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IDENTIFICATION AND SCREENING OF TOMPRO2 CDNA IN SOME TOMATO GENOTYPES AND THE MORPHO-PHYSIOLOGICAL AND YIELD RESPONSE TO SALT STRESS

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ABSTRACT

Four tomato genotypes were used in this investigation. They were the tolerant genotype of *Lycopersicon pennellii*, the seneitive genotype *Lycopersicon esculentum* and two Egyptian tomato cultivars, Strain B and Floradid. They were exposed to four salt concentration (0.0, 4000, 8000 and 15000ppm) to identify the response of the morphological, physiological and yield traits. The biochemical and molecular analyses were also screened to identify tomPRO2 gene. Plant height, plant fresh weight and root dry matter showed high response to salt, and the *L. pennellii* was found to be the most tolerant genotype whereas, the wild genotype *Lycopersicon esculentum* seemed to be the most sensitive one. The proline content of the genotype Floradid showed the highest increment. The wild type showed the least increments in proline content. For yield response, *L. pennillii* showed the most tolerable, meanwhile wild type *L. esculentum* was the most seneitive one. Protein banding patterns screened after 3 days treatment, indicated the presence of a unique dark stained band only with genotype Strain B. After 33 days of treatment, a newly synthesized protein was observed under salt concentrations of 4000 and 8000 ppm. The cathodal Peroxidase isozyme band C_6 at R.F. 0.36 cm was suggested to be a biochemical marker for salinity tolerance. A unique acid phosphatase isozyme band A_1 was also observed at 4000ppm salt Conc. A full length (~2145pb) tomPRO2 (P5CS) cDNA was amplified using RT-PCR from total RNA isolated from leaves of treated plants. The tomPRO2 gene was also screened and amplified from different tomato genotypes to test their tolerance to salinity which was found to present in some and absent in others. These results might be of great help to the tomato breeders in Egypt, which enable them to chose the best and tolerant genotypes to be involved in their breeding programs.

Keywords: Acid phosphatase, Morphological responses, Peroxidase, Proline, RT-PCR, SDS-PAGE, tomPRO2 gene, Salt stress, Tomato, and Yield response.

INTRODUCTION

Environmental stresses are among the most limiting factors for plant productivity. Among these, salinity is one of the most detrimental (Boyer 1982). Identification of these salt-regulated genes has allowed better understanding of the complexity of salt tolerance in higher plants (Cushman *et al.*1990). The effect of NaCl addition to nutrient solution has been found to cause a decrease in dry matter and fruit yield (Topculoglu *et al.* 2002). The physiological mechanism of salt tolerance in ten *Lycopersicon* species, all species accumulated proline in all organs in response to salinity but there

was no general relationship between the ability of these species to accumulate proline and their relative salt tolerance (Rajasekaran *et al.* 2000). Water stress triggers the accumulation of proline (pro) in a wide variety of species in all biological kingdoms (Csonka and Hanson 1999). The proteins were extracted from the roots of *Lycopersicon esculentum, L. peruvianm, L. pennellii* and *L. cheesmanii* exposed gradually to NaCl, the extraction being performed a short and long time after the end of NaCl addition. When the electrophoretic banding patterns of the proteins were studied, the most notable changes were: (1) those unique to the cultivated species, namely the decrease of a 37 KDa and increase of a 45.2 KDa protein after a short time only, and (2) those common to all 4 species, namely the decrease of a 22kD protein after a short and long time and the increase of a 26 KD protein after a long time only (Hazak *et al.* 1990). Two protein kinases with molecular masses of 48 and 40 KDa were activated in tobacco cells exposed to NaCl. The 48KDa protein kinase was identified as SIPK (Salicylic Acid –Induced Protein Kinase), a member of the tobacco MAPK(Mitogen – Activated Protein Kinase) family that is activated by various other stress stimuli. The activation of the 40KDa protein kinase was rapid and dependent, the activation of 40KDa protein kinase was specific for hyperosomotic stress. (Hoyos *et al.* 2000). Tomato cv. Pera seedlings grown hydroponically and treated with NaCl .Showed an increment in peroxidase (EC1.11.1.7) and associated with lignifications, in main roots. Its specific activity suggested that the enzyme participate in the synthesis of suberins. (Peyrano *et al.* 1997). The Peroxidase activity was recently reported for Arabidopsis and acid Phosphatase activity at alkaline pH. This activity was unaffected by potent inhibitors of acid Phosphatase activity (Gale *et al.* 2002) .Two tomato *Lycopersicon esculentum* cDNA clones, tomPRO1 and tomPRO2, were isolated and specified as delta 1- pyrroline–5- carboxylate synthetase (P5CS), the first enzyme of proline (Pro) biosynthesis. tomPRO1 is unusual because it resembles prokaryotic polycistronic operons, wherease tomPRO2 encodes a full-length P5CS.Treatment with 200 mM NaCl resulted in a >60-fold increase in Pro levels in roots and leaves. However, there was a < 3-fold increase in the accumulation of the tomPRO2 message and no detectable induction in the level of the tomPRO1 message in response to NaCl stress. Sequence comparison suggested that tomPRO1 is similar to prokaryotic P5CS loci, whereas tomPRO2 is closely related to other eukaryotic P5CS genes (Fujita *et al.*1998).

