IN VITRO SELECTION OF SALT-TOLERANT TOMATO PLANTS AND THE CHANGES IN GENE EXPRESSION UNDER SALINITY STRESS

Hamdy M. El-Aref

Dept. of Genetics, Fac. Of Agriculture, Assiut University, Assiut, Egypt.

Abstract: In vitro selection for salinity tolerance was studied in the two varieties Peto-86 and Roma VF of tomato (*Lycopersicon esculentum* Mill). From the two varieties, 420 cotyledons (explants) were subjected to selection at 6.0 g/l sodium chloride (NaCl). The progeny of the selected and unselected plants (R1-generation) as well as their donor parents were tested for salinity tolerance. In addition, soluble protein, and five different isozyme systems (peroxidase, esterase, ACP, GOT and MDH isozymes) were used to study the changes in gene expression under salinity stress. It was found that: (i) Seedlings of the selected tolerant somaclones showed better growth under salinity stress (6.0 g/l NaCl) when compared to the unselected somaclones and their parents; (ii) Mean salinity tolerance index, which is based on shoot and root lengths and dry weights, indicated that all somaclones selected from Pet-86, except PS-3, PS-4 and PS-14 were more tolerant than their donor parent and the unselected plants; (iii) S__alt tolerance was transmitted to the next generation in seed progeny of tolerant plants grown in the absence of exposure to salt; (iv) Under salinity stress, seven polypeptides were apparently suppressed (62.05, 45.82, 43.38, 31.98, 24.05, 21.87 and 14.58 kDa) whereas nine protein bands at molecular weight 91.24, 84.06, 81.15, 65.03, 54.86, 42.02, 36.59, 26.01 and 22.56 kDa were induced in all tested genotypes, as compared with the control treatment; (v) Salinity causes the induction of 5 isozyme bands in esterase and one band in peroxidase; and (vi) No quantitative variability in the isozymes of ACP, GOT and MDH was detected between tomato seedlings grown in the absence of NaCl and those irrigated with 0.6% NaCl.

Introduction

As excessive sodium concentration in the soil is one of the major problems for cultivated lands, much effort has been directed toward the development of salt tolerant plants. Attention has been devoted to the analysis of the responses to environmental stressful conditions of more or less sensitive genotypes (Boscherini *et al.*, 1999). Most crop plants, including the cultivated tomato, are sensitive to salinity, although differences between tomato

cultivars have been reported (Rus-Alvarez and Guerrier, 1994 and Cano *et al.*, 1996). One strategy to reduce the deleterious effects of soil salinity on tomato production is the development of salt-tolerant cultivars (Nabors *et al.*, 1980).

Cell and tissue cultures have been regarded as a useful tool to obtain and study lines tolerant to biotic and abiotic stresses, by exploiting the genetic variability arising during *in vitro* culture conditions (Buiatti *et al.*, 1984). Selection for salinity tolerance can be carried out *in vitro*, by culturing either explants, callus pices, cell suspension, protoplasts, embryos or microspores in the presence of screening agent, e.g. NaCl (Cano *et al.*, 1996). *In vitro* selection and screening for salinity tolerance have been reported in tomato by (Yusuf *et al.*, 1994; Cano *et al.*, 1998 and Mercado *et al.*, 2000).

On the other hand, changes in protein, isozyme and mRNA synthesis has been well documented under salinity stress (Singh *et al*, 1985; King *et al*, 1986; Claes *et al*, 1990; Ramagopal and Carr, 1991; Parcek *et al.*, 1998; El-Enany, 2000, and Ahmed *et al.*, 2001). In tomato, the alteration of gene expression by drought and salt stress was studied by Chen and Tabaeizadeh (1992) using two - dimensional polyacrylamide gel electrophoresis. They found that salinity stress accumulated two proteins in roots and induced different stress-specific proteins. It is suggested that the quantitative and qualitative changes in protein synthesis may contribute to stress-resistant or stress-injury mechanisms.

Under salinity stress, it was suggested that the newly synthesized proteins, together with amino acids and soluble nitrogenous compounds, act as components of a salt tolerance mechanism. They may function as compatible cytoplasmic solutes in osmotic adjustment to equalize the osmotic potential of the cytoplasm with the vacuoles under adverse conditions of salinity (Greenway and Munns, 1980 and Dubey and Rani, 1989).

The present investigation was carried out to focus on the utility of tomato tissue culture for *in vitro* selection of salt-tolerant tomato plants. In addition, to study the changes in gene expression under salinity stress as revealed by protein and isozyme analyses.

Materials and Methods

Plant materials: Two tomato (*Lycopersicon esculentum* Mill) varieties namely; Roma VF and Peto-86 were used in the present study. Seedlings from these varieties were grown *in vitro* on ¼ MS

(Murashige and Skoog, 1962) medium free of growth hormones.

Tissue culture method: The hypocotyls and cotyledonary explants from two weeks old seedlings of each variety were cultured in the regeneration medium. The regeneration medium consisted of MS medium supplemented with 2.0mg/l benzyladenine, 0.2mg/l indol acetic acid and 10.0mg/l adenine. The cultures were incubated in 16 hours light regime at 27°C. Callus and shoot regeneration were achieved on the same medium, however subculture to fresh medium was made when needed. After 6 – 8 weeks, the good developed shoots were excised and transferred to the rooting medium (MS-medium + 6.0 mg/l indol-3-acetic acid) and incubated under the same conditions for 4 weeks. The rooted shoots were transferred to hormone-free medium for further development. The good developed plantlets were transferred to the greenhouse for adaptation and further growth. The healthy plants were then transferred to the field (R0). At maturity, the tomato fruits were brought to the laboratory and the R1 seeds were collected.

In vitro selection procedure: preliminary experiment was done using the cultivar Peto-86 to determine the sub-lethal concentration of sodium chloride (NaCl). Explants were cultured on MS medium containing 0.0, 0.2, 0.4, 0.5, 0.6, 0.7, 0.8, and 1.0% (w/v) sodium chloride. The concentration 0.6% NaCl was found to be suitable for the in vitro selection for salinity tolerance. Hypocotyls and cotyledon explants taken from Roma VF and Peto-86 were cultured on the regeneration medium supplemented with 0.6% NaCl. The selected shoots were subjected for second round of selection by culturing its leaf explants on regeneration medium containing 0.6% NaCl. Shoot regeneration, rooting and plant hardening were attained under salinity stress. The good developed plantlets were transferred to the greenhouse and finally to the field to produce the R1-seeds. Control treatment from both cultivars was also taken in consideration.

