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Responses to NaCl Salinity of Tomato Cultivated and Breeding Lines Differing in Salt Tolerance in Callus Cultures

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ABSTRACT

Responses of three cultivated genotypes (Castlrock, Oriet and Super Marmande) and three breeding lines (BL-1076, BL-1077 and BL-1079) of tomato (*Lycopersicon esculentum*) to NaCl stress were studied in callus and regenerated plants. Hypocotyl segments and true leaves were chosen as explants for callus induction and regeneration *in vitro*, respectively. The six investigated tomato genotypes differed in their callus growth and regeneration capacities. Castlrock ande and BL-1079 performed better callus; whereas, Super Marmande and BL-1079 performed better organogensis in the specified tissue culture media. Based on the responses to NaCl, six tomato genotypes were ranked in the order BL-1079 > BL-1077 > Super Marmande > Oriet > BL-1076 > Castlrock, when callus fresh and dry weights, length and number of roots were considered as indicators for salinity tolerance *in vitro*. Chlorophyll *a* was less susceptable to NaCl than Chlorophyll *b* in all genotypes. It seems that genotypes with high organogenetic potential may be better able to grow in saline environment. It is evident that tissue cultur technique was able to evaluate several genotypes for salt tolerance under controlled environment with relatively little space and less time required comparing with such process studies at the whole plant level.

Key Words: Chlorophyll; Callus culture; Salt stress; Tomato

INTRODUCTION

Soil salinity is a major constraint to food production because it limits crop yield and restricts use of land previously uncultivated. Therefore, approaches should be developed to produce salt tolerant genotypes. Implementation of biotechnology strategies to achieve salt tolerance and yield stability is a promising approach. This requires substantial research to identify salt tolerance effectors and the regulatory components that control responses to salinity during stress episode (Hasgawa *et al.*, 2000). Cell and tissue culture could be good tools to screen the materials obtained in the different breeding programs since it permits the manipulation of large numbers of genotypes using relatively little space, reduction of time between generations, and controlled environment (Dracup, 1991). According to Hassan *et al*. (1999), the increase in soil salinity above 2-6 dSm^{-1} reduced tomato yield and vegetative growth, indicating the need to maintain low soil salinity level or introduce salt tolerant variety for maximum production.

Explant type is important factor affecting regeneration and the source of the explant seems to be important for the regeneration of the culture. For tomato, most plant parts had been used as explants in previous regeneration systems, e.g root (Norton & Bol, 1954), leaf from green house (Behki & Lesley, 1976), leaf from *in vitro* (Frankenberger *et al.*, 1981), stem internode (De Langhe & De Bruijne, 1976), and shoot apical meristem (Kartha, 1977). Ohki *et al.* (1978) studied shoot differentiation *in vitro* of tomato plants and transmission of this capacity into hybrids. Hypocotyls produced the most positive results. On the other hand, Frankenberger *et al*. (1981) found that, shoot forming capacities were generally higher from cotyledon explants than from hypocotyl explants. Duzyaman *et al.* (1994) compared the growth of hypocotyls, cotyledon and leaf explants of two tomato cultivars *in vitro*. The degree of shoot regeneration was in the order of leaves cotyledons ≥ hypocotyls, and there was no difference between the cultivars . Cano *et al.* (1998) investigated the possibility of using *in vitro* shoot apex culture to evaluate salt tolerance of cultivated (*Lycopersicon esculentum* Mill) and salt-tolerant breeding lines (L. *pennellii* Correll Darcy) tomato species. They concluded that rooting parameters are the most useful traits for rapid evaluation and screening of tomato species and segregating populations through *in vitro* shoot apex culture.

Mercado *et al*. (2000) report that the approach of *in vitro* shoot morphogenesis and shoot apex culture is not a reliable tool to evaluate salt tolerance in cultivated tomato. However, Rus *et al.* (2000) studied salt responses induced by long-term callus culture in leaf callus tissue of the cultivated tomato species (*Lycopersicon esculentum* Mill.) and its wild salt-tolerant relative (L. *pennellii* Correll Darcy). They concluded that the salt responses varied according to the precedence of calli (from control to saline

medium). The salt tolerance of the wild species was much higher than that of the cultivated species. They also observed that the salt tolerance increased according to the number of previous subcultures in salt medium and in the control as well. They concluded that *L. esculentum* is able to adapt to salinity, although the adaptation process to salinity is slower in the cultivated than in the wild tomato species. Santa-Cruz *et al*. (2001) were involved in determining whether the rootstock effect on the tomato (*Lycopersicon esculentum* Mill) salinity response depends on the shoot genotypes. Their results suggest that the saline ion accumulation in leaves was controlled predominately by the genotype of the rootstock. In addition, the characteristics of the rootstock able to induce salt tolerance to the shoot depend on that salt tolerance mechanism of the shoot genotype.

