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RESEARCH ARTICLE

POTASSIUM AND CALCIUM NITRATE AMELIORATES THE ADVERSE EFFECT OF NaCl ON *IN VITRO* INDUCED TOMATO (*Lycopersicon esculentum* Mill.)

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ABSTRACT

An *in vitro* tissue culture experiments were carried out to investigate the effects of supplementary potassium and calcium nitrate applied to basal media on morphogenesis of saline stressed tomato (*Lycopersicon esculentum* Mill. Cv. Omdurman). Shoot tip explants were cultured on MS media salinized with NaCl and supplemented with KNO3 and Ca $(NO_3)_2$. Treatments consist of four NaCl concentrations (0.0, 50, 75 and 100 mM) in combination with either KNO_3 (0.0, 5, 10, 15 and 20 mM) or Ca $(NO_3)_2$ (0.0, 5, 10, 15 and 20 mM). Salinity significantly reduced the root growth, shoot growth and whole plant growth. Root growth was more reduced by the presence of NaCl in growth media than shoot growth. Application of supplemental calcium nitrate (20 mM) and potassium nitrate (15 mM) resulted in mitigation of the harmful effect of NaCl on tomato growth. However Ca $(NO_3)_2$ at 20 mM has negative effect on tomato shoot tip rooting %. In non salinized media, the best root and shoot growth was observed at 10 mM Ca $(NO_3)_2$. These findings suggest that the additions of potassium and calcium nitrate can ameliorate the negative effect of salinity on the growth of tomato.

INTRODUCTION

Soil salinity is one of the most important a biotic stress that limit crop production (Debez *et al*., 2006; Koyro, 2006). Up to 20% of the irrigated arable land in arid and semiarid regions is already salt affected and is still expanding (Mühling and Läuchli, 2003). The reduction in growth due to salt stress may be attributed to; osmotic effects on water availability, reduction in net assimilation, specific ion effects, or ion imbalance due to interference with uptake of essential ions or a combination of any of those adverse factors (Bernstein *et al*., 1993; Ashraf, 2009). In Sudan, it was found that about 250 thousand hectares in the Northern Sudan were affected, to some degree, by sodicity and/or salinity (Ali and Fadil, 1977). The Soil Survey Department stated that the total area affected by salinity and/or sodicity was estimated at 2.5 million hectares. Saline soils occur in most productive land along both bank of the Nile and its attributes (Mustafa, 1986). The high clay content of 2:1 clay minerals makes complete reclamation of salt affected soils in Sudan impracticable. Moreover, the use of subsurface drainage system was not successful (Mustafa, 1986). Therefore, there is a need to test for crops tolerant to salinity.

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Study for salinity tolerance in the field is difficult due to special heterogeneity in soil chemical and physical properties and seasonal variation (Aghaei *et al*., 2008). *In vitro* plant tissue culture has been proposed as a useful, quick and economical tool to evaluate salt tolerance (Mercado *et al*., 2000).

Tomato (*Lycopersicon esculentum* Mill.) is moderately tolerant to salinity and is typically cultivated in regions that are exposed to soil salinization (Cuartero and Fernandez, 1999). In Sudan, tomato is important vegetable crop ranks second to onion among vegetable crops based on cultivated area (Ahmed *et al*., 2001). It is grown throughout the country where irrigation water and arable land are available (Abdelmageed *et al*., 2003). Its production is affected by various stresses such as disease, high temperature, draught, salinity and its vulnerability to frequent insect and pest attacks. Hence, there is a need to improve this crop using modern biotechnological approaches.

Calcium (Ca^{2+}) is an essential element for plant growth; it has many functions in plant physiological processes (Assmann, 1995; Marschner, 1995). Most plant physiologists believe that the deleterious effects of Na+ may be elevated by increasing the Ca^{2+} external concentration (Busch, 1995; Tuna, *et al.*, 2007). There is no agreement among researcher on the exact effect of calcium on plants grow under salinity induced by sodium chloride (Qing Song and Fujyiama, 1996), however

Rengel (1992) suggested that increasing calcium in external concentration may have ameliorative effective on NaCl stressed plants. This amelioration has been attributed to transport and discrimination (Subarrao *et al*., 1990), and to improved osmotic adjustment due to accumulation of osmoregulators in the root system as a result of the interaction between Na^+ and Ca^{2+} (Colmer *et al*., 1996).). Also it has role in reducing Na^+ uptake and increasing K^+ and Ca^{2+} uptake, resulting in an increase in plant growth (Rengel ,1992) or by decreasing Na^+ influx through nonselective cation channels (Shabala *et al*., 2006).