Salinity tolerance test: Tomato seeds of both selected and unselected clones (R1 generation) as well as their parents were cultured in pots containing a mixture of sand, peat and soil in equal sizes. When seedlings have one true leaf (after 2 weeks), they were irrigated three times a week with ¼ MS salt solution containing 0.0 and 0.6% NaCl. After three weeks, plants were harvested to measure the shoot and root lengths and dry weights. The experiment was designed in a randomized complete block design with three replicates. The relative salt tolerance index was determined

by calculating the mean salinity tolerance index, S.T.I (Reddy and Vaidyanath, 1986).

Electrophoresis: Soluble protein, and the isozymes of esterase (Est), peroxidase (Prx), malate dehydrogenase (Mdh), acid phospatase (ACP) and glutamate-oxalacetate-transaminase (GOT) were determined in order to study the changes in gene expression under salinity stress. Soluble proteins were extracted from NaCl-stressed (6.0 g/l) and control seedlings of two selected tolerant somaclones (Ps-7 and Rs-5), their unselected plants as well as the donor parents, Peto-86 and Roma VF, using equal volumes of extraction buffer (0.1 M Tris-Hcl + 2.0mM EDTA + 2 % glutathion, pH 7.8). Protein analysis by electrophoresis were carried out according to the method of Laemmli (1970) with 12% polyacrylamide and 1 % SDS (w/v) under denaturing conditions. The gels were stained for protein bands with Commassie Blue R. Then, gels were destained by repeated immersion in a mixture of methanol : acetic acid : water (1:1:8, by volume). The molecular weight of protein bands were determined against protein markers consisted of 94, 67, 48.1, 30 and 20.0 Kd using GS 365 electrophoresis data system program version 3.01 (Microsoft Windows @ version). For peroxidase and esterase analyses, samples were electrophorased on 7.5 % polyacrylamide gels under non-denaturing conditions. The activities of the enzymes were stained following the methods of Vallejos (1983) and Tanksley and Orton (1986).

Results and Discussion

Generally, the results revealed that embryogenic callus with globular structures was developed from both types of cultured explants (hypocotyle and cotyledon). The globular structures were then developed into shoots and roots (Fig. 1a). The regenerated shoots were subcultured on the same medium for further growth and development. When shoots were ca. 4 cm length, they excised and transferred separately to hormone-free medium for further growth (Fig. 1b). The good developed plants were transferred to the greenhouse (Fig. 1c) and finally to the field (Figs. 1d). At maturity, tomato fruits (fig. 1e) were collected and brought to the laboratory for seed collection (R1-seeds).

I- *In Vitro* Selection for Salinity Tolerance: Generally, the preliminary experiment (Table 1) revealed that the development of callus and its differentiation were decreased with the increment of NaCl level from 2.0 to 10.0 g/l. The formation of callus was decreased from 93.88% in non-salinized medium to be 17.86% under 6.0 g/l

NaCl. Similarly, the regeneration rate was reduced from 17 shoots per explant to 0.9 shoots/explant when the NaCl level increased from 0.0 to 6.0 g/l, respectively. At higher concentrations, almost all the cultured explants turned brown and the few developed calli (4.08% of cultured explants) failed to regenerate shoots. Therefore, it was decided to use the concentration 6.0 g/l NaCl as a selection stress to enhance salt tolerance in tomato.

The regeneration potential was adversely affected by culturing on medium containing 2.0, 4.0, 5.0, 6.0 g/l and beyond 6.0 g/l NaCl the inhibition was complete. A similar observation was found by Yusuf *et al.*, 1994; Cano *et al.*, 1998 and Mercado *et al.*, 2000 in tomato using tissue culture techniques for *in vitro* selection for salinity tolerance. Liu and Li (1991) found that callus was formed on 0.5% NaCl, but was less vigorous and the shoot-formation rate was decreased as compared with the control treatment.

The number of explants cultured on 0.6 % NaCl medium and number of selected shoots in comparison with the control treatment (unselected) are given in Table (2). From the two varieties, Peto-86 and Roma VF, a total of 420 cotyledons and hypocotyls were cultured under selection conditions. Averaged over the two varieties, plant regeneration was reduced under salinity stress to be 0.85 shoot/explant, as compared to 15.60 shoot/exp in the control treatment.

In vitro- selected cell lines, even when verified for salt tolerance, have often been found to lose their regeneration potential (Meredith, 1984; and McCoy, 1987). Perhaps for this reason, regeneration was drastically inhibited and the embryogenic callus failed to regenerate shoots when NaCl was found in the regeneration medium (Kirti *et al.*, 1991). In addition, Ben-Hayyin and Goffer (1989) reported that regeneration of plantlets from tolerant cell lines of *Citrus sinensis* did not succeeded in the presence of NaCl .

The regenerated shoots from each variety (resulted from the control and salinized medium) were excised and subjected for rooting. Table (2) show that (averaged over the two varieties) the perentage of root formation in the selected shoots (10.41%) was less than the unselected shoots (39.24%). These results also revealed the inhibitory effect of NaCl on root formation. Cano *et al.*, (1998) found that most apices and regenerated shoots of *L. esculentum* did not develop roots from low levels of NaCl, whereas those of *L. pennellii* were able to develop roots at different salt levels.

The good developed plants were then transferred to the greenhouse for adaptation and acclimatization before they were transferred to the field.

Table (1): The effect of NaCl concentration (g/l) on mean percentages of callus formation and regeneration rate (No. of shoots/explant) in tomato variety Peto-86 after 6 weeks of culture.

NaCl Levels (g/l)	No. of Explants	Callus formation (%)	Regeneration rate (Shoot/explant)
0.0	49	93.88	17.0
2.0	42	71.43	11.0
4.0	56	41.07	5.0
5.0	56	30.36	2.5
6.0	56	17.86	0.9
8.0	49	4.08	0.0
10.0	49	0.0	0.0

Table (2): The numbers of cultured explants and plants development on media supplemented with 0.0 and 6.0 g/l NaCl.

	Peto-86	Roma VF	MEAN
Medium With 0.0 % NaCl			
No of explants	70	77	73.5
No of shoots	1201	1081	1141
Shoot/explant	17.16	14.04	15.60
No. of rooted shoots	456	438	447
% of rooting	37.97%	40.52%	39.24%
Plants maintained in the field	128	148	138
Medium With 0.6 % NaCl			
No of explants	210	210	210
No of shoots	184	170	177
Shoot/explant	0.88	0.81	0.85
No. of rooted shoots	21	16	18.5
% of rooting	11.41%	9.41%	10.41%
Plants maintained in the field	14	12	13.0

From the unselected plants 128 and 148 plants of Peto-86 and Roma VF were maintained in the field, respectively. While, only 14 and 12 selected plants from Peto-86 and Roma VF were also maintained in the field (Table 2). The progeny of the selected and unselected plants (R1-generation) as well as their donor parents were tested for salinity tolerance as described in materials and methods. The mean values of the shoot and root lengths, and dry weights after three weeks of growth on control (non–salinized) and salinized (6.0 g/l NaCl) treatments are given in Tables (3 and 5), while tables 4 and 6 display the analyses of variance for these results.

