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In Vitro Selection of NaCl Resistant Mung Bean and Tomato Plants

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MUNG BEAN and tomato were *in vitro* selected on media containing 0, 25, 50, 100 and 150 mM NaCl for possible improving resistance to salinity. Moreover, for choice of better conditions of growth, cotyledons of mung bean were cultured on two different media (choosing hormone supplemented media, CB and hormone free media, MS) whereas cotyledons and shoot apices of tomato were cultured on MS media. The results reveal that NaCl decreased tissue viability, callus initiation, callus proliferation, shoot regeneration capacity, number and height of shoots per explant as well as root regeneration of both species. The magnitude of decrease was most pronounced with higher concentrations, the decrease was lesser in mung bean cultured on CB media than on MS media and in tomato cultured from shoot apices than from cotyledons. Moreover, in green house, NaCl inhibited shoot height and root length as well as fresh and dry weights of shoots and roots of both species grown in pots either *in vitro* selected plants under the different experimental conditions (types of media and explants) or the original intact plants. The effect of NaCl was being lesser on parameters of *in vitro* than those of intact plants that might point to an overcome, to some extent, of these selected plantlets to NaCl. These findings, therefore, could suggest that some resistance to NaCl were developed in *in vitro* selected plants. These observations were more obvious for mung bean selected on CB media and tomato selected from shoot apices concluding that these conditions are better for *in vitro* selection for salinity resistant mung bean and tomato, respectively.

As known, salts affect crop yield and plant development. Future expansion of agriculture must consider the cultivation of saline soils together or the use of water of high salinity content for irrigation. So, the need for salt tolerant crops increased as the growing world population seeks to feed itself. Among the various salts contributing to the general salinity of the soil, NaCl is the most limiting component directly related to the decrease in crop yield. However, tissue and cell culture techniques have been recognized as potentially valuable tools in crop improvement program (Carlson, 1975). *In vitro* selection schemes for the isolation of salt tolerant cell lines have been successful in various crop species (Beloualy & Bouharmont, 1992). *In vitro* culture, besides its use as a tool for obtaining salt tolerant plants, may offer potential for quick evaluation of germplasm against salt stress (Cano *et al.*, 1998).

Mung bean (*Vigna radiata* L.), a newly introduced summer crop in Egypt, is a short duration crop with relatively low fertilizer requirements (Abd El-Lateef *et al.*,1998) .

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Tomato (*Lycopersicon esculentum*), an economic vegetable plant, is a good dicotyledonous plant model for salt tolerance (Tal, 1984). Gulati & Jaiwal (1996) found that calli derived from species of *Vigna* grew better under NaCl stress than those from cultivated species. In confirmation, Kumar & Sharma (1989) reported that 300 mM NaCl proved to be completely inhibitory to growth of the calli of *Vigna radiata*. On incubation for 25 days, cells which could tolerate this concentration grew to form cell clones and the selected callus was capable to grow on medium containing NaCl at the inhibitory concentration. The tolerant callus lines of *Cymbogon martinii* which obtained by exposing to increasing concentration of NaCl (0-300 mM) grew better than the wild-type lines in all concentrations of NaCl tested. The selected lines retained their salt tolerance after 3-4 subcultures on salt-free medium indicating the stability of induced salt tolerance. Therefore, because of the seriousness of salinity problem especially NaCl, the present work was focused on increasing resistance to salinity of two economic crop plants: mung bean as a leguminous crop and tomato as an economic vegetable, through the *in vitro* selection techniques. In addition, the work was aimed also to choose the better media and better explant for selection of mung bean and tomato, respectively.

Material and Methods

Plant Material and Growth Conditions

Pure strain of mung bean (*Vigna radiata* L. Wilczeck Var. Kawmy-1) and tomato (*Lycopersicon esculentum* Var. Super Strain B), respectively supplied from National Research Center, Cairo and from Agriculture Research Center, Ministry of Agriculture, Cairo, Egypt, were used in both tissue culture and intact plant studies. For choice of better conditions of growth, two different media were used for *in vitro* selection of mung bean using cotyledon explants; MS media and CB media (according to Murashig & Skoog, 1962 and Gulati & Jaiwal, 1990, respectively) while two different explants were used for *in vitro* selection of tomato: cotyledons culture and shoot apices according to Iler *et al.* (1993) and Cano *et al.* (1998), respectively.

In vitro selection of NaCl resistant mung bean

Mung bean seeds were rinsed in 70% ethyl alcohol for 1 min, sterilized by 0.1% mercuric chloride solution for 5 min, washed by sterile distilled water several times and rinsed in sterile distilled water for 4 hr. The seeds were germinated in dark at 25±2 ° with 60% humidity on hormone free media, MS basal media (MS salts+B5 vitamins) containing 3% sucrose and 0.8% agar in 70x115 mm glass jars, each jar receives 50 ml media. All media were adjusted to pH 5.8 before autoclaving. Cotyledons of 2 days old germinated seeds were excised under aseptic conditions and cultured on hormone supplemented media (CB media *i.e.*, MS salts+B5 vitamins, 3% sucrose, 0.8% agar and 10^{-5} M benzyl adenine) or on hormone free media (MS basic media containing 3% sucrose, 0.8% agar with no hormone added). Each cotyledon was positioned with the proximal surface and embedded in the media. NaCl was simultaneously added to media to give a final concentrations of 0,

25, 50, 100 and 150 mM. The media were adjusted to pH 5.8 before autoclaving and the cultures were incubated at 25±2 ° for 4 weeks in 16 hr photoperiod (80 μmol m^{-2} s^{-1}). Ten replicates were used for each NaCl concentration and seven cotyledons were cultured per jar (50 jars and 350 cotyledons, for each type of media). After 28 days of incubation, tissue culture parameters (tissue viability, callus initiation, callus proliferation, shoot regeneration, shoot number, and shoot height) were recorded.

In vitro selection of NaCl resistant tomato

Tomato seeds were surface sterilized by 25% (v/v) commercial Clorox solution for 25 min and rinsed in sterile distilled water. The seeds were germinated on basal media (MS salts +B5 vitamins) containing 3% sucrose and 0.8% agar in 70x115 mm glass jars, each jar receives 50 ml media. The jars were kept in the dark at 25±2° with 60% humidity for 3 days then transferred to light (16 hr photoperiod, 76 μE m^{-2} s^{-1}). Two types of explants were used in tomato (cotyledons of 7 days old and shoot apices of 15 days old seedlings). Cotyledons were cultured on MS solid media (pH 5.8) containing 30 g l^{-1} sucrose, 8 g l^{-1} agar, 2.63 mg l^{-1} IAA, 4 mg l^{-1} kinetin. Each cotyledon was positioned with its adaxial surface facing upward. Ten 15x90 mm petri dishes were used for each concentration of NaCl (0, 25, 50, 100 and 150 mM) and eight cotyledons were cultured per a plate (50 plates and 400 cotyledons, in total). Shoot apices 1 cm in length were cultured upright into in 70x115 mm glass jars, each jar receives 50 ml MS basal media containing 30 g l^{-1} sucrose, 8 g l^{-1} agar, 1 mg l^{-1} indole butyric acid. The media were adjusted to pH 5.8 before autoclaving. The cultures were maintained in a growth cabinet at 25±2 ° with 16 hr/day and 600 μE m^{-2} s^{-1}. Tissue culture parameters were monitored after 32 days from incubation.