MATERIALS AND METHODS

Materials

Four Tomato (*Lycopersicon* sp. Mill) genotypes ; two cultivars and two wild types were used in this investigation. The two cultivars were Strain B (Tolerant to EC 4) and Floradid (Tolerant to EC 6) which were provided from the Horticulture Research institute Agriculture Research Center (ARC), Giza, Egypt. The two wild types; *Lycopersicon pennellii* and *Lycopersicon esculentum* P.I 246502 (Tolerant to EC 9) and PI 578495 (Salt susceptible)

were kindly provided by Prof. Dr. C. M. RICK, Tomato Genetic Resource Center, Department of Vegetable Crops, California University, Davis, California, USA . Another three tomato genotypes were only used in this study for screening their genomic DNA.They were Super strain B, Edkaye and Beto 86.

Methods

Effects of Salt Stress on the Tomato Plants:

The four tomato genotypes were grown in pots for one growing season in a sand culture. Three plants of each genotype were grown in each of the four replications under four salt concentrations; 0.0, 4000, 8000 and 15000ppm 3NaCl :1$CaCl_2$. The Plants were irrigated every two days, once with salt–Hoagland solution and once with free salt Hoagland solution. Measurements of three yield per plant and its attributes; plant height (cm) average fruit weight (gm) root dry weight (gm) and plant fresh weight (gm).

Proline Content:

Samples were taken as bulks from selected cultivars for physiological, biochemical and molecular analyses from both control and salt-stress treated plant . Proline was determined according to the method of Bates *et al.* (1973).

SDS Protein and Isozyme Electrophoresis:

SDS – Polyacrylamide gel electrophoresis (SDS – PAGE) was performed according to the method of Laemmli (1970). Starch gel electrophoresis was achieved to detect the biochemical genetic markers of tomato under salt stress. Two isozyme systems were used in this study. They were Peroxidase (Prx) E.C.1.11.1.7. and Acid Phosphatase (Acp) E.C.3.1.3.2. (Douglas and Pamela 1990). The buffer system and staining were applied according to Tanksley and Orton (1983).

Total RNA Isolation and RT-PCR for cDNA tomPRO2 gene:

Total RNA was isolated from leaf tissues using RNA isolation kit for laboratory use (SV Total RNA Isolation systems from Promega. USA). A tomPRO2 fragment (nucleotides 44-2197) containing a complete open reading frame was amplified by RT-PCR one step reaction kit from Promega. With using the primers 5'- AGA CAG TTC ATT CAA CTC-3' and 5'- ATC ACC CTT GCT GAC-3'. The PCR condition was 42 ^{0}C for 30 min for one cycle. Denaturation at 95 ^{0}C for 5 min, 55 ^{0}C for 1 min, polymerization at 72 ^{0}C for 1 min this for 32 cycles then 72 ^{0}C for 10 min, then hold at 4 ^{0}C.

Genomic DNA Isolation, Screening for tomPRO2 Gene and PCR of tomPRO2 (Delta 1-Pyrroline–5-Carboxylate Synthetase) :

Genomic DNA isolation using CTAB extraction method (Saghai-Maroof *et al* 1984).PCR reaction was conducted using two primers with the following sequences.P1 5´- AGA CAG TTC ATT CAA CTC-3´as forward primer. P2 5´- ATC ACC CTT GCT GAC-3´as reverse primer. The reaction conditions for both types of analyses were optimized and mixtures (50 ul total volume) . and

the following program was used; 95 °C/5 min (1cycle), then 94 °C/2min, 65°C/120 sec, 72°C/3min (35cycles), then 72 °C/10min (1 cycle).