Generally, when the tested plants were irrigated with salinized solution (6 g/l NaCl), seedlings derived from both selected and unselected genotypes exhibited a low rate of growth in comparison with these irrigated with NaCl- free solution. This was observed in all studied characters in the two genotypes (Tables 3 and 5). The analysis of variance for the studied characters revealed highly significant differences between all genotypes as well as between the two concentrations of NaCl (Tables 4 and 6).

The results in Tables (3) revealed that some selected somaclones exceeded their donor parents in salinity tolerance, while other somaclones did not reveal enhancement. Mean salinity tolerance index (S.T.I), which is based on shoot and root lengths and dry weights, indicated that all somaclones selected from Pet-86, except PS-3, PS-4 and PS-14 were more tolerant than their donor parent and the unselected plants (Table 3). Salinity tolerance index was ranged between 77.75 in PS-7 and 64.22 % in PS-3 for the selected plants, while it was 68.66 in Peto-86 and 67.99 % in the unselected plants (Table 3). No differences were found between the S.T.I of Peto-86 and each of unselected plants and the selected clone PS-4. The two somaclones PS-3 (S.T.I = 64.22 %) and PS-14 (S.T.I = 66.5%) showed less tolerance to salinity than their donor parent (S.T.I = 68.66%).

Mean salinity tolerance index (S.T.I.) in Roma VF (Table 5) revealed that the selected clone RS-13 followed by RS-5 and RS-7 possessed the highest S.T.I (72.01, 71.30 and 71.26 %, respectively). In addition, the selected clones RS-1, 6, 8, 9 and 11 (S .T .I = 67.58, 66.36, 68.86, 68.03 and 67.88 %, respectively) were also more tolerant to salinity than their donor parent (S.T.I. = 63.6 %). The two selected clones RS-4 and RS-12 did not show enhancement in their tolerance to salinity. While, RS-2 (S.T.I. = 60.71%) and RS-10 (59.48%) were more sensitive to salinity than their donor parent Roma VF.

Table (3): Mean values of shoot and root lengths (cm) and dry weights (DW) for the variety Peto-86 and both selected (PS-1 to PS-14) and unselected somaclones, on control (0.0 NaCl) and salinized (6.0 g/l NaCl) treatments.

Genotypes	NaCl g/l	Shoot length (Cm)	%	Root length (Cm)	%	Shoot DW (mg)	%	Root DW (mg)	%	S.T.I.[b]
Peto-86	0.0	12.7		8.3		82.7		21.0		68.66
	6.0	7.1	55.91	6.5	78.31	61.0	73.76	14.0	66.67	
Unselected	0.0	12.5		8.2		80.0		23.0		67.99
	6.0	8.7	69.6	5.3	64.63	58.0	72.50	15.0	65.22	
PS-1	0.0	13.4		11.3		83.0		30.0		73.22**
	6.0	9.8	73.13	8.2	72.57	53.0	63.85	25.0	83.33	
PS-2	0.0	14.9		8.2		87.0		21.0		73.03**
	6.0	10.6	71.14	6.3	76.83	55.0	63.22	17.0	80.95	
PS-3	0.0	12.8		11.4		80.0		28.0		64.22**
	6.0	8.3	64.84	7.2	63.16	61.0	76.25	20.0	52.63	
PS-4	0.0	15.1		12.6		89.0		33.0		68.93
	6.0	10.5	69.54	7.8	61.90	61.0	68.54	25.0	75.76	
PS-5	0.0	12.8		8.5		84.0		24.0		74.54**
	6.0	9.4	73.44	6.2	72.94	61.0	72.62	19.0	79.17	
PS-6	0.0	13.4		9.9		85.0		30.0		71.00*
	6.0	9.5	70.89	6.5	65.66	63.0	74.12	22.0	73.33	
PS-7	0.0	12.5		9.2		80.0		31.0		77.75**
	6.0	9.4	75.20	7.9	85.87	58.0	72.50	24.0	77.42	
PS-8	0.0	13.5		8.9		85.0		25.0		76.49**
	6.0	9.8	72.59	7.2	80.90	65.0	76.47	19.0	76.00	
PS-9	0.0	14.2		9.5		88.0		30.0		73.77**
	6.0	9.8	69.01	7.6	80.00	61.0	69.32	23.0	76.67	
PS-10	0.0	11.1		6.8		65.0		19.0		75.39**
	6.0	7.3	65.76	5.9	86.76	49.0	75.38	14.0	73.68	
PS-11	0.0	11.6		8.6		68.0		25.0		75.42**
	6.0	8.2	70.69	7.1	82.56	54.7	80.44	17.0	68.00	
PS-12	0.0	12.5		9.2		81.0		29.0		72.33**
	6.0	8.6	68.8	6.9	75.00	62.0	76.54	20.0	68.97	
PS-13	0.0	13.4		9.3		85.0		30.0		72.81**
	6.0	9.3	69.40	6.3	67.74	63.0	74.12	24.0	80.00	
PS-14	0.0	11.6		8.9		67.0		25.0		66.50
	6.0	9.6	82.76	6.5	73.03	39.0	58.21	13.0	52.00	
Average of selected plants	0.0	13.1		9.5		80.5		27.1		72.57
	6.0	9.3	70.99	7.0	73.68	57.5	71.43	20.1	74.17	
LSD 0.05		1.522		0.963		4.930		4.33		1.955
0.01		2.025		1.281		6.556		5.759		2.693

Where: a): % of its control, b): Salinity tolerance index (S.T.I.).

Table (4): The analysis of variance for mean values of shoot and root lengths and dry weights (DW) for the variety Peto-86 and both selected (PS-1 to PS-14) and unselected somaclones, on control (0.0 NaCl) and salinized (6.0 g/l NaCl) treatments.

Source	DF	Shoot length	Root length	Shoot DW	Root DW
Replicates	2	3.283	0.601	10.167	9.031
Genotypes (G)	15	5.804**	6.380**	257.272**	97.894**
Salinity (S)	1	361.150**	145.534**	12489.844**	1197.094**
G X S	15	0.931	1.668**	38.466**	5.694
Error	62	0.869	0.348	9.113	7.031

The use of plant tissue culture methods for screening and selecting salt-tolerant tomato plants has been reported and discussed by several authors (Tal, 1984; Stavarek and Rains 1984; Yusuf *et al.*, 1994; Cano *et al.*, 1998 and Mercado *et al.*, 2000). A positive correlation between the responses of whole plants and callus derived from them was found in the genus *Lycopersicon* (Tal, 1984). Therefore, the salt tolerance, which expressed at the cellular level, could be expected to be expressed in whole plants in *Lycopersicon*, and breeding tomato cultivars for salt tolerance might be conducted at callus culture level effectively (Liu and Li, 1991).