The *in vitro* response of tomato genotypes during the regeneration phase to salinity was studied by El-Anany (1997) and Sancho-Carrascosa *et al*. (2000). Shoot regeneration from cotyledon, hypocotyls and leaves of tomato was inhibited by NaCl in both studies. Sancho-Carrascosa *et al.* (2000) found that root formation was the parameter most affected by salt in both cultivars.

Bhivare and Nimbalkar (1984) stated that plant species differ in their response to salinity with respect to chlorophyll contents. Chlorophyll contents in the leaves of *Phaseolus* plants decreased under K^+ and Na^+ salinities. This decrease was due to the reduction in leaf Mg induced by salinity (Mohamed & Abd El-Hadi, 1985). Treating spinach with NaCl reduced chlorophyll *a* and chlorophyll *b* contents 17% relative to the controll plants (Guenther & Melis, 1989). In callus cultures, salinity suppressed the accumulation of chlorophyll *a* in pea seedlings grown under NaCl stress (Alina *et al.,* 1985). However, in Chinese cabbage chlorophyll contents increased by 2-3 folds in calli grown on either NaCl or Na_2SO_4 (Paek *et al*., 1988). Radish cotyledons treated with NaCl displayed a significant decline in chlorophyll (Dily *et al*., 1993). Guerrier (1996) reported a decrease in chlorophyll contents in both cultivated and wild tomato species in response to NaCl. Delfine *et al.* (1999) found a strong reduction in total chlorophyll content and an increase in chlorophyll *a/b* ratio in salt-stressed and rewatered spinach leaves.

This study was conducted to examine the responses of six tomato genotypes in callus and regenerated plants to NaCl stress, explant type for callus induction and organogenesis, chlorophyll content and genotype capacity for callus growth and regeneration, were determined for the six genotypes in absence and presence of NaCl to evaluate their salt tolerance using tissue cultur technique.

MATERIALS AND METHODS

Plant material. Salt responses were studied in callus tissues and regenerated shoot clumps of six tomao cultivars (*Lycopersicon esculentum*). Three cultivated tomato cultivars (Castlrock, Oriet, and Super Marmande) were obtained from the open seed market in Egypt. Three salt-tolerant tomato breading lines (BL-1076, BL-1077, and BL-1079) were kindly provided by Asian Vegetable Research and Development Center (AVRDC, Shanhua, Taiwan, ROC.)

Plant cultivation. Seeds were surface sterilized by dipping in aqueous HgCl solution (0.25 g/L) before being rinsed thoroughly five times in sterilized deionized water, each for 3 minutes. Seeds were then germinated *in vitro* on agar solidified (7 g/L) medium containing Murashige and Skoog basic medium (MS) (Murashige & Skoog, 1962). The medium pH was adjusted to 5.7±1 before the addition of agar and subsequent autoclaving at 121°C and 15 psi for 20 min. Cultured seeds were maintained at 24°C for three weeks under 16/8 h photoperiod of 3000 lux and 70% humidity.

Explant selection. This experiment was conducted to select suitable explant materials for callus initiation and regeneration. three different explant types: cotyledon (5 × 5 mm), hypocotyl (5 mm) and true leaf (5 × 5 mm) were excised aseptically and placed on the corresponding growth medium. True leaf and cotyledonary explant segments were taken including the mid-rib, trimmed all-round the margins, and then placed abaxial side down facing the medium.

Selection of explant type for callus induction and organogensis. Three explant sources (cotyledon, hypocotyl, true leaf) of cultivated tomato genotypes (Castlrock, Oriet) were induced for callus and proliferation on medium containing MS macro and micronutrients and supplemented with myo-inositol (100 mg/L), sucrose (30 g/L) and solidified with agar (7 g/L). Plant growth regulators were 0.4 mg/L NAA and 2 mg/L kinetin (for callus induction) or BA (for organogensis).

For callus induction, explant cultures were mintaind in dark for the first two weeks, then allowed to grow under light for another two weeks. After four week, culture treatments were evaluated for each explant on the basis of a) callus fresh weight, b) callus dry weight, c) callus compactness, d) callus colour e) per cent explants forming callus and f) percent explants forming roots.

