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Research Article

Improving Salt Tolerance in Tomato *Lycopersicon esculentum* Mill. by *in Vitro* Mutation Technique

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Abstract: This research was conducted to produce salt tolerant tomato plants by using mutation and tissue culture techniques. Seeds of two Tomato cultivars (Super Regina and Trescantos) were treated with 2.0 mM of Sodium Azide NaN_3 for 4 h and sterilized with Sodium Hypochlorite (NaOCl) of (0.0, 1.0 or 3.0)%. The treated seeds were germinated on (MS) medium and shoot tips (1 cm) were used to induce calli on media supplemented with different hormones combinations. The produced calli were cultured on media of (6.5, 8.5, or 10.5) dSm^{-1} sodium chloride. Plants were then regenerated from the survival calli. The results showed that 3% NaOCl was the best sterilization treatment. Moreover the best growth regulator combinations were either 2 mg /L BA with 2 mg/L IAA or 0.5 mg of BA with 1 mg/L IAA. Since there was no significant difference between them, the last was recommended. Furthermore, salinity had negative effect on callus growth and (6.5 dSm^{-1}) treatment showed significantly higher callus fresh and dry weights than the other treatments. No plants were produced from 10.5 dSm^{-1} treatment and Super Regina showed higher percentage of plant regeneration than Trescantos. Calli grown on 10.5 dSm^{-1} contained the highest Sodium and Chloride ions. Trescantos cultivar showed significantly higher calli sodium ions content compared with Super Regina. However, no significant differences between the two cultivars in the Chloride ion content were found. In conclusion it has been possible to induce and select salt tolerant tomato plants via *in vitro* mutation technique.

Keywords: BA, IAA, shoot tip, sodium azide, sodium chloride

INTRODUCTION

Tomato (*Lycopersicon esculentum* Mill.) is one of the important vegetable crops of the Solanaceae family. Tomato is grown throughout the world due to its high contents of vitamins, minerals and antioxidants. Several breeding methods were used to improve its quantity and quality (Flowers, 2004; Foolad, 2004; Estan *et al*., 2005; Cuartero *et al*., 2006). However its yield is still severely affected by environmental stresses such as high temperature, drought, salinity, insects and various diseases.

High salinity stress is the most severe environmental stress, which affect crop production on at least 20% of irrigated land worldwide. Also salinity is the major problem in Iraq which has great impact on tomato production. Thus there is a need to improve the ability of tomato plants to tolerate soil salinity using any available breeding methods.

The fast method is through the selection from the genetic pool of this plant. However increasing the variations via chemical and physical mutation among the available cultivars will broaden the gene pool. Moreover tissue culture provides uniform environment for the selection under any stress. Combination of chemical mutation and *in vitro* selection has been used successfully by many researchers to select plants with desirable characteristics (Al-Hattab *et al*., 2002; Aslid *et al*., 2006; Shlahy and Al-Hattab, 2009a; Shlahy and Al-Hattab, 2009b). Therefore the current experiment was conducted to select tomato plants with good salt tolerance ability using *in vitro* chemical mutation and selection method.

MATERIALS AND METHODS

Two tomato cultivars (Super Regina and Trescantos) were used in this experiment. Mature dry seeds were treated with 2.0 mM Sodium Azide (NaN_3) in phosphoric buffer of pH 3 for 4 h. The seeds were sterilized for one mint with 70% ethanol and then with

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Table 1: Effect of sodium hypochlorite concentration on contamination percentage of the seeds of two tomato cultivars

Sodium hypochlorite %	Contamination % cultivar Trescantos	Super Regina	Mean
0.0	100.0	100.0	100
1	62.0	46.0	54.0
3	0.0	0.0	0.0
Mean	54.0	48.7	

L.S.D (0.05) cultivar = 4.3 Interactions = 7.5 sodium hypochlorite = 5.4

Sodium Hypochlorite (0, 1, or 3 %) for 15 min and rinsed three times with distilled water 5 min each. The sterilized seeds were cultured on hormone free MS medium (Murashige and Skoog, 1962) supplemented with 30 g/L sucrose and 7 g/L agar to produce seedlings. Percentage of contamination was recorded after one week in culture. Shoot tips (1 cm) were excised from fourteen days old seedlings and used for callus induction. Shoot tips were cultured on MS media supplemented with different combinations of Benzyl adenine (BA) of (0.0, 0.5, 1.0, or 2.0 mg/L) and Indol Acetic Acid (IAA) of (0.0, 1.0, or 2.0 mg/L) with ten replications per treatment. Growth regulators effect on callus fresh weight was analyzed to select the best hormone combination.