Statistical Analyses:

Analysis of variances among replication means for both treated and untreated individual tomato plants at the level of replications and also the least significant deferences were calculated according to Snedecor and Cochran (1972).

RESULTS AND DISCUSSION

Morphological, Physiological and Yield Responses to Salt Treatments:

Plant Height Response to Salt Treatment:

Data in Table 1 showed that under 0.0ppm salt treatment (3NaCl : $1CaCl_2$), the tomato variety *L. esculentum* Floradid had the highest plant height mean values after 90 days of planting followed by the wild type *L. esculentum* (45.3cm), while the *L. pennellii* genotype showed the least plant height mean value (41.4cm). These results indicated that *L. pennellii* was the most tolerable genotype to salt treatments in relation to the other three genotypes. While the wild type *L. esculentum* was the most susceptible one.

Plant Fresh Weight Response to Salt Treatments:

The *L. pennellii* genotypes showed a highly significant difference of its plant fresh weight value as a result to salt treatment concentration of 15000ppm. Meanwhile, the other genotypes showed to have significant differences in response to both concentrations of 8000ppm and 15000ppm. These results indicated that *L. pennellii* is the most tolerable genotype to salt treatments comparing to the other three genotypes, while the wild type *L. esculentum* was the most sensitive one (Table 1).

Root Dry Matter Response to Salt Treatments:

All genotypes showed highly significant lower values than their respective ones as a result to salt treatment concentration of 15000ppm except the tomato variety *L. esculentum* Floradid which showed to have this lower significant value as a response to the salt treatment concentration of 8000ppm (Table 1). However, none of the root dry matter values of any other genotypes showed any significant lower values which differ from their respective average when treated with the same or lower salt treatments. Similar results were obtained by Topculoglu *et al.*, (2002).

Proline Content Response to Salt Treatment:

Results in (Table 1) showed the effect of salt treatments on proline content (ug) of four tomato genotypes after 90 days of sowing. The tomato variety *L. esculentum* Floradid had the highest average proline content. The tomato variety *L. esculentum* Floradid showed the highest increments in proline content as a result to each increase in salt treatments. This response was lower in case of *L. esculentum*. Meanwhile, *L. pennellii* showed to have the least increments in proline content as the result to the increase in salt

stress treatments. In this concern Rajasekaran *et al.* (2000) and Csonka and Hanson (1999) concluded that water stress triggers the accumulation of prolene in different plant species.

Yield Response to Salt Treatments:

Plant fruit weight was taken as a measure to indicate the yield response to salt treatments and presented in (Table 1). The *L. pennellii* treated with 15000ppm, showed to have a highly significant difference of its fruit weight value, comparing with the average value which was taken as the lowest value over the four treatments. Meanwhile, the rest genotypes showed to have significant differences. These findings indicated that *L. pennellii* considered the most tolerable genotype to salt treatments in relation to the other three genotypes. However, the wild type *L. esculentum* is found to be the most susceptible one, it had the least fruit weight value/plant among all genotypes at the treatment of 15000ppm concentration. Generally, salt stress was the most detrimental to plant yield (Boyer 1982). Also, Topculoglu *et al.* (2002) concluded that, in general, NaCl in nutrient solution decreased fruit yield.

The analysis of variances for the five characters of four tomato genotypes treated with four salt concentrations is presented in (Table 2). Both the four genotypes and the four salt concentrations caused highly significant differences in all the five studied traits; either morpho-physiological or yield traits. In addition, the interaction between genotypes and salt concentration also showed highly significant differences in four out of the five traits (Table 2).

Biochemical Analysis in Relation to Salt Stress of Tomato Genotypes.

SDS-PAGE were achieved to screen the water soluble leaf proteins extracted from tomato plants under salinity and control treatments either after 3 days or after 33 days of salinity treatments and presented in Figures (1) and (2), respectively. The protein banding patterns in Figure (1) showed a maximum number of thirty-seven bands which have characterized with molecular weights (Mw) ranged from 97-14.4 KDa. All salt treated genotypes were characterized by the accumulation of two common protein bands of 53.3 and 14.4 KDa. Meanwhile, lower intensity bands were observed in lanes 5 and 9 which represent *L. esculentum* wild type treated with 4000 and 8000ppm salt. Moreover, another lighter intensity bands (53.3 and 14.4 KDa) were found in lane 7 for the genotype *L. esculentum* strain B which was treated with 4000ppm salt. However, a unique dark-stained band with a molecular weight of 17.4 KDa was found only as a response of the *L.esculentum* strain B to the salt concentration of 4000 ppm (Fig.1).