For organogenesis, explant cultures were maintaind in the dark for three weeks at 24°C and 70% R.H. After eight weeks, clean and healthy cultures were removed from the culture jars for growth analysis. Culture treatments were evaluated on the basis of a) number of shoots per regenerated clump, b) culture fresh weights, and c) culture dry weight, d) shoot length (cm), e) per cent explant forming shoots, f) percent explants forming roots, and percent explants forming callus. Data were recorded as means of 10 replications (Jars).

Determination of genotype capacity for callus growth and regeneration. The hypocotyl segments (for callus growth) and true leaf explant segment (for regeneration) of the six tomato genotypes (Castlrock, Oriet, Super Marmande, BL-1076, BL-1077, and BL-1079) were

maintained in the media as those described in the the previous section . For callus growth, cultures were left in the dark for four weeks at 24°C and 70% R.H. The following measurments were taken: callus fresh and dry weights, per cent explants forming callus, per cent explant forming roots, root length and number of roots per callus mass. For regeneration capacity, after eight weeks the following measurments were carried out: the number of regenerated shoots per explant, culture fresh and dry weights, and viability. All treatments were replicates 10 times.

To test the genotype capacity for callus growth and regeneration in presence of NaCl, a group of homogenious cultures were selected to cotinue growth or shoot clumps produced previously were subcultured on regeneration media, in presence of different NaCl concentrations (0, 1, 2, 3, 4, 5 or 6 g/L), respectively, for eight weeks. The same measurments, as previously described, for callus growth and regeneration were determined. The experimental design was 6 × 7 (genotypes × NaCl concentrations) factorial experiment in a randomized complete blocks with 6 replications.

Estimation of chlorophyll pigments. Chlorophyll *a*, chlorophyll *b*, and total chlorophyll contents were estimated in the fresh leaves of regenerated plantlets from each genotype X salinity treatment according to the procedure described by Sadasivam and Manickam (1991). The amount of chlorophyll present in the extract (mg chlorophyll per g tissue) was calculated.

Statistical analyses. Differences among tomato genotypes were tested by the analysis of variance (ANOVA) and means significance differences were tested by LSD.

RESULTS

***In vitro* selection of explant type.** For the two tested cultivars, the use of hypocotyls segments as explant materials produced better callus fresh and dry weights than cotyledonary or true leaf explants (Table I). Explants from hypocotyls segments regenerated roots from callus and produced the highest percentage of callus induction. Accordingly, hypocotyls segments were chosen for subsequent experiments as explants for callus induction. Table II indicated that the utilization of true leaf sections as explant materials produced larger shoot clumps in terms of clump fresh weight and dry weight for Castlrock and Oriet as compared with hypocotyls or cotyledonary explants. True leaf explant also produced higher number of shoots and better shoot length. The percentage of explant regenerated shoots was higher from true leaves: 33 and 85% for Castlrock and Oriet, respectively. Based on these results, true leaf discs were chosen as explant materials for subsequent regeneration trials under salt stress *in vitro.*

***In vitro* callus and regeneration capacities of tomato genotypes.** results shown in Table III indicated that the tomato breeding line BL-1079 had the highest callus growth potential in terms of its callus fresh weight and dry weight as compared with the other genotypes. Among the cultivated tomato genotypes, Super Marmande had higher callus fresh weight and dry weight as compared with Castlrock and Oriet. Both Super Marmande and BL-1079 produced longer roots from callus, but the produced root numbers were almost equal in all genotypes (Table III). The percentages of rooted callus were higher (88 - 100%) in the cultivated tomato genotypes than in the selected salt-tolerant inbred lines (66 – 77%).

The tomato line BL-1079 had the greatest organogenic potential in terms of its clump fresh weights, dry weights and the percent regeneration (66%) (Table IV). The number of regenerated shoots per explant were almost equal in the four genotypes Oriet, Super Marmande, and BL-1079 followed by BL- 1077, while Castlrock and BL-1076 had the lowest mean shoot number/explant (Table IV).

Salinity Stress and *in vitro* Callus Growth Potential of Tomato Genotypes

Fresh weight. The mean callus fresh weight generally decreased with the increase in NaCl level in the medium, from 2.1 g at 1000 ppm NaCl to 1.2 g at 6000 ppm NaCl (Table V). The tomato line BL-1079 had the highest significant callus FW (2.6 g) when tested over all salinity levels, followed by the two lines BL-1076 and BL-1077. Among the three cultivars, Super Marmande produced higher callus fresh weight (1.4 g) that the oriet (1.3 g) and Castlrock (1.3 g). The increase in callus fresh weight was 43% over the control at 1000 ppm NaCl, then declined to 35, 31, 31, 22 and 8% at 2000, 3000, 4000 and 5000 ppm NaCl, respectively. The six tested tomato genotypes could be ranked based on fresh weight as indicator for their salinity tolerance in the order: BL. 1079> BL.1077> Super Marmande> Oriet> BL-1076> Castlrock.