Ten replications with (100 mg) /tube of the produced calli from all the above treatments were transferred to the selected medium with the addition of Sodium Chloride (NaCl) of (6.5, 8.5, or 10.5 dSm^{-1}). After 6 weeks in culture, callus fresh and dry weights were recorded. The amounts of Sodium and Chloride ions in the dry calli of three replications were estimated according to Perez-Alfocea *et al*. (1994). Survival calli were transferred to regeneration medium supplemented with Sodium Chloride of the corresponding concentration. Numbers of regenerated plants from all the treatments were calculated.

The experiments were setup using (C.R.D) and the means were compared using Least Significant Differences (L.S.D) at $p \leq 0.05$ level.

RESULTS AND DISCUSSION

The results showed (Table 1) that 3% Sodium Hypochlorite was the best sterilization treatment that reduced seed contamination to 0% in both cultivars Trescantos and Super Regina. Therefore 3% Sodium Hypochlorite was used throughout this experiment. Sterilization is the most important step to start successful culture. The disinfectant should not affect the viability of the explants and provide 100% sterilization.

The results (Table 2) showed that the growth regulators had significant effect on the fresh weight of the two cultivars. The medium with 2 mg/L BA showed significantly higher callus fresh weight in both cultivars compared with the other treatments. Moreover 1 and 2 mg/L of IAA gave significantly higher fresh weights than the control and there were no significant differences between them in both cultivars. Whereas the interaction analysis showed that the combination of 2 mg/L of both BA and IAA was the best for callus induction for Trescantos and Super Regina cultivars. However this treatment was not significantly different than the combination of 0.5 mg/L BA and 1 mg/L IAA. Therefore from this result it has been recommended to use the last treatment for callus induction. The results of the current study are in agreement with what was reported by other researchers that the cultivars have different callus induction response in the presence of different hormone combinations (El-Kaaby *et al*., 2012; Shlahi, 2003).

The saline medium had negative effect on callus proliferation in both cultivars (Table 3). The control medium of 6.5 dSm^{-1} showed significantly higher fresh weight compared with the medium of 8.5 dSm^{-1} and 10.5 dSm^{-1} salt levels. Moreover the control gave significantly higher dry weight compared with the other media. However Trescantos cultivar gave higher fresh and dry weights compared with Super Regina but they were not significant. Salt tolerant is complicated trait and controlled by multiple genes. Thus the ability of the cultivars to tolerate the salt level depends on the number of salt tolerant genes that present in their genomes. The mutation effect of sodium azide might induce some genetic changes in the seeds which are reflected by the variation among the ex-plans in survival ability of the callus under the salt stress.

Table 2: Effect of growth regulators on callus fresh weight (mg) of two Tomato cultivars

	Trescantos				Super Regina			
	IAAmg/l				IAAmg/l			
BAmg/L	0	1	2	Mean	0	1	2	Mean
0	0	126.9	67.4	64.8	0	133.5	67.1	66.9
0.5	59.0	216.0	170.5	148.5	57.0	220.5	176.9	151.5
1	98.4	133.4	185.6	139.1	105.3	138.0	195.5	146.3
2	122.4	182.9	230.5	178.6	127.2	187.6	235.9	183.6
Mean	69.9	164.8	163.5		72.4	169.9	168.9	

Trescanto L.S.D (0.05) IAA = 16.14 BA = 18.64, Interaction = 32.29, Super Regina L.S.D (0.05) IAA = 16.30 BA = 18.82 Interaction = 32.60

Table 3: Effect of salt level on fresh and dry weight (mg) of two tomato cultivars after 6 weeks in culture

Salt levels dSm^{-1}	Fresh weight (mg) Trescantos	Fresh weight (mg) Super Regina	Mean	Dry weight (mg) Trescantos	Dry weight (mg) Super Regina	Mean
6.5	356.6	321.4	339.0	34.1	30.2	32.1
8.5	272.9	266.9	269.9	29.0	26.5	27.8
10.5	218.7	221.4	220.0	18.8	18.2	18.5
Mean	282.7	269.9		27.3	25.0	

FW L.S.D (0.05) Cultivars = 16.8 Salt Level = 20.6 Interaction = 29.2, DW L.S.D (0.05) Cultivars = 2.8 Salt Level = 3.5 Interaction = 4.9

Table 4: Sodium ions (Na^+) concentration (mg/g dry weight) in the Tomato calli grown under salt stress

Salt level dSm^{-1}	Na^+ Concentration mg/g, Tomato cultivars: Trescantos	Super Regina	Mean
6.5	3.80	2.55	3.1
8.5	8.13	6.41	7.2
10.5	22.18	14.61	18.3
Mean	11.30	7.80	