Root formation

Well developed mung bean shoots of control and NaCl-treated cultures obtained from CB media were transferred to rooting media (MS basal media supplemented with $5x10^{-6}$ M IAA) containing the corresponding concentration of NaCl. In the same time, plantlets selected on MS media were transferred to an elongation MS basal media supplemented also with the corresponding concentration of NaCl for more elongation. For tomato, well developed shoots of control and NaCl-treated cultures regenerated from cotyledons were transferred to solid-hormone free media (MS basal media). Fifty jars were used (ten replicates for each concentration containing seven shoots per jar). Plantlets regenerated from shoot apices were transferred to MS basal media containing also the same corresponding NaCl concentration for more elongation. Cultures of both species were kept in a growth cabinet as previously mentioned. Root regeneration and root length were recorded after 30 days of incubation.

Plant acclimatization

Acclimatization is a very important step towards the production of normal and mature regenerated plants. The plantlets obtained by *in vitro* selection of mung bean (regenerated on the two types of media) and tomato (regenerated from the two types of explants) were washed under running tap water. The bags were removed after one

week. The regenerated plantlets were placed in aquarium condition using Hoagland nutrient solution (Hoagland & Arnon, 1950) containing macronutrients (potassium nitrate 6 mM, calcium nitrate 4 mM, ammonium phosphate 1 mM, magnesium sulphate 2 mM), and micronutrients (boric acid 25 μM, manganese sulfate 20 μM, copper sulfate 0.4 μM, zinc sulfate 0.7 μM, ammonium molybdate 0.2 μM). The plantlets were kept in Hoagland solution till they formed a good rooting system (7 days) before being transplanted in peat-moss pots. After that, plantlets were cultured in plastic pots (25 cm in diameter) containing a mixture of peat-moss and sand (2/1 v/v), covered with transparent polyethylene bags, to keep constant high humidity (90%) and irrigated with Hoagland solution.

Green House Studies

The adapted plants after being irrigated with Hoagland solution for a week, Hoagland solution supplemented with the corresponding levels of NaCl was then used. For intact plant studies, original seeds mung bean and tomato were surface sterilized, thoroughly washed several times and then maintained in distilled water for 4 hr (for mung bean only). Seeds were allowed to germinate in sandy soil in plastic pots (30 cm diameter), each pot contained 5 Kg pre-washed soil. Soil was washed with HCl for removal of salts then washed thoroughly with distilled water several times. The pots were kept under controlled conditions at 32±1° with a 14-h photoperiod (580 $\mu E\ m^{-2}\ s^{-1}$) and 75% relative humidity. Distilled water (50 ml) was applied daily to each pot for the first three days. Thereafter, the pots were irrigated with Hoagland solution daily. When the seeds were in the two-true leaves stage (7 days for mung bean and 21 days for tomato), the seedlings were thinned to 5 plants per pot. The intact seedlings as well as plantlets obtained from *in vitro* selection were irrigated with half-strength Hoagland solution containing 0, 25, 50, 100 and 150 mM NaCl. The *in vitro* selected and intact plants were flushed with an excess of the appropriate solution every two days for three weeks, while half-strength Hoagland solution was used to restore the soil to field capacity. After 21 days from NaCl treatments, all plants were harvested one time, separated into shoots and roots and taken for measurements of growth parameters.

All data of tissue culture parameters and growth parameters were statistically analyzed using the least significant differences (LSD) method (Snedecor & Cochran, 1980).

Results

Changes in tissue culture parameters

As shown in Figure 1, tissue viability appeared alike in untreated mung bean explants either cultured on CB media or on MS media. The same was also shown for tomato cultured from cotyledons or shoot apices. However, CB media resulted in higher magnitudes of callus initiation and callus proliferation of mung bean than did MS media. In the same pattern, greater was the callus initiation and callus proliferation

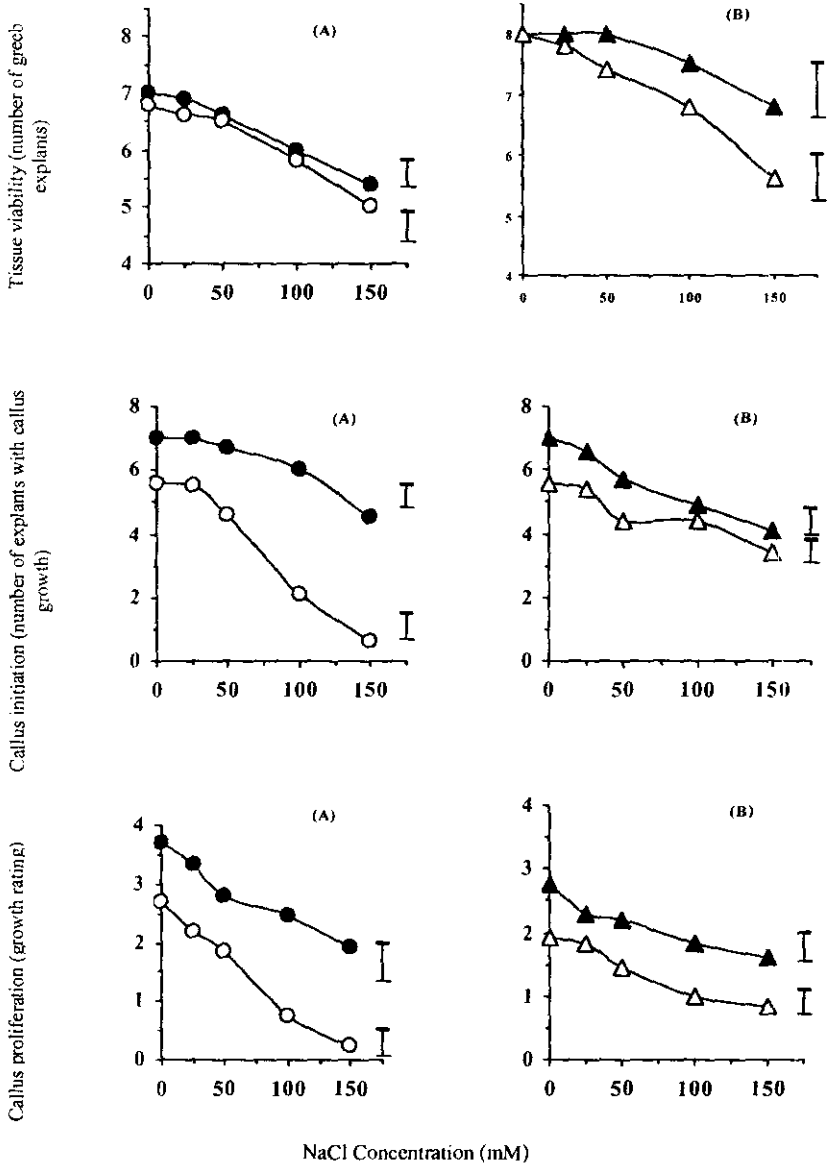

Fig. 1. Effect of NaCl on tissue viability, callus initiation and callus proliferation of *in vitro* cultured mung bean (A) and tomato (B). Two media were used for mung bean; CB (•) and MS media (○) and two explants for tomato; cotyledons (▲) and shoot apices (Δ). Data are means of two experiments. Vertical bars represent LSD values at 5% level.

