f-ı	0.40f-ı	0.97ghı
	5	8.33de	0.87fgh	0.43fgh	1.10fgh
	10	10.57cd	0.99ef	0.49ef	1.32ef
	15	12.82cd	1.02def	0.51def	1.38e
	20	13.01cd	1.19cde	0.59cde	1.71d
	25	13.93c	1.24cd	0.62cd	1.81cd
150	0	2.19f	0.51j	0.25j	0.73ı
	5	2.93ef	0.58ij	0.29ij	0.86hı
	10	3.16ef	0.61ij	0.30ij	0.90ghı
	15	3.58ef	0.64hij	0.32hij	0.96ghı
	20	4.02ef	0.70g-j	0.35g-j	1.10g
	25	3.88ef	0.67g-j	0.33g-j	1.01gh
	F	0.01	0.01	0.01	0.01

supplementation of Ca^{2+} and K^+ is known to be useful for overcoming the negative impact of high salinity where the growth medium or may become saline at some time during the crop growth cycle.

Effect of potassium: In this study, potassium was showed ameliorative effect on salinity stress of cucumber embryos. At the 100 mM NaCl treatment, increasing K^+ improved root characters (rooting percent, root number, root length and root fresh weight) and shoot characters (shoot length, shoot fresh weight, leaf fresh weight and whole plant fresh weight) of embryos compared with absence of K^+. When NaCl concentration was increased to 150 mM, K^+ positively affected root and shoot characters but this effect was not as much as in 100 mM NaCl concentration. At both NaCl concentrations, the greatest ameliorative concentrations of K^+ was 10 mM for root and shoot characters (Table 2 and 3). On the other hand, indexes of root/shoot length and root/plant fresh weight was positively affected by added K^+ for both 100 and 150 mM NaCl concentrations. Callus induction in salt stressed embryos caused clear augmentation according to control. Even callus induction certain increased in high salinity level (150 mM NaCl). Increasing potassium applications firstly decreased callus induction from 0 to 10 mM level and then increased from 18 to 28 in moderate (100 mM NaCl) and high (150 mM NaCl) salinity (Table 4).

Table 7: Effect of Ca^{2+} ($Ca(NO_3)_2$) on callus induction and root/plant indices characters of cucumber embryos stressed with NaCl(Values comparisons between means were made with Fischer's least significant test within each columns. Values followed by a common letter are not significantly different, P<0.05)

NaCl (mM)	Ca^{2+} (mM)	Callus induction (%)	Root/shoot length index	Root/plant FW index
0	0	12.42efh	0.639ab	0.630a
	5	9.09fgh	0.884abc	0.446abc
	10	1.00h	0.932ab	0.469abc
	15	2.56gh	0.965ab	0.459abc
	20	5.12gh	0.995ab	0.389bcd
	25	8.58fgh	1.083a	0.437abc
100	0	74.44ab	0.409d	0.204d
	5	61.11a-d	0.447cd	0.270cd
	10	43.98cd	0.450cd	0.338bcd
	15	38.14cde	0.478cd	0.348bcd
	20	35.89def	0.581bcd	0.448abc
	25	32.07d-g	0.956ab	0.456abc
150	0	88.88a	0.406d	0.443abc
	5	75.00ab	0.406d	0.481ab
	10	67.22abc	0.412d	0.474ab
	15	54.92bcd	0.453cd	0.481ab
	20	50.00bcd	0.483cd	0.579a
	25	52.38bcd	0.488cd	0.502ab
	F	0.01	0.05	0.01

It has been reported by previous investigations[10,27,28] that an improve has obtained by potassium supplementation on characters of plants and explants cultivated *in vivo* and *in vitro* by salt stressed. In addition to these investigations conducted *in vivo*, there are some studies that reported to ameliorative effects of potassium *in vitro* for chinese cabbage and cucumber[29,30].

Effect of calcium: Similarly to potassium experiment, calcium supplementation of growth media was made cure effect on root and shoot characteristics of embryos stressed with NaCl. Characters of cucumber embryos cultivated in calcium added media alleviated against to salt stress. Tolerance ability of embryos was more successful than the control applications.

Increasing Ca^{2+}alleviated rooting percent, root number, root length and root fresh weight at the 100 and 150 mM NaCl accordance with absence of Ca^{2+}. The best levels were 25 mM and 20 mM $Ca^{2+}$for root characters at 100 mM and 150 mM NaCl, respectively (Table 5). Similarly, shoot characters as shoot length, shoot fresh weight, leaf fresh weight and whole plant fresh weight of embryos relieved with adding of Ca^{2+}compared with absence of Ca^{2+} for these NaCl concentrations. When 20 mM $Ca^{2+}$and 25 mM Ca^{2+}append to basal media successful results were obtained for shoot characters at 100 and 150 mM NaCl, respectively (Table 6). Indexes of root/shoot length and root/plant fresh weight was alleviated by added Ca^{2+}comparison with absence of Ca^{2+}at both 100 and 150 mM NaCl concentrations. Callus induction increased with increasing salinity from 100 to 150 mM NaCl compared with non-salinity. Calcium addition was evidently decreased callus induction at both 100 and 150 mM NaCl levels (Table 7).

Our results agree with previous studies in bean[28], in tomato[31], in cucumber [32] at the *in vivo* condition. In salt-affected plants Ca^{2+}improved germination and plumula emergence, root elongation and shoot growth[33].

Because, $Ca^{2+}$is important factor in the resistance of plants to salinity. The protective effect of Ca^{2+}in salinized plants is due to its role in maintaining membrane integrity and suggested that one of the primary effects of salinity is a disruption of membrane integrity caused by displacement of $Ca^{2+}$from the cell surface by Na^{+}[34].

In conclusion, we observed that inhibition effects of salinity and ameliorative effects of supplementation of 10 mM potassium and 20 mM calcium to basal media on growth of salt stressed cucumber embryos *in vitro* condition.

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