Potassium is major macronutrient, that required by plants in large amount. It has many functions in plant; enzyme activation, protein synthesis and photosynthesis (Marschner, 1995). Application of $Ca(NO_3)_2$ at 10 mM had a beneficial effect on growth and metabolism of NaCl treated guava seedlings (Ebert *et al*., 2002) and increase fresh and dry weight of ray grass under sodium chloride salinity (Tabatabaei and Fakhrzad , 2008). The objectives of this study are to evaluate *in vitro* tissue culture technique to test salinity tolerance and the ameliorative effect of potassium and calcium on salinity induced by sodium chloride in tomato plant.

MATERIALS AND METHODS

Plant material

Seeds of tomato (*Lycopersicon esculentum* Mill., cv Omdurman) used in this study were obtained from the National Institute for Promotion of Horticultural Exports, University of Gezira, Sudan.

Surface sterilization and seed germination

Seeds were washed under continuously running tap water for 15 minutes then washed by sterile distilled water. Under laminar flow cabinet seeds were disinfected with Clorox (0.5 % free chlorine) at concentration of 15% v/v for 15 mints then rinsed three times with sterile distilled water. After surface sterilization, ten seeds were directly transferred to culture bottle contain 30 ml of half- strength Ms Media (Murashige and Skoog, 1962) supplemented with 0.6% (w/v) agar and 3% sucrose and incubated for 10 days at 25°C±2 with a 16 h photoperiod. *In vitro* raised explants (Shoot tip of 1-1.5 cm length from 10-15 days – old seedling was used as explants).

Effects of potassium nitrate and calcium nitrate on *in vitro* shoots induction and rooting under salinity stress

Explants were inoculated on culture bottle containing 25 ml MS medium supplemented with, 4.0 mgl^{-1} Kin and different NaCl concentrations(0. 50, 75 and 100 mM) in combination with either KNO_3 (0.0, 5, 10, 15 and 20 mM) or Ca $(NO_3)_2$ (0.0, 5, 10, 15 and 20 mM). To investigate the effect of KNO_3 and Ca $(NO_3)_2$ on tomato rooting under saline stress, shoot-tip were excised and cultured on half-strength MS media supplemented with 0.5 mgl^{-1} NAA and different NaCl concentrations (0. 50, 75 and 100 mM) in combination with either KNO_3 (0.0, 5, 10, 15 and 20 mM) or Ca $(NO_3)_2$ (0.0, 5, 10, 15 and 20 mM).

Culture condition and data analysis

The media were adjusted to pH 5.7 before autoclaving at 120 ^oC for 15 min. The cultures were incubated in growth room at 25 ^oC $\pm$ 2 under 16-h photoperiod provided by fluorescent light. The treatments consist of six bottles and three explants were used for each bottle. At the end of the experiment the physiological parameters including; shoot length, shoot fresh weight, whole plant fresh weight, rooting % and root length were measures. Data were statically analysis using ANOVA and means were separated using Duncan multiple range test (Duncan, 1955).

RESULTS AND DISCUSSION

Shoots and roots growth of tomato plants were significantly reduced by the increase in NaCl concentrations in the growth media not supplemented with Ca^{+2} or K (Tables 1, 2, 3 and 4). This results in agreement with that reported by Akinci and Semsek (2004) on the effect of salinity on cucumber under *in vitro* conditions. The results also showed that the increase in NaCl concentration in growth media (50, 75 and 100 mM) decreased shoot length by 19.7, 30.5 and 53.9 % and shoot fresh weight by 15.7, 34.2 and 74.3% respectively (Table, 1). Root number and root length were severely affected by increases in NaCl concentration. Root number was reduced by 55.6, 86.9 and 99.9% at 50, 75 and 100 mM respectively. Similar trend was detected for root length (Table, 2). It is clear salinity effect on root growth was higher compared to its effect on shoot growth. Similar results were reported by Cano *et al.* (1998) and Mercado *et al*. (2000) on tomato and by Morpurgo (1991) and Martínez *et al*. (1996) on potato.