that formed as cotyledons of tomato were used rather than shoot apices. Nevertheless, increasing NaCl concentrations resulted in gradually and progressively decreases in tissue viability, callus initiation and callus proliferation of mung bean selected on CB media and MS media and also of tomato selected from cotyledons or shoot apices. The higher the NaCl concentration used, the greater was the magnitude of decrease in these parameters. However, the decreased values in these parameters in mung bean were greater as MS media was used relative to CB media. Similarly, tissue viability, callus initiation and callus proliferation of tomato were more affected by NaCl when shoot apices were used for selection relative to cotyledons.

In Figure 2, shoot regeneration and shoot number of mung bean were more expressed on CB media than on MS media. This response was reversed regarding shoot height. On the other hand, cotyledons of tomato resulted in greater shoot regeneration and taller shoots than did shoot apices. The latter explants, on the contrary, gave great numbers of shoots than that given by cotyledons. However, salinity induced significant decreases in shoot regeneration, shoot height and shoot number of both mung bean and tomato under the different conditions. The magnitude of decrease augmented with increasing NaCl concentration. The trends of decrease in shoot regeneration and shoot height appeared alike for both media in mung bean and also for both explants in tomato. On the other hand, shoot number appeared to be unchanged by salinity either in mung bean selected on MS media or in tomato selected from shoot apices. In spite of the great reduction in shoot number of mung bean selected on CB media or of tomato selected from cotyledons, the values still remained higher than the respective condition of either species.

On the other hand, root regeneration and root length of both species were not greatly differed as the media changed for mung bean or as explants changed for tomato (Fig. 3). Root regeneration and root length were, however, significantly decreased by NaCl. The higher concentration of NaCl induced the greatest decrease in root regeneration and the shortest roots.

Growth parameters of in vitro-selected and intact plants

Figure 4 shows shoot height and root length of the regenerated mung bean and tomato plantlets selected from *in vitro* selection under the different conditions. Untreated mung bean plants regenerated on MS media have shoots and roots slightly taller than those regenerated on CB media. Similarly, cotyledons of tomato resulted in slight taller shoots and roots than those regenerated from shoot apices. Treatments of regenerated mung bean and tomato with concentrations of NaCl higher than 25 mM and 50 mM, respectively caused significant decreases in shoot height and root length as compared with the respective control. However, the trends of responses of shoot height and root length in mung bean obtained from the two media and also tomato obtained from the two explants to salinity appeared alike.

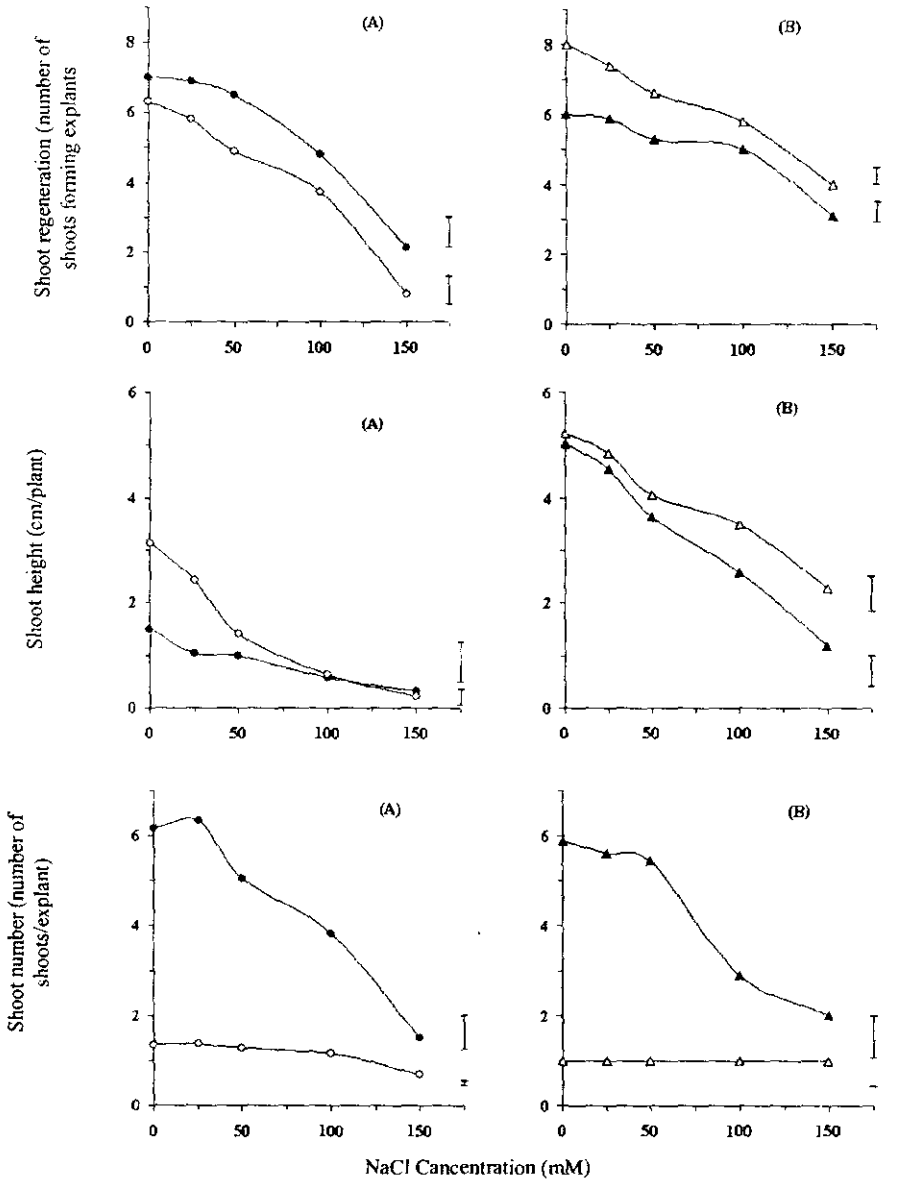

Fig. 2. Effect of NaCl on shoot regeneration, shoot height and shoot number of *in vitro* cultured mung bean (A) and tomato (B). Two media were used for mung bean; CB (•) and MS media (○) and two explants for tomato; cotyledons (▲) and shoot apices (Δ). Data are means of two experiments. Vertical bars represent LSD values at 5% level.