L.S.D $_{(0.05)}$ cultivars = 2.1 interaction = 6.2 salt levels = 3.5; *Each number represents mean of three replicates

Table 5: Chloride ions concentration (mg/g dry weight) in the Tomato dry calli grown under salt stress

Salt level dSm^{-1}	Cl^- Concentration mg/g, Tomato Cultivars: Trescantos	Super Regina	Mean
6.5	2.41	2.58	2.49
8.5	4.05	3.91	3.98
10.5	5.17	6.68	5.92
Mean	3.88	4.39	

L.S.D $_{(0.05)}$ cultivars = 0.65 interaction = 1.13 salt levels = 0.80; *Each number represents mean of three replicates

Table 6: Percentage of plant regeneration from tomato grown under salt stress

Salt level dSm^{-1}	Tomato cultivars: Trescantos	Super Regina	Mean
6.5	23.6	28.4	26.0
8.5	17.0	17.8	17.4
10.5	0.0	0.0	0.0
Mean	13.5	15.4	

L.S.D $_{(0.05)}$ cultivars = 1.9 interaction = 3.3 salt levels = 2; *Each number represents mean of plant regeneration percentage of ten replicates

The results in Table 4 and 5 showed significantly higher accumulation of Na^+ and Cl^- ions in the dry calli as the salinity level increased in the medium. The 10.5 dSm^{-1} showed the highest concentration of Na^+ compared with the calli grown on medium with 6.5 dSm^{-1}. Moreover Trescantos cultivar showed significantly higher accumulation of Na^+ compared with Super Regina. Also the interaction analysis showed that Trescantos cultivar grown on medium with 10.5 dSm^{-1} salinity level contained the highest Na^+ concentration (Table 4). This result indicated the ability of Trescantos cultivar to accumulate Na^+ in the cells with less effect on callus growth which is one of the mechanisms used by the plants to avoid the effect of high salinity levels in the growth environment.

The results in Table 5 showed significant increase in Cl^- concentration in the dry calli grown in medium supplemented with 10.5 dSm^{-1} salinity level compared with the control. Although Super Regina cultivar showed higher concentration of Cl^- compared with Trescantos but it was not significant. For the interaction between the cultivars and the salinity level Super Regina dry calli showed significantly higher Cl^- concentration on the 10.5 dSm^{-1} salinity level. At 8.5 dSm^{-1} salinity level, Trescantos dry calli contend higher Cl^- concentration than Super Regina.

Fig. 1: (A) Tomato induced calli (B) Plant regeneration

Calli from both cultivars grown on medium with 10.5 dSm^{-1} salinity level lost the ability to regenerate plants, whereas the control medium gave the highest percentage of plant regeneration (26%) (Table 6, Fig. 1A). Super Regina gave slightly significant higher percentage of plant regeneration than Trescantos (Fig. 1B).

Plants regenerated from salt tolerant calli might be salt tolerant too. The calli was induced from seeds treated with NaN_3 which has mutation affect, therefore it is possible some of the calli have genetic changes or due to the somaclonal variations that occur in culture. Al-Qurainy and Khan (2009) discussed the mutagenic effect of sodium azide and its application in crop improvement. In addition several studies showed that NaN_3 is effective in economical crop improvements *in vitro* such as tomato (Adamu and Aliyu, 2007), peas (Aslid *et al*., 2006) and sugarcane (Ikram *et al*., 2011).

Combination of tissue culture and mutation techniques were used to induce genetic changes to improve different crops (Irshad *et al*., 2010; Saif-Ur-Rashid *et al*., 2001). Selection of salt tolerant plants is difficult under field conditions, but plant tissue culture techniques are performed under aseptic and controlled environmental conditions. These advantages of plant tissue culture allow various opportunities for researcher to study the complex responses of plants against environmental stresses and select plants with desired traits (Lokhande *et al*., 2010; Lokhande *et al*., 2011; Safarnejad, 2004; Sakthivelu *et al*., 2008).

All the regenerated plants were transferred to the field and produced fruits. The seeds of the second generation will be used to test the salt tolerant ability of the regenerated plants under field conditions.

CONCLUSION

Sodium Hypochlorite at 3% concentration for 15 min is the best sterilization treatment for tomato seeds. Moreover, combination of 0.5 mg/L BA and 1 mg/L IAA is recommended for callus induction. Trescantos cultivar has the ability to accumulate Na^+ and Cl^- ions in the cells with less effect on callus growth which is one of the mechanisms used by the plants to avoid the effect of high salinity levels in the growth environment. However, Super Regina gave higher percentage of plant regeneration than Trescantos. Tomato plants regenerated from salt tolerant calli were transferred to the field and grown to maturity.

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