However, an important point remain to be verified, i.e., is that any such new trait that has been selected at the cellular level can be expressed at the whole plant level and then transferred to the next generation. In the present study, the cotyledon and hypocotyl explants of tomato were cultured on the selective medium containing 0.6% NaCl then the consequential calli were subcultured on the same medium for regeneration and development of the selected plants until the regenerants were able to be transferred to the greenhouse. Then, the progeny (R1generation) of such plants were tested and showed salt tolerance. Thus, it could be concluded that salt tolerance that has been expressed at the cellular level and the mechanism(s) of enhanced salt tolerance in tomato is stable during the course of plant development in the field and transmitted to the progeny of the selected plants. Orton (1980) suggested relatively simple genetic bases for salinity tolerance in barley, which may be transferable by hybridization and selection, and that genes for tolerance may be additive

and therefore amenable to concentration by selection. Meanwhile, Nabors *et al.*, (1980), Tal (1984) and Bressan *et al.*,

Table (5): Mean values of shoot and root lengths and dry weights (DW) for the variety Roma VF and both selected (RS-1 to RS-12) and unselected somaclones, on control (0.0 NaCl) and salinized (6.0 g/l NaCl) treatments.

Genotypes	NaCl g/l	Shoot length (Cm)	%	Root length (Cm)	%	Shoot DW (mg)	%	Root DW (mg)	%	S.T.I.[b]
Roma VF	0.0	11.5		8.0		76.0		29.0		63.60
	6.0	7.4	64.35	5.0	62.50	55.0	72.37	16.0	55.17	
Unselected	0.0	11.0		7.0		71.0		22.0		65.53
	6.0	6.5	59.09	5.0	71.43	45.0	63.40	15.0	68.18	
RS-1	0.0	10.0		6.0		55.0		19.0		67.58**
	6.0	5.8	58.00	4.5	75.0	35.0	63.64	14.0	73.68	
RS-2	0.0	11.0		9.0		72.0		28.0		60.71
	6.0	6.5	59.09	6.0	66.67	38.0	52.78	18.0	64.28	
RS-3	0.0	12.8		6.0		82.0		19.0		72.01**
	6.0	8.5	66.41	5.0	83.33	53.0	64.63	14.0	73.68	
RS-4	0.0	13.5		10.0		89.0		30.0		63.79
	6.0	8.7	64.44	6.0	60.00	57.0	64.04	20.0	66.67	
RS-5	0.0	11.1		7.0		70.0		21.0		71.30**
	6.0	7.5	67.57	5.0	71.43	49.0	70.00	16.0	76.19	
RS-6	0.0	11.5		8.0		74.0		25.0		66.36*
	6.0	7.8	67.83	5.5	68.75	48.0	64.86	16.0	64.00	
RS-7	0.0	10.8		7.5		76.0		24.0		71.26**
	6.0	7.2	66.67	6.0	80.00	45.0	59.21	19.0	79.17	
RS-8	0.0	12.5		7.0		79.0		25.0		68.86**
	6.0	8.8	70.40	5.0	71.43	55.0	69.62	16.0	64.00	
RS-9	0.0	12.2		7.0		75.0		21.0		68.03**
	6.0	7.8	63.93	5.0	71.43	49.0	65.33	15.0	71.43	
RS-10	0.0	10.5		6.0		68.0		19.0		59.48**
	6.0	6.1	58.09	4.0	66.67	34.0	50.00	12.0	63.16	
RS-11	0.0	10.6		6.5		68.0		21.0		67.88**
	6.0	6.7	63.21	5.0	76.92	44.0	64.70	14.0	66.67	
	6.0	10.5		7.2		70.0		23.0		
RS-12	0.0	6.3	60.00	5.0	69.44	38.0	54.28	15.0	65.22	62.23
	6.0	11.0		9.0		72.0		28.0		
Average of selected plants	0.0	11.4		7.3		73.2		22.9		67.89**
	6.0	7.9	69.30	5.2	71.23	45.4	62.02	15.8	69.00	
LSD 0.05		2.383		1.389		6.388		3.425		2.185
0.01		3.041		1.772		8.152		4.371		3.043

Where: a): % of its control, b): Salinity tolerance index (S.T.I.).

(1987) reported that inheritance patterns for salinity tolerance were complex and not strictly Mendelian, suggesting multiple mutaional events.

In the present investigation, most of the selected clones showed significant enhancement in their growth under salinity treatment, which revealed higher percentages of salinity tolerance indices, as compared with their donor parent. These results indicating the feasibility and effectiveness of

Table (6): The analysis of variance for mean values of shoot and root lengths and dry weights (DW) for the variety Roma VF and both selected (RS-1 to RS-12) and unselected somaclones, on control (0.0 NaCl) and salinized (6.0 g/l NaCl) treatments.

Source	DF	Shoot length	Root length	Shoot DW	Root DW
Replicates	2	1.232	3.816**	32.250	9.250
Genotypes (G)	13	6.979**	3.847**	476.349**	46.154**
Salinity (S)	1	312.506**	91.354**	12752.679**	1203.857**
G X S	13	1.474	1.031	56.217**	8.703
Error	54	1.932	0.656	13.880	3.991

screening and selection for salt tolerant genotypes via tissue culture in salt stressed medium. Salt tolerant plants was demonstrated stable, inheritable tolerance have been regenerated from tolerant selected cultures of tomato (Yusuf *et al.*, 1994; Cano *et al.*, 1998 and Mercado *et al.*, 2000).

Tomato plants selected from Peto-86 and Roma VF showed better shoot and root growth on salt treatment when compared to the control treatment. Delane *et al.* (1982) reported that the primary detrimental effect of NaCl on salt tolerant barley grown in hydroponic experiments was on shoot growth

rather than on root growth. In contrast, El-Sharkawi and Salama (1977) reported that the root volume play the major role in tolerance to salinity in wheat and barley plants.

The results also revealed that a number of salt-selected clones such as PS-4, RS-4 and RS-12 did not show enhancement in their tolerance to salinity at R1 generation, as compared with their donor parent

(Tables 3 and 5). These results indicating that the tolerance displayed by their original R0-selected plants was likely due to the physiological adaptation during the course of selection and not as the result of genetic changes. Similar results and conclusion were also found by Chandler and Vasil (1984). Chaleff (1983) summarized a number of reasons why a trait selected at the cellular level may not be expressed in regenerated plants. These reasons are; (1): many variants selected *in vitro* are not the result of genetic change, but rather a change in gene expression or biochemical activity, (2): other variants may be the result of genetic changes of an unstable nature, e.g. gene amplification, and (3): the absence of expression of selected trait may be due to the metabolic complex of higher plants (Chaleff, 1983).