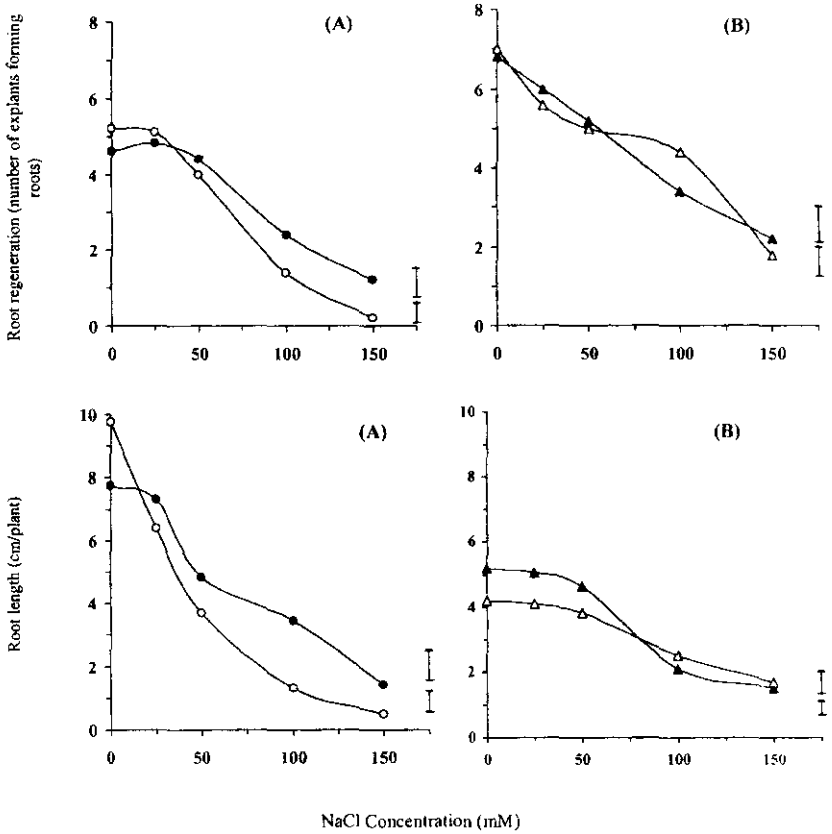

Fig. 3. Effect of NaCl on root regeneration and root length of *in vitro* cultured mung bean (A) and tomato (B). Two media were used for mung bean; CB (•) and MS media (○) and two explants for tomato; cotyledons (▲) and shoot apices (Δ). Data are means of two experiments. Vertical bars represent LSD values at 5% level.

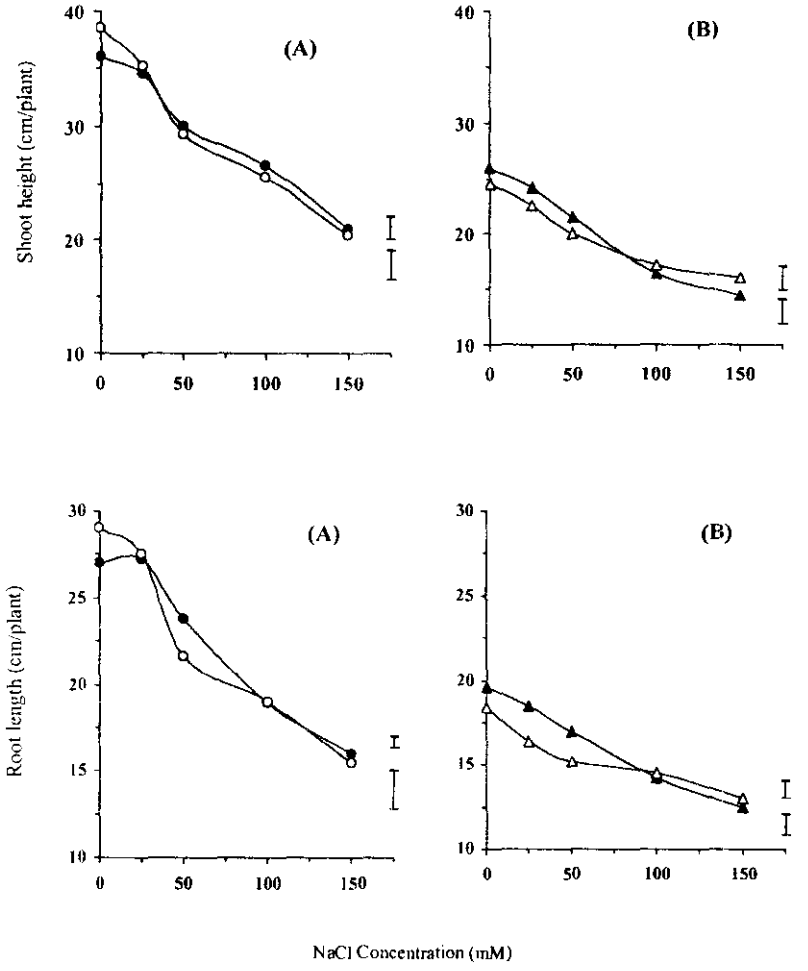

Fig. 4. Effect of NaCl on shoot height and root length of *in vitro* selected of mung bean (A) and tomato (B). Mung bean plantlets were selected on CB (•) and MS media (○) while tomato were selected from cotyledons (▲) and shoot apices (Δ). Data are means of two experiments. Vertical bars represent LSD values at 5% level.

In the same manner, NaCl resulted in significant reductions in fresh weights of shoots and roots of *in vitro*-selected mung bean on CB or MS media from 50 mM onward and of *in vitro*-selected tomato from cotyledons or shoot apices from 100 mM NaCl onward (Fig. 5). The magnitude of reduction increased with increasing NaCl concentrations as compared with the respective control. However, fresh weight of mung bean plants cultured on MS media was being more affected by NaCl than plants cultured on CB media at all used NaCl concentrations. Similarly, fresh weight of shoots and roots of *in vitro*-selected tomato regenerated from shoot apices rather than cotyledons was being more reduced by NaCl.