Dry weight. The increase in NaCl level in the media had

Table I. Callus induction potential of cultivated tomato genotypes (Castlrock and Oriet) in response to different explant types. Values are means of 10 callus masses

Genotype G Genotype	Explant	Parameter					
		Fresh Wt. (g)	Dry Wt. (g)	Compactness	Color	Callus (%)	Root (%)
Castlrock	Hypocotyl	1.812	0.183	Compact	greenish	88	100
	Cotyledon	1.444	0.145	Compact	Beige	40	61
	True Leaf	1.316	0.130	Friable	Beige	70	63
Oriet	Hypocotyl	1.821	0.055	Compact	Greenish	100	100
	Cotyledon	0.754	0.025	Compact	Beige	100	58
	True Leaf	0.715	0.030	compact	beige	100	66

resulted in significant decrease in callus dry weight (Table V). When tested over all genotypes, the mean callus dry weight was the highest at 1000 – 3000 ppm NaCl (0.184-0.195 g), then declined to the lowest value at 6000 (0.1301 g). However, as previously noted for callus fresh weight, callus derived from non-saline media had less dry weight than those obtained from media containing 1000–3000 ppm NaCl. The tomato line BL-1079 had the highest callus dry weight followed by BL-1077, BL-1076 and Oriet which were not significantly different.

The highest dry weight was that obtained from hypocotyl explant of the tomato line BL –1079 at 2000 ppm NaCl, while the lowest one was that obtained from Castlrock explant grown on a medium with 6000 ppm NaCl. Callus dry weight of BL–1079 was always higher on all NaCl levels than the control treatment. This trend was also true for the cultivar Super Marmande. In addition, callus dry weight was markedly decreased at 6000 ppm NaCl for the cultivar Castlrock, Oriet, as well as BL-1076 (Table V). When compared with the control, these reductions were 27, 35 and 8%, respectively. However, the reduction in callus dry weight at 6000 ppm NaCl was only 4% for Super Marmande and BL-1077. More interestingly, callus dry weight was 25% higher at 6000 ppm NaCl than the control for the tomato line BL-1079.

Root length. Callus of the cultivar Oriet had produced the highest root length (12.2 cm), while that of BL-1077 had the shortest root (Table VI). Callus on NaCl–free medium had the longest root (8.76 cm) followed by callus grown on 4000 ppm NaCl. The differences among salinity treatments within the range 1000 - 3000 ppm were not significant for root length, but produced shorter roots than the control, 4000 ppm or 5000 ppm NaCl. The highest NaCl level in the medium (6000 ppm) had resulted in callus with the lowest root length.

The cultivar Oriet had initiated longer roots from callus at all salinity levels except at 6000 ppm NaCl as compared with the control (Table VI). The root length of Super Marmande grown on 1000 and 2000 ppm NaCl was lower than the control, but increased significantly over the control at higher NaCl levels. For the cultivar Castlrock, all salinity treatments produced less root length than the control. The same trend was also true for the two lines BL-1076 and BL-1077. However, callus of BL-1079 had initiated longer roots than the control at all salinity levels up to 5000 ppm NaCl. Root length at 6000 ppm was always lower for all genotypes except Super Marmande and BL-1079. The highest reduction in root length (91%) was detected for BL-1076 followed by BL-1077 (57%), Castlrock (22%) and Oriet (8.4%). For Super Marmande,

Table II. Organogenic potential of cultivated tomato genotypes (Castlrock and Oriet) in response to different explant types. Values are means of 10 regenerated clumps.

Genotype	Explant	Parameter						
		clumpFresh Wt. (g)	clumpDry Wt. (g)	Shoot Length (cm)	Number of Shoots	Shoot (%)	Root (%)	Callus (%)
Castlrock	Hypocotyl	1.003	0.070	2.75	1.0	6	0	73
	Cotyledon	1.276	0.126	3.70	3.0	26	0	100
	True Leaf	1.342	0.132	3.00	2.0	33	0	20
Oriet	Hypocotyl	1.041	0.068	3.50	2.0	0	22	83
	Cotyledon	1.076	0.086	3.20	6.0	33	0	73
	True Leaf	1.812	0.179	3.70	5.7	85	0	47

Table III. *In vitro* callus growth capacity of the six tomato genotypes. Values are means of 10 replicates