II- The Changes in Gene Expression Under Salinity Stress:

Since proteins comprise the majority of stable functional genetic products, the changes in gene expression by salinity stress, as revealed by protein and isozyme analysis were studied in the two varieties Peto-86 (S.T.I = 68.66%) and Roma VF (S.T.I. = 63.60%), their unselected plants (S.T.I= 67.99, 65.53%) and the two selected salt-tolerant somaclones PS-7 (S.T.I = 77.75%)and RS-5(S.T.I. = 71.30%).

a) Protein analysis:

Electrophoretic changes in protein patterns of the two varieties Pearson improve and Midi-A, and their unselected and selected (PS-7 and RS-5) plants grown under control and salinity treatments (0.0 and 0.6 % NaCl) are summarized in Table (7) and illustrated in Fig. (2). All tested genotypes exhibited different protein patterns. They manifested a maximum number of 46 protein bands, which were not necessarily being present in all tested genotypes (Table 7).

Generally, the results revealed marked changes in protein patterns as a result of salinity treatment. Under salinity stress, several polypeptides were apparently suppressed whereas others were induced (Table 7). The results revealed that nine bands at molecular weight 91.24, 84.06, 81.15, 65.03, 54.86, 42.02, 36.59, 26.01 and 22.56 kDa were induced under salinity stress in all tested genotypes, as compared with the control treatment (Table 7). The induction of these bands differed from one genotype to another. In this respect, 3 bands were induced in Peto-86, while its unselected plants and selected clone Ps-7 induced 2 and 3 bands, respectively. Only one band in Roma VF, two bands in the unselected plants and four bands in the selected clone Rs-5 were induced

Table (7): Molecular weights of protein bands detected in the donor parents Peto-86 and Roma VF, and their unselected and selected plants grown under salinity stress (S) and control treatment (C). Data were obtained by GS 365 electrophoresis data system program version 3.01.

No.	M.W. KDa	Peto-86 genotypes						Roma VF genotypes					
		Peto-86		Un-selected		Selected		Roma VF		Un-selected		Selected	
		C	S	C	S	C	S	C	S	C	S	C	S
1	99.706	+	+	+	+	+	+	+	+	+	+	+	+
2	93.724	+	+	+	+	+	+	+	+	+	+	+	+
3	91.240	-	-	-	-	-	+	-	-	-	-	-	-
4	86.879	+	+	+	+	+	+	+	+	+	+	+	+
5	84.062	-	-	-	-	-	-	-	-	-	-	-	+
6	81.155	-	-	-	-	+	+	-	-	-	-	-	+
7	79.614	+	+	+	+	+	+	+	+	+	+	+	+
8	76.857	+	+	+	+	+	+	-	-	-	-	-	-
9	69.081	+	+	+	+	+	+	+	+	+	+	+	+
10	65.031	+	+	+	+	+	+	-	-	-	+	-	-
11	62.054	+	+	+	+	+	+	+	-	+		+	+
12	57.966	+	+	+	+	+	+	+	+	+	+	+	+
13	54.865	-	+	+	+	+	+	+	+	+	+	+	+
14	53.865	-	-	-	-	+	+	-	-	-	-	-	-
15	52.780	+	+	+	+	+	+	-	-	-	-	-	-
16	47.558	+	+	+	+	+	+	+	+	+	+	+	+
17	45.82	+	-	-	-	-	-	-	-	-	-	-	-
18	44.992	+	+	+	+	+	+	+	+	+	+	+	+
19	43.389	+	+	+	-	-	-	-	-	-	-	-	-
20	42.02	-	-	-	-	-	+	-	-	-	-	-	-
21	40.612	+	+	+	+	+	+	+	+	+	+	+	+
22	38.258	+	+	+	+	+	+	+	+	+	+	+	+
23	36.592	-	-	-	-	-	-	-	-	-	-	-	+
24	33.877	+	+	+	+	+	+	+	+	+	+	+	+
25	31.981	-	-	-	-	-	-	+	-	-	-	-	-
26	30.338	+	+	-	-	-	-	-	-	-	-	-	-
27	29.635	+	+	+	+	+	+	+	+	+	+	+	+
28	28.380	+	+	+	+	+	+	+	+	+	+	+	+
29	26.102	-	+	-	+	-	+	-	+	-	+		+
30	25.454	+	+	+	+	+	+	+	+	+	+	+	+
31	24.78	+	+	+	+	+	+	+	+	+	+	+	+
32	24.053	+	-	+	-	-	-	-	-	-	-	-	-
33	23.272	+	+	+	+	+	+	+	+	+	+	+	+
34	22.564	-	+	-	+	+	+	+	+	+	+	+	+
35	21.876	+	-	+	-	+	-	+	-	-	-	+	-
36	21.278	-	-	-	-	-	-	+	+	+	+	+	+
37	20.389	+	+	+	+	+	+	+	+	+	+	+	+
38	19.125	+	+	+	+	+	+	+	+	+	+	+	+
39	18.444	+	+	+	+	+	+	+	+	+	+	+	+
40	17.825	+	+	+	+	+	+	+	+	+	+	+	+
41	16.935	+	+	+	+	+	+	+	+	+	+	+	+
42	16.090	+	+	+	+	+	+	+	+	+	+	+	+
43	14.187	+	+	+	+	+	+	+	+	+	+	+	+
44	14.586	+	-	+	-	+	+	-	-	+	-	-	-
45	13.205	+	+	+	+	+	+	+	+	+	+	+	+
46	12.574	+	+	+	+	+	+	+	+	+	+	+	+
No. of bands		35	34	34	32	35	37	31	29	30	30	30	33
Induced			3		2		3		1		2		4
Reduced			4		4		1		3		2		1

under salinity stress. It is interesting to note that a 26.01 kDa protein band was induced under salinity stress in all tested genotypes. Singh *et al* (1985) found that salinity stress induced the synthesis of several novel proteins in tobacco cells, including the predominant 26 Kd protein. Since, 26 Kd protein is specifically synthesized and accumulated in cells undergoing osmotic adjustment to salt or desiccation stress, this protein was named "osmotin" by Singh *et al*, 1987.

The results showed that salinity-stress induced the synthesis of 9 new protein bands in all tested genotypes. In rice, Claes *et al.*, (1990) reported that 8 proteins were induced under NaCl-treatment. In a suspension culture of sugarcane, Ramagopal and Carr (1991) found that the expression of 15 proteins and 18 mRNAs were induced or enhanced by salinity. In *Brassica juncea*, Jain *et al.*, (1993) found that salt stress induced the expression of four new polypeptides (56.1-70.8 kD) at 60 mM NaCl. In tomato, Chen and Tabaeizadeh (1992) found that salinity accumulated two proteins in roots and induced different stress-specific proteins. It is suggested that the quantitative and qualitative changes in protein synthesis may contribute to stress-resistant or stress-injury mechanisms.