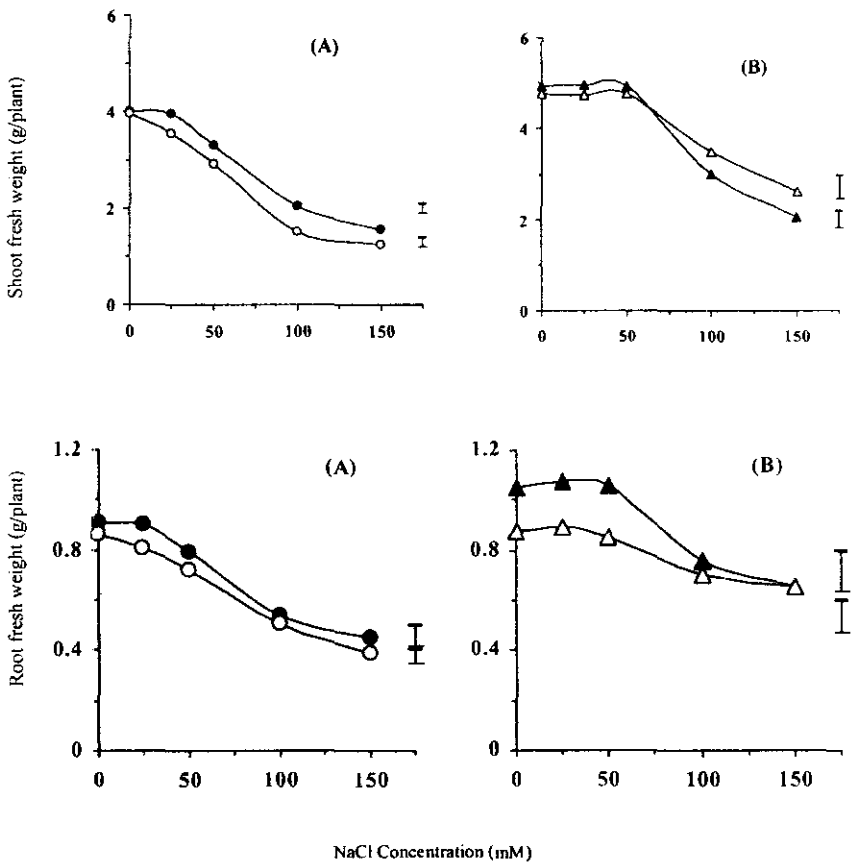

Fig. 5. Effect of NaCl on fresh weight in shoots and roots of *in vitro* selected mung bean (A) and tomato (B). Mung bean plantlets were selected on CB (•) and MS media (○) while tomato were selected from cotyledons (▲) and shoot apices (△). Data are means of two experiments. Vertical bars represent LSD values at 5% level.

As Figure 6 shows, dry weight of shoots and roots of the *in vitro*-selected mung bean on CB or MS media and *in vitro*-selected tomato from cotyledons or shoot apices were significantly decreased by higher NaCl concentrations (100 and 150 mM). The behaviour of dry weight response seemed alike for mung bean plants selected on the two different media and also for tomato selected from both explants. Anyway, dry weight of shoots and roots appeared higher in mung bean selected on CB media and in tomato selected from cotyledons.

Growth parameters (shoot height and root length as well as fresh and dry weights of shoots and roots) of intact mung bean and tomato are shown in Figure 7. It clear from the figure that these parameters of growth in mung bean and tomato were significantly decreased by NaCl. Shoot height of both species particularly mung bean was progressively decreased with increasing NaCl concentrations. Similar decreases but to a lesser extent were observed in root length. On the other hand, there was a gradual and progressive decrease in fresh and dry weights of shoots of both species as NaCl increased while a steady, if any, change in both parameters in roots was detected.

Percentage of changes in growth parameters of intact plants and in vitro-selection of two species under 150 mM NaCl medium

Inspection of Table 1 shows that the percentages of reduction in mung bean shoot height selected from *in vitro* on CB or MS media with the presence of 150 mM NaCl were respectively 42% and 47% relative to 58% in intact plants. In tomato, the percentages of shoot reduction of plants regenerated from cotyledons or shoot apices were respectively 44% and 35% against 41% in intact plants. On the contrary, roots of *in vitro* selected plants appeared shorter than that of intact plants. However, the decreases in root length of selected mung bean on both media or tomato on both explants were respectively 41% and 47% or 36% and 29% against 38% or 34% in the respective intact plants. Moreover, the table clearly indicates that shoot fresh weight of mung bean selected on CB or MS media in presence of 150 mM NaCl was decreased by 55% or 63%, respectively against 72% in intact plants. In tomato, shoot fresh weight of plants selected from cotyledons and shoot apices was decreased by 52% and 45%, respectively against 59% in intact plants. Regarding roots, the percentages of decrease were 41% and 47% in mung bean relative to 55% in intact plants and 34% and 22% in tomato opposite to 42% in intact plants. These observations support the greater reductions in growth of intact plants rather than in *in vitro* selected plants as NaCl was applied. In support, 150 mM NaCl reduced shoot dry weight of *in vitro* selected mung bean by 42% and 45% for both media versus 59% in intact plants. Also, NaCl reduced shoot dry weight of *in vitro* selected tomato by 33% and 26% for both explants against 51% in intact plants. The same was also shown in roots. The reduced dry weights by NaCl decreases in root dry weight were 37% and 43% against 58% in mung bean and 30% and 25% against 43% in tomato.

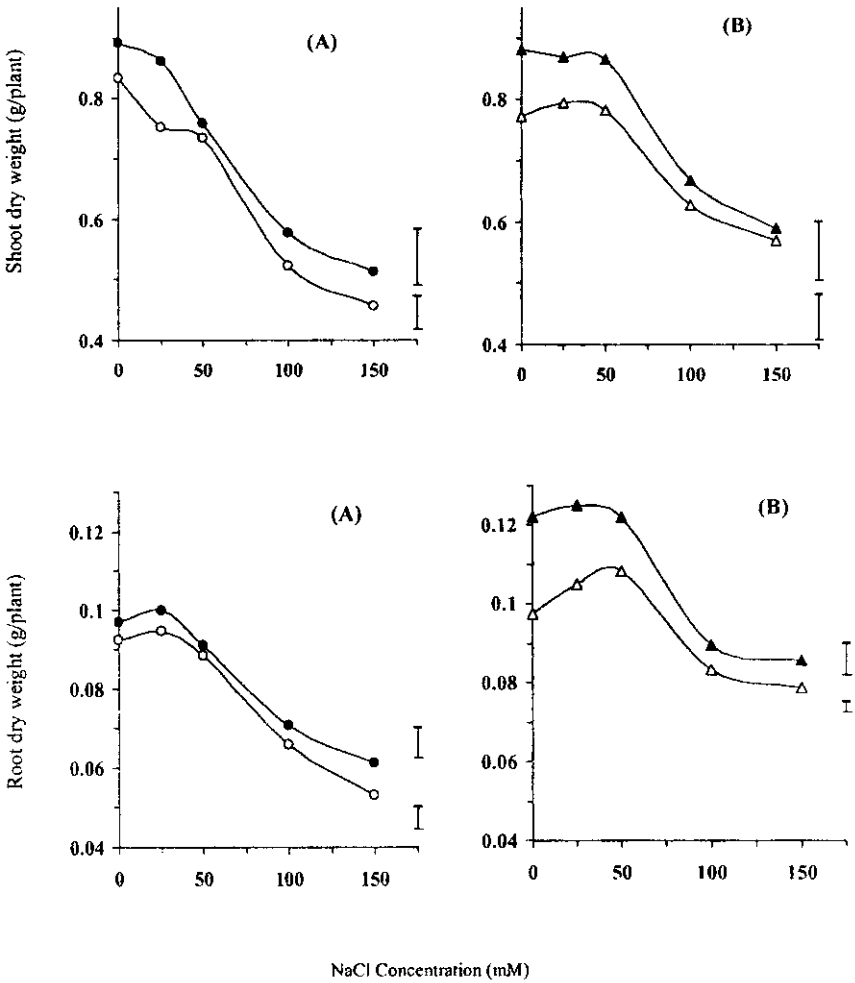

Fig. 6. Effect of NaCl on dry weight in shoots and roots of *in vitro* selected mung bean (A) and tomato (B). Mung bean plantlets were selected on CB (•) and MS media (○) while tomato were selected from cotyledons (▲) and shoot apices (△). Data are means of two experiments. Vertical bars represent LSD values at 5% level.