Genotype	Parameter					
	Fresh Wt. (g)	Dry Wt. (g)	Root Length (cm	Callus Induction (%)	Number of Roots	Rooting (%)
Castlrock	1.826	0.0545	3.24	88	4.5	100
Oriet	0.821	0.0549	3.62	100	4.1	88
Super Marmande	0.878	0.0671	4.12	80.6	3.6	100
Bl-1076	0.608	0.052	6.50	76	3.5	60
Bl-1077	0.829	0.0657	6.00	83.3	4.0	62
BL-1079	1.359	0.0851	12.2	87	4.0	77

Table IV. *In vitro* regeneration potential of the six tomato genotypes. Values are means of 10 replicates

Genotype	Parameter			
	Fresh Wt. (g)	Dry Wt. (g)	Number of Shoots	Regeneration (%)
Castlrock	1.040	0.0686	2.0	60
Oriet	1.058	0.0766	3.0	42
Super Marmande	1.077	0.0846	3.0	62
Bl-1076	0.418	0.0363	2.0	33
Bl-1077	1.040	0.1043	3.0	44
BL-1079	1.176	0.1073	2.6	66

Table V. Callus fresh weigh and dry weight (g) of cultivated and breeding lines of tomato genotypes grown on different NaCl callus induction media

Salt level (ppm)	Genotype											
	Cultivated						Breeding lines					
	Castlrock		Oriet		Super Marmande		BL-1076		BL-1077		BL-1079	
	Fresh wt.	Dry wt.	Fresh wt.	Dry wt.	Fresh wt.	Dry wt.	Fresh wt.	Dry wt.	Fresh wt.	Dry wt.	Fresh wt.	Dry wt.
0	1.243	0.1198	1.288	0.1774	1.121	0.1085	1.677	0.1253	1.543	0.1344	1.682	0.0737
1000	1.751	0.2139	1.666	0.1674	1.692	0.1131	1.518	0.1591	2.256	0.1590	3.376	0.2893
2000	1.950	0.2331	1.020	0.1235	1.710	0.2060	1.450	0.1491	1.812	0.1404	3.653	0.3189
3000	1.146	0.1039	1.418	0.1933	1.691	0.1690	1.695	0.2060	2.506	0.2168	2.798	0.2637
4000	1.116	0.1024	1.653	0.1511	1.552	0.1480	1.521	0.1378	1.765	0.1310	2.589	0.2490
5000	0.982	0.0925	1.186	0.1286	0.947	0.1218	1.481	0.1408	1.675	0.1370	2.477	0.2340
6000	0.910	0.0870	1.073	0.1145	0.930	0.1041	0.852	0.1148	1.664	0.1290	1.973	0.2314

For fresh wt.: LSD 0.05 salt = 0.077 genotype = 0.071 salt x genotype = 0.208
For dry wt. : LSD 0.05 salt = 0.014 genotype = 0.013 salt x genotype = 0.037

Table VI. Root length (cm) and number of roots from differentiated callus of cultivated and breeding lines of tomato genotypes grown on different NaCl callus induction media

Salt level (ppm)	Genotype											
	Cultivated						Breeding lines					
	Castlrock		Oriet		Super Marmande		BL-1076		BL-1077		BL-1079	
	Length	Number	Length	Number	Length	Number	Length	Number	Length	Number	Length	Number
0	8.48	3.0	10.20	6.0	6.62	4.0	14.50	10.0	1.55	1.5	6.20	10.0
1000	7.76	5.0	11.00	5.0	3.44	4.0	07.25	9.0	3.56	1.0	7.32	15.0
2000	7.80	2.0	12.20	4.0	4.20	5.0	05.77	5.6	4.22	8.0	7.00	15.0
3000	5.56	3.0	13.24	2.0	8.16	3.0	05.25	5.3	4.36	5.3	6.55	4.5
4000	6.22	2.0	14.36	2.0	8.00	3.0	04.32	4.1	5.22	4.2	7.42	4.0
5000	6.73	2.0	15.20	2.0	7.84	2.0	01.44	2.1	2.10	3.1	9.20	4.0
6000	6.62	1.0	09.34	2.0	9.22	1.0	01.30	2.0	3.00	0.0	6.24	4.0

For root length : LSD 0.05; salt = 0.598; genotype = 0.554; salt x genotype = 1.53
For root number: LSD 0.05; salt = 0.76; genotype = 0.68; salt x genotype = 1.13

Table VII. Plantlet survival percentage of cultivated and breeding lines of tomato genotypes grown on different NaCl regeneration media

Salt Level (ppm)	Genotype					
	Cultivated			Breeding lines		
	Castlrock	Oriet	Super Marmande	BL-1076	BL-1077	BL-1079
0	100	100	100	100	83	66
1000	100	100	100	66	75	83
2000	100	76	100	76	88	100
3000	100	75	100	75	87	100
4000	100	75	100	50	75	100
5000	100	75	100	50	75	100
6000	66	75	100	50	50	50

root length at 6000 ppm NaCl was 28% higher than the control, while for BL-1079 the root length was almost as similar to the control.