In addition to these newly synthesized proteins, salinity stress also suppressed or reduced the production of 7 different proteins in the unselected and selected tomato plants as well as their donor parent. Most of these reduced proteins were of low molecular weights and differ from one genotype to another. These reduced protein bands are 62.05, 45.82, 43.38, 31.98, 24.05, 21.87 and 14.58 kDa (Table 7). The protein band 21.87 kDa was commonly reduced under salinity stress. Under salinity stress, 4 bands in each of Peto-86 and their unselected plants were reduced, while 3 bands in Roma VF and 2 bands in their unselected plants were also suppressed. Only one band (21.87 kDa) was reduced in plants selected from Peto-86 or Roma VF. Ramagopal and Carr (1991) found that salinity stress reduced the expression of 3 proteins and 8 mRNAs in suspension cultures of sugarcane.

Evidently, the presence of different patterns of soluble protein in tomato genotypes having different degrees of salt tolerance strengthens the view that salt tolerance or sensitivity depends on the genetic and biochemical makeup of the genotype. Similar conclusion was also reported by Dubey (1994), Rashed *et al.*, (1994), Pareek *et al.* (1998), and Ahmed *et al.* (2001).

b) Isozyme analysis:

Protein extracts from NaCl-stressed (6.0 g/l) and control seedlings of two selected tolerant somaclones (Ps-7 and Rs-5), their unselected plants as well as the donor parents, Peto-86 and Roma VF were subjected for esterase (Est), peroxidase (Prx), malate dehydrogenase (Mdh), acid phospatase and glutamate-oxalacetate-transaminase (GOT) analyses (Figs. 2 and 3).

In esterase zymogram (Fig. 2), quantitative differences were observed between control and NaCl-stressed treatments of the tested genotypes. In Roma VF, salinity treatment induced the synthesis of esterase bands No. 12 and 13 in both selected (Rs-5) and unselected seedlings. Salinity stress also induces the expression of esterase band No. 5 in tomato seedlings selected (Ps-7) from Peto-86. Salinity also induced the two bands No. 10 and 11 in the unselected plants from Peto-86, while these two bands were also expressed in the selected plants (Ps-7) even under control or salinized treatments.

In peroxidase zymograms (Fig.2, PRX), few differences were observed between the control and stress treatments in the expression of peroxidase isozymes. In this respect, one band (No.1) in the selected plants (Rs-5) of Roma VF was induced under salinity stress. That band (No.1) was also detected in the selected plants (Ps-7) of Peto-86 under both control and stress treatments. In addition, the selected and unselected plants from the two varieties revealed the common expression of two peroxidase bands, No. 2 and 4, not present in their donor parents under both control and salt treatments.

Except the differences in isozyme activity, no quantitative variability was detected between tomato seedlings grown in the absence of NaCl and those irrigated with 0.6% NaCl in the isozymes of acid phosphatase (ACP), glutamate-oxalacetate transaminase (GOT) and malate dehydrogenase (MDH) in all tested genotypes (Fig. 3). Meanwhile, genetic differences in ACP isozymes were found between the tested genotypes. In this respect, the selected and unselected plants of Peto-86 as well as their donor parent revealed two bands of ACP. While the variety Roma VF and its selected and unselected plants were characterized by one band of ACP in their zymogram.

The results revealed that salinity causes induction of isozyme bands, depending on the nature of the isozymes and the tested genotypes.

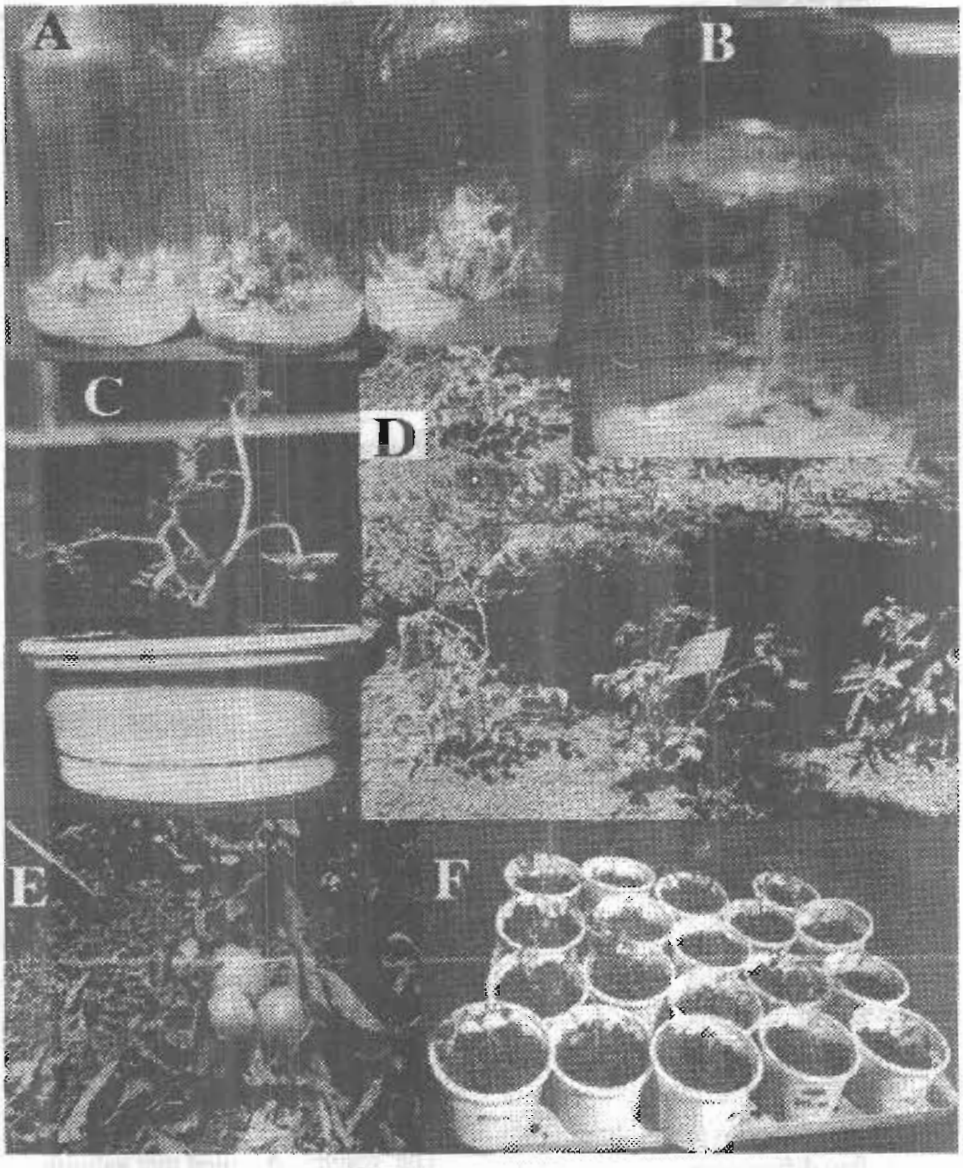

Fig. (1): (A) The regeneration of tomato plantlets from tissue culture. (B): Regenerated tomato plant having shoot and roots. (C): Regenerated tomato plant grown in pot. (D and E): The regenerated tomato plants (R1) grown in the field at the flowering (D) and fruiting (E) stages. (F): Salt selected seedlings of R1 generation during salinity test.