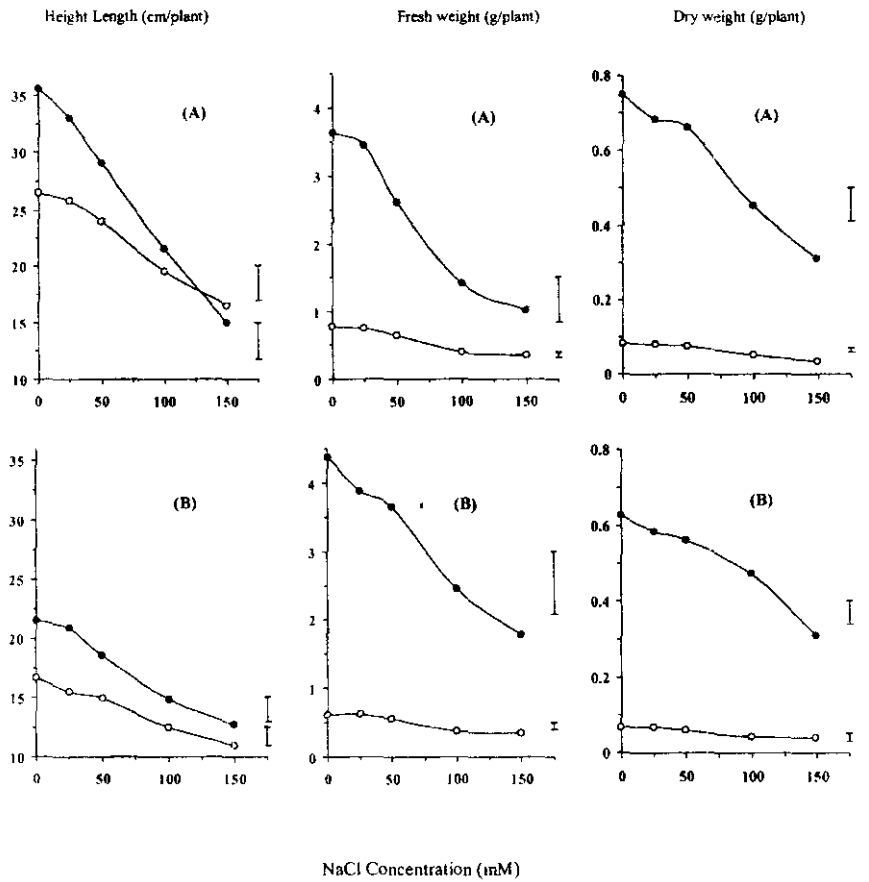

Fig. 7. Effect of NaCl on shoot height and root length as well as fresh and dry weights of intact mung bean (A) and tomato (B) shoots (•) and roots (○). Data are means of two experiments. Vertical bars represent LSD values at 5% level.

Discussion

Growth was used to characterize the overall tolerance of plants to salinity. The present results revealed that NaCl treatments decreased the survival explants in mung bean and tomato. CB media had the capability to produce callus mung bean than MS media. Also, cotyledons of tomato expressed higher tissue viability than shoot apices. Callus initiation and callus proliferation of both species were decreased due to salinity treatment. In the mean time, shoot regeneration was greatly decreased

in mung bean on both tested media by 50 mM NaCl onward and in tomato from cotyledons or shoot apices by 100 mM NaCl onward. In this respect, Gulati & Jaiwal (1993) reported that tissue viability was decreased with increasing NaCl concentrations and only 33.3 % of the explant survived at 200 mM NaCl. Similarly, Gulati & Jaiwal (1993) reported that the amount of callus produced at the proximal end of explants of mung bean decreased by increasing the NaCl above 25 mM. Also, Mercado *et al.* (2000) found that at higher NaCl levels, the explants of two tomato cultivars were necrotic and died after few weeks of culture. In support, Gulati & Jaiwal (1995) reported that the growth of non-selected and selected cells of mung bean was declined sharply at 100 mM NaCl and decreased further with increase in NaCl concentration.

TABLE 1. The percentage of changes, relative to the respective control, in shoot height and root length as well as in fresh and dry weights of shoots and roots of either intact or *in vitro* selected mung bean and tomato as a consequence of treatment with 150 mM NaCl. Two media were used for *in vitro* selection of mung bean using cotyledon explants (hormone supplemented media, CB and hormone free media, MS) while two explants were used for *in vitro* selection of tomato (cotyledons and shoot apices).

	Mung bean			Tomato		
	Intact	*In vitro*		Intact	*In vitro*	
		CB	MS		Cotyledons	Shoot apices
Shoot height	-58	-42	-47	-41	-44	-35
Root length	-38	-41	-47	-34	-36	-29
Shoot fresh weight	-72	-55	-63	-59	-52	-45
Root fresh weight	-55	-41	-47	-42	-34	-22
Shoot dry weight	-59	-42	-45	-51	-33	-26
Root dry weight	-58	-37	-43	-43	-30	-25

In addition, Cano *et al.* (1996) reported that the callus fresh weights of two tomato cultivars were reduced by salinity. Yusuf *et al.* (1994) also found that callus differentiation was inhibited progressively as salinity increased. Barakat & Abdel-Latif (1995) found that the relative growth of callus was significantly influenced by NaCl concentrations. Moreover, Barakat & Abdel-Latif (1996) reported that the growth of callus on medium containing 12 g/l NaCl became necrotic after five subcultures and after placing it on regeneration media without producing a shoot

regeneration. Sabbah & Tal (1995) reported that addition of NaCl to the media reduced growth of callus in three genotypes of Solanum (*Solanum tuberosum*) and the wild species *Solanum kurzianum*.

Decline of the callus growth due to NaCl stress is described as a usual phenolmenon in rice and other plant tissues subjected to stress (Basu *et al.*, 1997). They suggested that this retardation of growth may be due to the fact that certain amount of the total energy available for tissue metabolism is channeled to resist the stress. They further indicated that the regeneration was low in 85 mM NaCl but a concentration of 128 mM was inhibitory to regeneration. In addition, Gulati & Jaiwal (1993) reported that at 150 mM NaCl, out of 90% explants that survived only 16.6% regenerated an average of 2 shoots per culture and NaCl beyond 150 mM caused complete inhibition of shoot organogenesis. Li &Heszky (1986) reported that the ability for redifferentiation of the salt selected callus lines of rice was strongly inhibited by the presence of NaCl in the regeneration medium. In confirmation, the high regeneration potential of rice (59.6%) on salt free medium decreased rapidly with increasing concentration of salt in the regeneration medium, roots developed very well in salt free condition, less in 0.5 and poorly in 0.75% NaCl (Binh *et al.*, 1992). These observations are in accordance with the results depicted herein. In fact, Shoots regeneration capacity in terms of explants forming shoots of both species decreased with increasing NaCl concentration. Consequently, the regenerated shoots on NaCl medium were stunted with small leathery and light-green leaves. Moreover, NaCl not only affected the amounts of callus produced at the proximal end of explants, but also delayed the differentiation of shoots by 7-10 days.