Table 1. Effect of K^+ (KNO^3) on *in vitro* shoot induction of tomato shoot tip cultured on MS medium supplemented with 4.0 mg/l Kin and different concentration of NaCl.

NaCl (mM)	K^+ (mM)	Shoot length (mm)	Shoot FW (g)	Whole plant FW (g)
0.0	0.0	7.6 abc	0.7abc	0.9abcde
	5	7.7 ab	0.7ab	0.97abcd
	10	8.6 a	0.9a	1.09a
	15	8.4 a	0.79a	1.08ab
	20	8.3 a	0.79a	1.033abc
50	0.0	6.1def	0.59bcd	0.8cde
	5	6.4cde	0.59bcd	0.85bcde
	10	6.8 cde	0.61bcd	0.85bcde
	15	7bcd	0.68abc	0.9abcde
	20	6.5 bcd	0.7abc	0.9abcde
75	0.0	5.3 efg	0.46d	0.726667de
	5	5.5 efg	0.52cd	0.74de
	10	5.5 efg	0.56bcd	0.75de
	15	5.9defg	0.57bcd	0.76de
	20	5.9defg	0.57bcd	0.77de
100	0.0	3.5 i	0.18f	0.23f
	5	4.1hi	0.18f	0.28f
	10	4.9 gh	0.19f	0.29f
	15	5.1 fgh	0.26ef	0.35f
	20	5.1fgh	0.44de	0.69e

Data represent the mean of 5 replicates with 15 explants for each treatment. Means followed by same letter do not differ statistically at p=0.05 according the Duncan's multiple range test.

Reduction in growth with increasing salinity in growth media may be attributed to water deficit or ion toxicity associated with excessive ion uptake particularly of Na^+ and Cl^- (Satti and Lopez, 1994). Nutrients imbalance as a

result of depressed uptake, shoot transport and impaired internal distribution of minerals especially K^+ and Ca^{+2} may also explained the reduction in plant growth (Munns, 1993).

Addition of KNO_3 to growth media significantly ameliorates the adverse effect of NaCl on shoots and roots growth of tomato (Table 1 and 2). In non saline growth media (0 mM NaCl) the highest shoot length, shoot fresh weight and whole plant growth was observed at 10 mM KNO_3. However, more increase in KNO_3 concentration (15 and 20 mM) resulted in none significant decreases in shoot length, shoot fresh weight and whole plant fresh weight. While at 50 mM NaCl the highest shoot length was at 15 mM KNO_3. At 75 and 100 mM NaCl in growth media 15 mM and 20 mM KNO_3 produced the same higher ameliorate effect on shoot length. For shoot fresh weight and whole plant weight, the highest value at 100 mM NaCl was recorded in growth media supplemented with 20 mM KNO_3. Therefore, it is more likely that with the increases in salinity in growth media, there is a need to increase the concentration of KNO_3 in growth media.

Table 2. Effect of Ca^+ ($CaNO^{3)}$ on *in vitro* shoot induction of tomato shoot tip cultured on MS medium supplemented with 4.0 mg/l Kin and different concentration of NaCl

. NaCl (mM)	Ca (mM)	Shoot length (mm)	Shoot FW (g)	Whole plant FW(g)
0.0	0.0	6.6b	0.63abc	0.97bcd
	5	6.7b	0.63abc	0.97bcd
	10	8.6a	0.77a	1.5a
	15	8a	0.72ab	1.21b
	20	8a	0.70abc	1.1bc
50	0.0	5.6bc	0.45def	0.68defg
	5	5.7bc	0.45def	0.77def
	10	6.1bc	0.54cde	0.78def
	15	6.4b	0.55cde	0.87cdef
	20	6.5b	0.58bcd	0.92bcde
75	0.0	3.6ef	0.23ghi	0.31hi
	5	4.2de	0.23ghi	0.39ghi
	10	5.1cd	0.25ghi	0.58fgh
	15	5.1cd	0.35fgh	0.61efg
	20	5.2cd	0.4efg	0.67defg
100	0.0	1.1h	0.11i	0.15i
	5	1.2gh	0.11i	0.18i
	10	1.4gh	0.12i	0.21i
	15	2.4fg	0.14hi	0.21i
	20	2.7f	0.19hi	0.30hi

Data represent the mean of 5 replicates with 15 explants for each treatment. Means followed by same letter do not differ statistically at p=0.05 according the Duncan's multiple range test.