Number of roots. Callus of BL-1079 had the highest significant mean number of roots (8.0) as tested overall salinity levels, while Castlrock had the lowest significant one (2.5) (Table VI). Mean root number decreased markedly from 5.75 in the control to 2.0 at 6000 ppm NaCl. However, the number of roots at zero, 1000 and 2000 ppm NaCl was not significantly different. Compared with control, the reduction percent in root number at 6000 ppm NaCl were 66, 66 and 80% for Castlrock, Oriet and BL-1076, respectively. However, these reductions were 50, 33 and 60% for Super Marmande, BL-1077 and BL-1079, respectively (Table VI).

Salinity Stress and *in vitro* Regeneration Potential of Tomato Genotypes

Survival. Super Marmande and BL-1076 produced callus from the base of regenerated shoots after prolonged exposure to the culture medium. High percentage of survival under salinity stress (50 – 100%) was achieved for the most genotypes (Table VII). Super Marmande was the best genotype that survived all salinity levels, followed by Castlrock and BL-1079.

Fresh weight. Mean clump fresh weight of Super Marmande was the highest significant over all tested genotypes (Table VIII) followed by Castlrock and Oriet, which were not significantly different. Among the imported

Table VIII. Regenerated clump fresh and dry weights (g) of cultivated and breeding line of tomato genotypes grown on different NaCl callus induction media

Salt level (ppm)	Genotype											
	Cultivated						Breeding lines					
	Castlrock		Oriet		Super Marmande		BL-1076		BL-1077		BL-1079	
	Fresh wt.	Dry wt.	Fresh wt.	Dry wt.	Fresh wt.	Dry wt.	Fresh wt.	Dry wt.	Fresh wt.	Dry wt.	Fresh wt.	Dry wt.
0	3.163	0.352	3.271	0.254	3.388	0.235	1.476	0.118	1.610	0.194	1.639	0.131
1000	2.301	0.257	2.988	0.353	2.795	0.275	1.407	0.139	1.281	0.141	1.422	0.142
2000	2.178	0.259	2.290	0.331	2.543	0.315	1.114	0.080	1.056	0.087	1.431	0.115
3000	1.920	0.186	2.202	0.113	3.470	0.355	1.175	0.088	1.019	0.080	1.992	0.108
4000	2.223	0.195	1.942	0.089	4.304	0.354	0.996	0.077	1.003	0.073	1.113	0.096
5000	2.810	0.225	1.915	0.079	3.311	0.303	0.901	0.074	0.916	0.071	0.915	0.093
6000	2.752	0.219	1.906	0.064	3.152	0.252	0.886	0.060	0.861	0.069	0.861	0.087

For fresh weight: LSD 0.05; salt = 0.393; genotype = 0.363; salt x genotype = NS
For dry weight: LSD 0.05; salt = 0.0016; genotype = 0.0015; salt x genotype = 0.0041

Table IX. Leaf chlorophyll content (mg/g fresh weight) of the cultivated and breeding lines of tomato genotypes grown under different NaCl levels *in vitro*