In addition, Mercado *et al.* (2000) found that the presence 86 mM of NaCl in the regeneration media strongly inhibited shoot regeneration and the number of regenerated shoots per explant in two tomato cultivars. The results reported herein, moreover, emphasized the influence of NaCl on roots regeneration of both species. Root regeneration of NaCl treated plants was significantly decreased by higher concentrations (100 mM and 150 mM). Root regeneration was more expressed in mung bean selected on CB media and in tomato selected from shoot apices. However, root length of both species was decreased by increasing salt concentration. In this connection, Cano *et al.* (1998) found that rooting of *Lycopersicon esculentum* was reduced by salinity thus only about 35% of species developed roots at 70 mM NaCl.

Regarding green house study, NaCl also induced reductions in shoot height and root length of mung bean and tomato of the *in vitro* selected plantlets. The magnitude of reduction was being most pronounced in respone to higher levels of NaCl (100 mM and 150 mM). Supporting these findings, Misra *et al.* (1996) found that, shoot and root length and fresh mass of mung bean decreased with salinity (0, 0.5, 1, 2 and 3%). Similar results were also found in mung bean (Hafeez *et al.*, 1988) *& Brassica* (He & Cramer, 1996). As a whole, the reduction of shoot height of *in vitro*-selected mung bean and tomato by NaCl appeared lower than that of intact non selected plants.

Also, NaCl particularly with the higher levels significantly reduced fresh and dry weights of shoot and root *in vitro*-selected plant of both species. In accordance with these results, Younis *et al.* (2003) found that shoot height, root length and dry weight of *Vigna sinensis* and *Zea mays* were mostly suppressed by salinization using artificial seawater mixture. In addition, Imamul-Huq & Larher (1983) noted that *Phaseolus aureus* showed decreases in fresh and dry weights in their shoots and roots in the presence of NaCl. Ashraf & Rasul (1988) reported that increasing salt concentration significantly reduced lengths, fresh and dry weights of shoots and roots of two cultivars of mung bean. Gill (1990) reported that increasing salinity levels decreased the fresh weight:dry weight ratio of *Vigna radiata.* He attributed the deleterious effect of salinity on shoot height, root length and fresh and dry weight to increasing the osmotic pressure. Also Abd El-Samad (2002) found that there is significant drop in fresh and dry matter of shoots and roots of tomato with increasing salinity. Sudhakar *et al.* (1990) found that dry weight of *Vigna radiata* shoots and roots was decreased by NaCl and Na_2SO_4. On the other hand, fresh weight of *Chenopodium quinoa* was reduced by salinity in both embryonic axes and cotyledons (Prado *et al.*, 2000).

The present results clearly indicate that the magnitude of decrease in fresh and dry weight contents as well as shoot height and root length of intact plants of both species by NaCl were greater than those of *in vitro* selected plants. These results could indicate a delay or a retraction in the effects of NaCl on growth of mung bean and tomato following *in vitro* selection. The delay of NaCl effect following *in vitro* selection might point to an overcome, to some extent, of these selected plantlets to the effect of NaCl. These findings, therefore, might lead to a suggestion that some resistance to NaCl were developed in *in vitro* selected plants. These observations were obvious for mung bean regenerated on CB media and tomato regenerated from shoot apices. Consequently, it could be concluded that CB is better than MS media and also shoot apices explants are better for *in vitro* selection of mung bean and tomato, respectively.

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References

Abdel-Lateef, E.M.; Ashour, N. I. and Farrage, A.A. (1998) Effect of foliar spray with urea and some micronutrients on mung bean [*Vigna radiata* (L.) Wilczek] growth, yield and seed chemical composition. *Bull. NRC, Egypt* **23** (2), 219.

Abd El-Samad, H.M. (2002) Salt tolerance and interaction with abscisic acid of tomato cultivars. *Bull. Fac. Sci. Assiut Univ.* **31**(I-D), 205.

Ashraf, M. and Rasul, E. (1988) Salt tolerance of mung bean (*Vigna radiata* (L.) Wilczek) at two growth stages. *Plant and Soil* **110** , 63.

Barakat, M.N. and Abdel-Latif T.A. (1995) *In vitro* selection for salt tolerant lines in wheat. I. Effect of salt on the embryogenic cultures. *Alex. J. Agric. Res.* **40**, 77.

Barakat, M.N. and Abdel-Latif, T.H. (1996) *In vitro* selection of wheat callus tolerant to high levels of salt and plant regeneration. *Euphytica* **91**, 127.

Basu, S.; Gangopadhaya, G.; Mukherjee, B. B. and Gupta, S. (1997) Plant regeneration of salt adapted callus of indica rice (*Var. Basmati* 370) in saline conditions. *Plant Cell, Tissue and Organ Cult.* **50**, 153.

Beloualy, N. and Bouharmont, J. (1992) NaCl tolerant plants of *Poncirus trifoliata* regenerated from tolerant cell lines. *Thear. Appl. Genet.* **83**, 509.

Binh, D.Q.; Heszky, L. E.; Gyulai, G. and Csillag, A. (1992) Plant regeneration of NaCl-pretreated cells from long-term suspension culture of rice (*Oryza sativa* L.) in high saline conditions. *Plant Cell, Tissue and Organ Cult.* **29**, 75.

Cano, E.A.; Perez-Alfocea, F.; Moreno, V.; and Bolarin, M.C. (1996) Responses to NaCl stress of cultivated and wild tomato species and their hybrids in callus cultures. *Plant Cell Reports* **15**, 791.

Cano, E.A.; Perez-Alfocea, F.; Moreno, V.; Caro, M. and Bolarin, M. C. (1998) Evaluation of salt tolerance in cultivated wild tomato species through *in vitro* shoot apex culture. *Plant Cell, Tissue and Organ Cult.* **53**,19.

Carlson, P.S. (1975) Crop improvement through techniques of plant cell and tissue cultures. *Bioscience* **25**,747.

Gill, K.S. (1990) Effect of saline irrigation at various growth stages on growth, yield attributes accumulation pattern in green gram (*Phaseolus radiatus*). *Indian J. of Agric. Sci.* **60** (4), 280.

Gulati, A. and Jaiwal, P.K. (1990) Culture conditions effecting plant regeneration from cotyledon explants of *Vigna radiata* (L.) Wilczek. *Plant Cell, Tissue and Organ Cult.* **23**, 1.

Gulati, A. and Jaiwal, P.K. (1993) *In vitro* selection of salt-resistant *Vigna radiata* (L.) Wilczek plants by adventitious shoot formation from cultured cotyledon explants. *J. Plant Physiol.* **142**, 99.