Tabatabaei and Fakhrzad (2008) attributed the improvement in tolerance for salinity of the perennial ryegrass at 10 mM potassium nitrate concentration to the reduction in Cl concentration and increased proline and K/Na ratio. Salinity induced by NaCl decrease rooting % only at 100 mM NaCl, at other concentrations rooting % was not affected. 100% rooting was observed at 0.0, 50 and 75 mM NaCl (Table 2). However, root number and root length were significantly decreased with increasing NaCl concentration in growth media. Akinci and Semsek (2004) reported that NaCl decreased root number, root length and root fresh weight on cucumber under *in vitro* conditions. Supplementing the growth media with KNO_3 improved root number and root length particularly at higher NaCl concentration (75 and 100 mM) in growth

Table 3. Effect of K^+ ($KNO^{3)}$ on *in vitro* rooting of tomato shoot tip cultured on half strength MS medium supplemented with 0.5mg/l NAA and different concentration of NaCl.

NaCl (mM)	K^+ (mM)	Rooting (%)	Root number	Root length (cm.)
0.0	0.0	100	25a	2.93b
	5	100	17.5b	2.4cd
	10	100	17.6b	2.47c
	15	100	16.26bc	4.93a
	20	40	22.9a	2.07de
50	0.0	100	9.9ef	1.47fg
	5	100	11.1de	1.83ef
	10	100	13.4cd	1.93e
	15	100	13.7cd	2.0e
	20	100	13.40cd	2.06de
75	0.0	100	3.27hi	0.7hi
	5	100	4.27gh	0.7hi
	10	100	7.27fg	0.77hi
	15	100	7.47f	1.1gh
	20	100	7.5f	1.21g
100	0.0	0.01	0.01i	0.01k
	5	46.6	0.4i	0.046k
	10	80	0.67i	0.24ijk
	15	93.3	0.87i	0.40ijk
	20	86.7	2.2hi	0.60ij

Data represent the mean of 5 replicates with 15 explants for each treatment. Means followed by same letter do not differ statistically at p=0.05 according the Duncan's multiple range test.

media. At all salinity level there was an increase in root growth with increase in KNO_3 in growth media and the best ameliorative effect was observed at 20 mM KNO_3.

The ameliorative effect of Ca $(NO_3)_2$ on the growth of tomato shoot tip cultured on salinized half strength Ms media is shown on Table 3 and 4. Similar to potassium experiment, calcium supplementation to the growth media improved roots and shoots characteristics of shoot tip stressed with NaCl. However, the best ameliorative effect of Ca $(NO_3)_2$ was observed at 20 mM Ca $(NO_3)_2$ in NaCl stressed media. This in agreement with result reported by Akinci and Semsek (2004) on ameliorative effect of calcium on the salinity stressed embryo culture of cucumber. In non salinized media the highest shoot and whole plant growth were observed at 10 mM Ca $(NO_3)_2$ (Table, 3). Similar trend was observed for root growth except root % where 20 mM Ca $(NO_3)_2$ resulted in reduction in rooting % (Table, 4). *Jafari et al.* (2009) reported application of 20 mM $CaCl_2$ as well as 20 m*M* KCl to the root medium ameliorated salinity effects on sorghum, especially at moderate and high stress. Franco *et al*. (1999) suggested that Ca^{2+} could have a protective effect in root tips, which is of fundamental importance for the maintenance of root elongation in NaCl

stressed seedlings. 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^{+} (Lynch and Läuchli, 1988).

In conclusion, in salt affected soil fertilization of tomato plants with either potassium nitrate or calcium nitrate could offer an economical and simple solution to the production problems in aridisol caused by high salinity. However, further studies are required in order to determine the efficiency of these materials under natural field condition

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