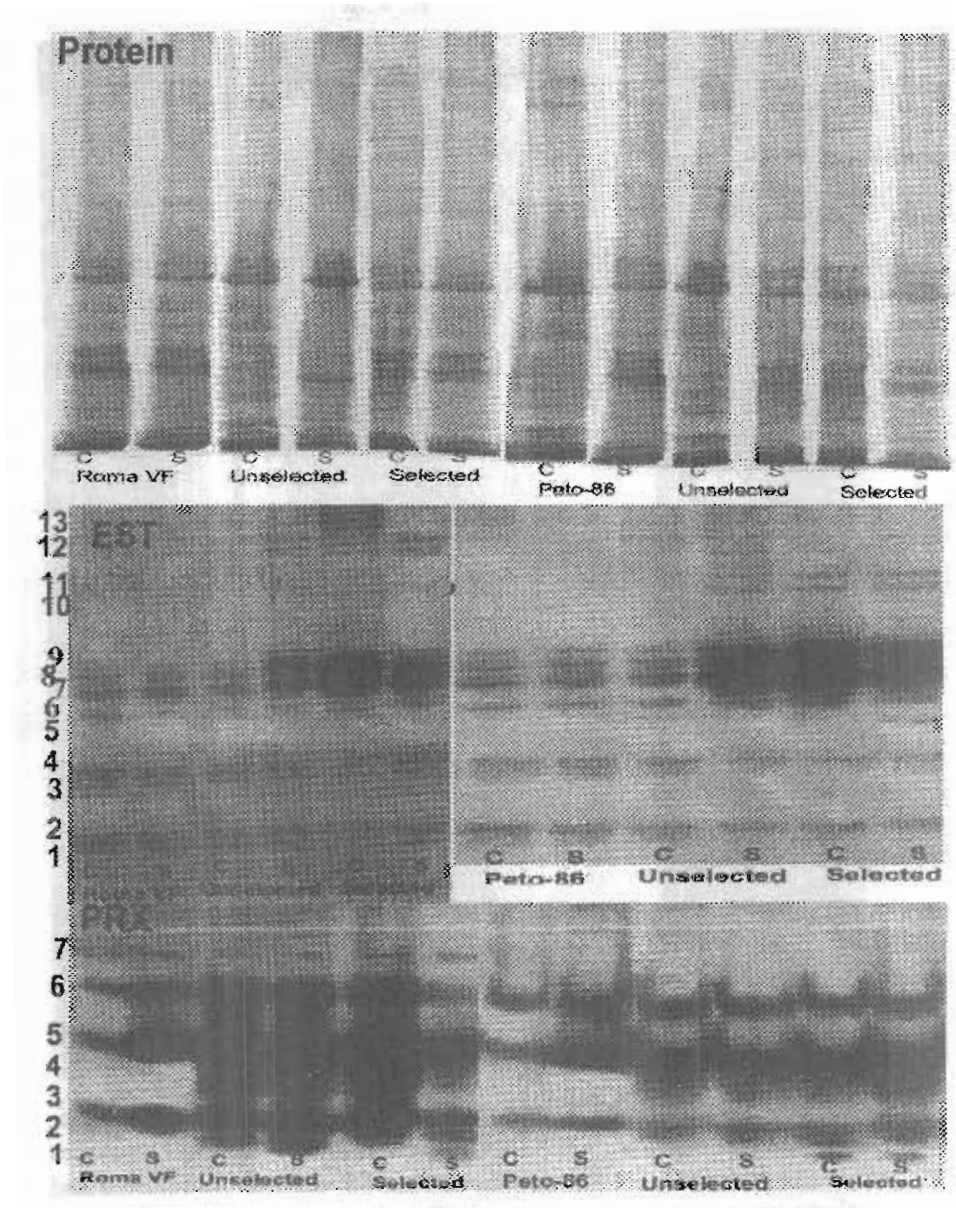

Fig. (2): Electrophoretic patterns of Protein profile and both esterase and peoxidase isozymes detected in the donor parents Roma VF and Peto-86, and their unselected and selected plants grown under salinity stress (S) and control treatment (C).