Gulati, A. and Jaiwal, P.K. (1995) Changes in growth, ions and metabolites in two callus lines of *Vigna radiata* which differ in salt tolerance. *J. Agronomy and Crop Science* **175**, 325.

Gulati, A. and Jaiwal, P.K. (1996) Effect of NaCl on nitrat reductase, glutamate dehydrogenase and glutamate synthase in *Vigna radiata* calli. *Bio. Plant.* **38** (2), 177.

Hafeez, F.Y.; Aslam, Z. and Malik, K.A. (1988) Effect of salinity and inoculation on growth, nitrogen fixation and nutrient uptake of *Vigna radiata* (L.) Wilczek. *Plant and Soil* **106**, 3.

He, T. and Cramer, G.R. (1996) Abscisic acid concentrations are correlated with leaf area reductions in two salt-stressed rapid cycling *Brassica* species. *Plant and Soil* 179.

Hoagland, D.R. and Arnon, D.I. (1950) The water culture method for growing plants without soil. Calif. *Agric. Exp. Sta. Circ.* **347**, 32.

Iler, S.E.; Swanton, C.J. and Peter Pauls, K. (1993) *In vitro* selection of Imazthapyr-tolerant tomato (*lycopersicon esculentum* mill). *Weed Sci.* **41**,12.

Imamul-Huq, S.M. and Larher, F. (1983) Osmoregulation in higher plants: Effect of NaCl salinity on non-nodulated *Phaseolus aureus* L. I. Growth and mineral content. *New Phytol.* **93**, 203.

Kumar, V. and Sharma, D.R. (1989) Effect of exogenous proline on growth and ion content in NaCl stressed and non stressed cells of mung bean, *Vigna radiata* Var. *Radiata. Indian J. Exp. Biol.* **27**(9), 813.

Li, S.N. and Heszky, L.E. (1986) Testing of salt (NaCl) tolerance and regeneration in callus culture (n, 2n) of rice. In "*Genetic Manipulation in Plant Breeding* ", W. Horn, JC. Jensen; W. Odenbach and O. Schieder (Ed.), pp. 617-619, Walterde Gruyter and Co, Berlin-New York.

Mercado, J. A.; Sancho-Carrascosa, M. A.; Jimenez, B. S.; Peran-Quesada, R.; Pliego-Alfaro, F. and Quesada, M. A. (2000) Assessment of *in vitro* growth of apical stem sections and adventitious organogenesis to evaluate salinity tolerance in cultivated tomato. *Plant Cell, Tissue and Organ Cult.* **62**, 101.

Misra, A.N.; Murmu, B.; Singh, P. and Misra, M. (1996) Growth and proline accumulation in mung bean seedlings as affected by sodium chloride. *Biol. Plant.* **38**(4), 531.

Murashige, T. and Skoog, F. (1962) A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol. Plant.* **15**, 472.

Prado, F.E.; Boero, C.; Gallardo, M. and Gonzalez G. A. (2000) Effect of NaCl on germination, growth, and soluble sugar content in *Chenopodium quinoa* willd. *Seeds Bot. Bull. Acad. Sci.* **41**, 27.

Sabbah, S. and Tal, M. (1995) Salt tolerance in *solanum kurzianum* and *S. tuberosum* cvs Alpha and Russet Burbank. *Potato Res.* **38**, 319.

Snedecor, W. and Cochran, G. (1980) *"Statistical Methods"*, 7th ed., The Iowa State Univ. Press, USA.

Sudhakar, C.; Reddy, P. S. and Vecraneyulu, K. (1990) Effect of salt stress on dry matter production and mineral content during growth of horsegram (*Macrotyloma uniflorum* (Lam.) Verdc.) and green gram (*Vigna radiata* L. Wilczek). *Plant Physiol. Biochem.* **17**(2), 88, *New Delhi* .

Tal, M. (1984) Physiological genetics of salt resistance in higher plants: Studies on the level of the whole plant and isolated organs, tissues and cells. In "*Salinity Tolerance in Plants*", R.C. Staples and G.H. Toenniessen (Ed.), pp. 301-20, New York, John Wiley & Sons .

Younis, M.E.; El-Shihaby, O.A.; Nemat Alla, M. M. and El-Bastawisy, Z. M. (2003), Kinetin alleviates the influence of water logging and salinity on growth and affects the production of plant growth regulators in *Vigna sinensis* and *Zea mays*. *Agronomie* **23**, 277.

Yusuf, A.; Li, S. J. and Li, S. X. (1994) *In vitro* flowering, fruiting and differentiation of callus in different genotypes of tomato in the presence of NaCl. *Sarhad J. Agri.* **10** (1), 59.

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استنباط نباتات فول المانج والطماطم مقاومة لكلوريد الصوديوم باستخدام تقنية زراعة الأنسجة

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يتضمن البحث دراسة تحسين مقاومة نباتى فول المانج (سلالة قومى ١) و الطماطم (سلالة سوبر سترين ب) للملوحة باستخدام تقنية زراعة الأنسجة. تم زراعة فلقات فول المانج على وسطين غذائيين مختلفين أحدهما يحتوى على الهرمون والأخر خالى منه وذلك لاختيار أنسب ظروف النمو فى وجود تركيزات مختلفة من كلوريد الصوديوم. كما تم استخدام كلا من الفلقات أو القمم النامية لزراعة الطماطم على أوساط غذائية تحتوى على تركيزات مختلفة من كلوريد الصوديوم. أوضحت النتائج أن كلوريد الصوديوم أحدث نقصا فى حيوية الأنسجة ووجود الكالوس ومعدل نموه و كذلك تمايز السيقان و الجذور و طولهما لكلا النباتين. و كان هذا النقص أوضح ما يكون فى حالة استخدام التركيزات العالية من كلوريد الصوديوم. و كان هذا النقص أقل لعينات فول المانج المزروعة على الوسط الغذائى المحتوى على الهرمون منه عن المزروعة على الوسط الغذائى الخالى من الهرمون. وكذلك نمت عينات الطماطم الناتجة من القمم النامية بصورة أفضل من تلك الناتجة من الفلقات. أما فى الصوبة فقد أدت المعاملة بكلوريد الصوديوم الى نقص فى طول كلا من الساق والجذر وكذلك الوزن الغض و الوزن الجاف للسيقان والجذور فى كلا النباتين سواء النباتات النامية فى الأصص او المنتخبة فى البيت الزجاج تحت الظروف المختلفة. وكان تأثير كلوريد الصوديوم أقل على دلالات النمو للنباتات المنتخبة فى الزجاج عنه فى النباتات النامية فى الأصص. وتدل هذه النتائج على استطاعة هذه النباتات المنتخبة فى الزجاج على مقاومة الملوحة. وكانت هذه المقاومة أوضح لنباتات فول المانج النامية على الوسط الغذائى المحتوى على الهرمون و كذلك الطماطم الناتجة من القمم النامية مما يؤكد على أنسب ظروف نمو هذين النباتين فى الزجاج.