Genotype	NaCl concentration (ppm)							Mean
	0.0	1000	2000	3000	4000	5000	6000	Genotype
Chlorophyll a								
Castlrock	0.22	0.20	0.15	0.15	0.09	0.06	0.05	0.12
Oriet	0.21	0.18	0.10	0.10	0.08	0.07	0.07	0.11
Super Marmande	0.18	0.10	0.19	0.20	0.07	0.07	0.07	0.13
BL-1076	0.23	0.07	0.07	0.05	0.05	0.05	0.04	0.08
BL-1077	0.26	0.26	0.15	0.09	0.08	0.08	0.05	0.11
BL-1079	0.23	0.20	0.20	0.10	0.09	0.08	0.06	0.12
Mean Salt	*0.22*	*0.16*	*0.13*	*0.11*	*0.076*	*0.068*	*0.056*	--
Chlorophyll b								
Castlrock	0.30	0.12	0.07	0.05	0.03	0.03	0.02	0.09
Oriet	0.32	0.27	0.18	0.18	0.14	0.13	0.12	0.19
Super Marmande	0.36	0.19	0.08	0.07	0.14	0.13	0.12	0.16
BL-1076	0.11	0.03	0.04	0.03	0.02	0.02	0.02	0.04
BL-1077	0.12	0.12	0.06	0.03	0.03	0.02	0.02	0.06
BL-1079	0.08	0.05	0.21	0.17	0.04	0.04	0.02	0.05
Mean Salt	*0.21*	*0.13*	*0.08*	*0.09*	*0.06*	*0.06*	*0.05*	--
Total Chlorophyll								
Castlrock	0.52	0.34	0.22	0.21	0.12	0.09	0.07	0.224
Oriet	0.53	0.45s	0.29	0.27	0.22	0.21	0.19	0.308
Super Marmande	0.56	0.29	0.27	0.27	0.20	0.20	0.19	0.297
BL-1076	0.34	0.10	0.10	0.08	0.08	0.07	0.06	0.112
BL-1077	0.39	0.39	0.21	0.12	0.11	0.10	0.07	0.198
BL-1079	0.31	0.25	0.23	0.17	0.13	0.12	0.08	0.184
Mean Salt	*0.43*	*0.30*	*0.22*	*0.18*	*0.14*	*0.13*	*0.11*	--

tomato inbred lines, BL-1079 had relatively better clump fresh weight as compared with BL-1076 or BL-1077. The main effect of salinity was also significant. Clump fresh weight declined with the increase in the NaCl level, and reached 28% of the control at 6000 ppm NaCl (Table VIII). The reduction in clump fresh weight at 6000 ppm NaCl relative to the control was the minimum for Super Marmande (7%) followed by Castlrock (13%), but reached more than 40% for the other tested genotypes.

Dry weight. The mean clump dry weight of Super Marmande and Castlrock were significantly higher than all other tested genotypes (Table VIII). Although mean clump dry weight of BL-1079 was relatively better than either BL-1076 or BL-1077, the three genotypes did not differ significantly. Super Marmande showed increased clump dry weight at higher salinity levels (2000 – 5000 ppm NaCl) as compared with the control, while for Castlrock and BL-1077, clump dry weights were always less than the control at any NaCl level from 1000 to 6000 ppm (Table VIII). At the highest NaCl level, clump dry weight of Super Marmande increased 6% over the control, while all other genotypes had reduced clump dry weight in varying degree. These reductions were about 30% for Castlrock and BL-1079, but were 75, 49 and 64% for Oriet, BL-1076 and BL-1077, respectively.

Chlorophyll content. The contents of chlorophyll in the regenerated plantlet leaves of both the cultivated and salt-tolerant inbred lines were affected by NaCl (Table IX). Chlorophyll *a* was more tolerant to NaCl than chlorophyll *b*. For the cultivated genotypes, the relative reductions in chlorophyll *a* were 77% in Castlrock, 66% in Oriet, and 61% in Super Marmande. The Super Marmande showed an

increase in chlorophyll *b* content at 4000 and 5000 ppm NaCl. For the salt-tolerant breeding lines, BL-1076 and BL-1077 showed less tolerance to salt stress than BL-1079. BL-1079 had less reeducation in chlorophyll contents at the highest salinity levels than BL-1076 and BL-1077.

DISCUSSION

For callus induction, hypocotyls segments outperformed those from cotyledon or leaf explants. Differences in hormonal levels and gradients in different explant tissues may explain the observed differences among these explants for callus growth. In accordance with our results, Gunay and Rao (1980) also found that hypocotyls explant. Cassells (1979), and De langhe and De Bruijne (1976) report that endogenous levels of auxin in explant tissues depend and may vary on the mother plant from which they are derived. Optimum regeneration capacity was obtained using leaf disc explants. The use of less mature tissues and/ or the presence of optimal endogenous auxin/cytokinin balance in leaf tissue may explain their increased regeneration potential, as previously indicated by Behki and Lesley (1976), Ohki *et al*. (1978) and Abdel–Hamid (1995).

For callus and regeneration potentials**,** Super marmande and BL – 1079 among the cultivated and breeding liness were relatively the better. In other *in vitro* studies that included different genotypes, it was demonstrated that genotype differences in callus growth and shoot regeneration capacities were found among genotype (Kurtz & Lineberger, 1983; Stomel & Sinden, 1991; Abdel–Hamid, 1995). Reish and Bingham (1980) and Padmanabhan *et al.* (1974) indicate that the regeneration capacity of plant tissue is genetically controlled and specific for each genotype.