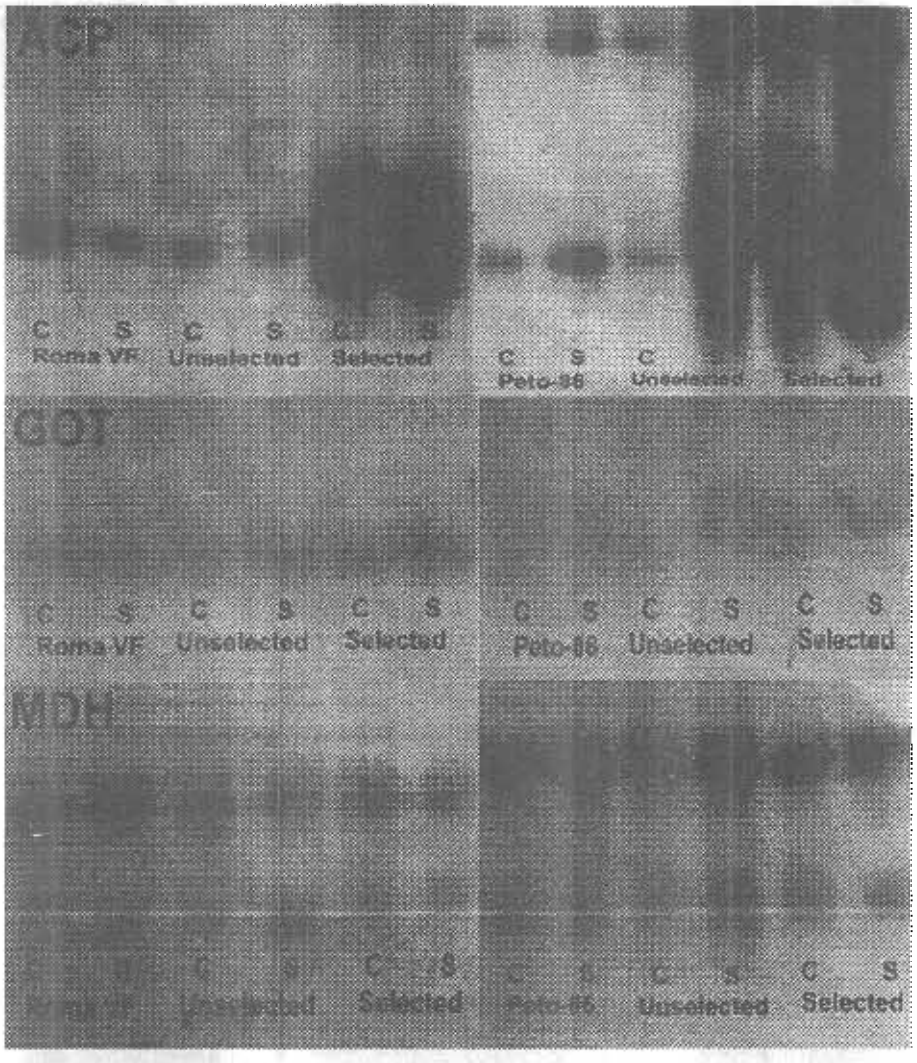

Fig. (3): Electrophoretic patterns of acid phosphatase (ACP), glutamate-oxalacetate transaminase (GOT) and malate dehydrogenase (MDH) isozymes detected in the donor parents Roma VF and Peto-86, and their unselected and selected plants grown under salinity stress (S) and control treatment (C).

The induced bands were; 5 bands in esterase (No. 5, 10, 11, 12 and 13) and one band (No. 1) in peroxidase (Figs. 2 and 3). It has been reported that the peroxidases play an important role in the mechanism of salt tolerance in plants including tomato (Bradley *et al.*, 1992 and Sancho *et al.*, 1996). In tomato plants, enhanced expression of a peroxidase gene was reported in the roots of salt-stressed plants (Botella *et al.*, 1994a). Mittal and Dubey (1991) found that when rice seedlings were raised under increasing levels of NaCl salinity, certain new isoforms of peroxidase appeared and the intensities of some of the preexisting isozymes increased. They suggested that peroxidase isozymes can be useful markers in the analysis of gene functions and metabolic regulations including salt tolerance characteristics.

It is worthy to mention that the selected plants involved in the isozyme analysis showed enhanced tolerance to salinity, as compared to their donor parents. In addition, the selected plants revealed the expression of newly isozyme bands not present in their donor parents. These differences between the tested genotypes strengthen the view that salt tolerance or sensitivity depends on the genetic and biochemical makeup of the genotype. The differences in the number of isozyme and protein bands observed among salt treated seedlings and their control might reflect a differential gene expression as a consequence response to salinity stress. Similar modifications in gene expression during salinity treatments had also been observed by Singh *et al.*, 1985; King *et al.*, 1986; Claes *et al.*, 1990; and Ramagopal and Carr, 1991. In addition, the extra bands of isozymes and soluble protein which appeared in tested genotypes (with different degrees of salinity tolerance) suggested that the genetic program in tomato was altered by salinity stress to induce the production of these proteins for specific pathways involved in the tolerance to salinity. Singh *et al.*, 1987 reported that the osmotin which induced under salinity stress providing the osmotic adjustment to the cells either by facilitating the accumulation of solutes or by providing certain metabolic alterations in the cells, which may be helpful in osmotic adjustment.

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استخدام مزراع الأنسجة لانتخاب نباتات طماطم تتحمل الملحية والتغير في التعبير الجيني تحت إجهاد الملوحة

د. حمـــدي محـــمد العـــارف

قسم الوراثة – كلية الزراعة – جامعة أسـيوط – أسـيوط – مصـر

تم إجراء الانتخاب في مزارع الأنسجة لصفة تحمل الملحية في صنفي الطمـاطم بيتـو ٨٦ ، روما في.أف. حيث تم زراعة ٤٢٠عدد ورقة فلقية من كلا الصنفين علي بيئة انتخابية تحتـوي على ٦,٠ جرام/لتر كلوريد صوديوم. ثم بعد ذلك تم اختبار نسل النباتات المنتخبة وغير المنتخبة وكذلك آبائها (صنفي الطماطم) بالنسبة لصفة تحمل الملحية. وبالإضافة إلى ذلك استخدم كل مـن الطرز الحزمية للبروتينات وخمسة نظم إنزيمية في دراسة التغير في التعبير الجيني تحت إجـهاد الملحية. أوضحت النتائج ما يلـــي: (١) أظهرت بادرات النباتات المنتخبة نموا أفضـل تحـت إجهاد الملحية مقارنة ببادرات النباتات غير المنتخبة وآبائها. (٢) أشار دليل تحمـل الملحيـة ، والمبني علي أساس الطول والوزن الجاف لكل من المجموع الخضري والجذري ، إلى أن كـل الكلونات المنتخبة من الصنف بيتو ٨٦ ما عدا الكلونات PS3, PS4, PS14 كانت اكثر تحمـلا للملحية من آبائها المستخدمة والنباتات غير المنتخبة. (٣) أوضحت النتائج أن صفة تحمل الملحية تنتقل إلى الجيل التالي في نسل النباتات المنتخبة والنامية في غياب إجهاد الملحيـة. (٤) تحـت إجهاد الملحية تم تثبيط تعبير ٧ حزم بروتينية (٦٢,٠٥ ، ٤٥,٨٢ ، ٤٣,٣٨ ، ٣١,٩٨ ، ٢٤,٠٥ ، ٢١,٨٧ ، ١٤,٥٨ كيلودالتون) كما تم تحفيز ٩ حزم بروتينيـة ذات وزن جزيئـي ٩١,٢٤ ، ٨٤,٠٦ ، ٨١,١٥ ، ٦٥,٠٣ ، ٥٤,٨٦ ، ٤٢,٠٢ ، ٣٦,٥٩ ، ٢٦,٠١ ، ٢٢,٥٦ كيلودالتون فـي جميع التراكيب الوراثية المختبرة مقارنة بمعاملة الكنترول. (٥) حفز إجهاد الملحية أيضا ظـهور تعبير خمسة حزم إنزيمية من الاستريز وحزمة إنزيمية واحدة من البيروكسيديز ، ولم تظـهر أي تغيرات كمية في تعبير حزم كل من إنزيمات اسيد فوسفاتيز و جلوتاميت اوكسال اسيتيت ترانـس امينيز و ماليت ديهيدوجينيز بين بادرات الطماطم النامية في غياب الملحية (كلوريد الصوديـوم) وتلك التي تم ريها بمحلول يحتوي على ٠,٦% كلوريد صوديوم.