All tomato genotypes tested were able to produce callus and initiate roots from callus under different concentrations of NaCl in callus induction media, and to regenerate shoots in NaCl added to regeneration media. However, these capacities were different, depending on genotype and salt level. In this regard, callus derived from the cultivar Super Marmand and the breeding lines BL–1079 and BL–1077 were more able to adapt to higher salinity levels than other genotypes. These three genotypes had less reduction in callus fresh weight, callus dry weight, and the root length and root number at 6000 ppm NaCl. Callus dry wight and the root length of Super marmande were higher at 6000 ppm NaCl than the control, indicating superiority over the other tested genotypes for salt tolerance *in vitro*. These results are in agreement with thos of Rahman and Kaul (1989) and El Bahr *et al.* (1993) who show that salt adapted tomato lines exibited better callus growth in high saline media. Cano *et al.* (1996, 1998) found that rooting parameters were the most useful traits for rapid evaluation, which is in agreement with our results that under higher NaCl level, callus of the proposed salt tolerant genotype, Super Marmande had roots longer than the control. Rus *et al*. (2000) also found that adaptation capacity to salinity varies with the genotype's degree of tolerance. The study of Sancho–Carrascosa *et al.* (2000) also proved that at specific NaCl concentration in the regeneration media, tomato shoot clump growth and regeneration frequency were reduced, and the magnitude of reduction varied, depending on the degree of tolerance of the cultivars tested. In contrast to our results Mercado *et al.* (2000) found that genotype responses of the six tomato genotypes under salinity stress were almost similar both during callus induction and regeneration stages. In strawberry plants, cultivars that were ranked as salt tolerant during the proliferation stage of micropropagation showed the same response during the rooting as well as during the *ex vitro* growth periods (Mohamed, 2002).

The obtained results are in agreement with the previous findings concerning the physiological responses of tomato cultures to salt treatments; marked differences in the behavior of both susceptible and tolerant tomato genotypes were evident (Maliwal & Paliwal, 1970; Patolia, 1983; Lynegar *et al*., 1984; Cruz *et al*., 1990; Cano *et al*., 1996; Rus *et al*., 2000). Yet, an understanding of the mechanisms that plants use to cope with high salinity is necessary to select and develop tomato plants that are more tolerant to salinity. Under salinity stress, high percentage of survival (50-100%) was achieved for most of the genotypes after 45 days in culture. Super Marmande was the best genotype that survived all salinity levels and produced better regenerated clump fresh and dry weights, followed by Castlrock and BL-1079. Moreover, the reduction in clump fresh weight at the highest salinity level (6000 ppm) was minimum for Super Marmand. Super Marmande showed a 6% increase in the dry weight at the highest NaCl level (6000 ppm); and the lowest reduction in dry weights (30%) were reported for Castlrock and BL-1079.

The obtained results indicated reduced chlorophyll contents in all the tested tomato genotypes under *in vitro* salinity stress.The report of Guerrier (1996) also indicated a decrease in chlorophyll accumulation in both cultivated (salt-sensitive) and wild (salt-tolerant) tomato species in response to NaCl. Super Marmande and BL-1079 were ranked as salt tolerants since they had less regenerated reduction in chlorophyll contents than those ranked as salt sensitive (Castlrock & BL-1076), which agrees with the results of Sinel nikova *et al.* (1988) where chlorophyll *a* and *b* as well as total chlorophyll were decreased under salt stress more in susceptible tomato cultivars than in tolerant ones. The decrease in chlorophyll accumulation in tissues of the tested tomato genotypes with the increase in NaCl levels in the media is in agreement with the results of Guerrier (1985) on tomato and Guenther and Melis (1989) on Spinach. In callus cultures, salinity also supepressed the accumulation of chlorophyll *a* in pea seedlings (Allina *et al*., 1985) and total chlorophyll in spinach (Delfine *et al.,* 1999). The reduction in chlorophyll *b* (76%) was higher than

chlorophyll *a* (72%) at 6000 ppm NaCl which agrees with the finding of Delfine *et al.* (1999). For chlorophyll contents in the regenerated plantlet leaves, Super Marmande and BL-1079 were the most tolerant genotypes .

CONCLUSIONS

Differences among tomato genotypes for regeneration capacity under *in vitro* salt stress could be used as rapid way to detect salt tolerant genotypes. Survival, regenerated clump fresh and dry weights indicated the possibility of ranking the tested tomato genotyps into salt tolerant: Super Marmande and BL-1079, or salt sensitive: Castlrock and BL-1076. Oriet and BL-1077 seem to be intermediate in terms of sensitiveity to salt stress.

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