In Figures (2) a band with a MW of about 17.4 KDa could be considered as a marker associated with salt sensitivity. Where it was highly expressed under treatment (8000 and 4000ppm) in all cultivars, except it was present in lower intensity in cultivars (P) and (E). In addition the band at a molecular weight of 58.4 KDa showed to be highly expressed In low level of salt treated plants but not in the high level of salt treated plants.

In this concern, Claes *et al* (1990) found that stress induced damage to proteins is a likely consequence of salinity and desiccation and provides an explanation for the induction of this protein. Tabaeizada *at al* (1995) found that a 65-KDa protein was accumulated gradually in tomato cv. Starifre leaves during water stress. He-Dy and Yu (1995) found that new proteins of MW 21.8, 22.5, 40.7 and 53.3 KDa were present in the callus. The 53.3 KDa protein was increased in rice callus under salinity stress. El-Enany (1997) reported that SDS-PAGE analyses of extracted proteins revealed that in cultures growth of 25 mM NaCl plus proline an extra polypeptides of MW 190, 58 and 26 KDa accumulated as NaCl concentration was increased in the medium and a protein of MW 67 KDa also accumulated. Hassanein (1999) stated that the SDS-PAGE analysis revealed that tomato plants grown under NaCl showed induction (127 and 52 KDa) or repression (260 and 38 KDa) in the synthesis of some polypeptides. Hyperosmotic stress caused a rapid activation of 40KDa protein kinase (Hoyos *et al.*, 2000), and a 26 KDa protein after a long time period of root treatment (Hazak *et al.*, 1990).

Isozyme Polymorphism:

Electrophoresed starch gels for salt treated tomato samples were stained with specific substrates to detect the isozymes of each Peroxidase (Prx, E.C.1.I I.1.7) and Acid Phosphatase (Acp, E.C.3.1.3.2) and were used to detect the biochemical markers for salt tolerance in tomato genotypes

Peroxidase Isozymes.

The electrophoretic patterns of Peroxidase isozymes were detected in both cathodal (C) and anodal (A) migration directions. A total number of fourteen isoperoxidase were detected. Eight bands were exhibited in the anodal direction (A_1-A_8) and six ones towards the cathodal direction (C_1-C_6) with different intensities. However, a maximum total number of ten isozymes were observed for Floradid genotype treated with 15000ppm salt (Fig.3). The anodal bands from A_1 to A_5 were found to be polymorphic. Moreover, band number (A_1) was detected in the profiles of a group of genotypes under salt treatment 4000ppm, and in *L. esculentum* under salt treatment 8000ppm. The isozyme A_2 was detected in *L. pennellii*, *L. esculentum* strain B, and *L. esculentum* Floradid at 8000ppm salt treatment. Meanwhile, at the salt concentration of 15000 ppm, the band number (A_3) appeared in genotype Floradid. Band number (A_4) was found as a common band in all the genotypes at all treatments except in Floradid genotype at salt treatment concentration 8000 ppm. The band number (A_5) was only detected in the profile of Floradid genotype under salt treatment concentration 8000 ppm. The bands number (A_6, A_7 and A_8), were found to be monomorphic and considered as a common bands in all tomato genotypes, even though, it appeared in different intensities.

The cathodal bands were observed among the profiles of the studied genotypes, which all were not necessarily present in one profile. The bands number ($C_1$and C_2) were monomorphic and considered as common bands to all tomato genotypes under all treatments. Although, they appeared in different intensities. The other four bands were polymorphic; i.e., present in

some and absent in other genotypes, with substantial differences in their intensities. Moreover both the isozymes C_3 and C_5 were detected in the profile of the four tomato genotypes under salt treatment concentration 15000 ppm. The band number C_4 was detected in the profiles of all genotypes under salt treatment concentrations of 0.0 ppm, 4000 ppm, and 8000 ppm. However, it was absent only from strain B at 8000ppm treatment (Fig.3). Band number C_6 was detected in the profiles of all genotypes except *L. esculentum* under salt treatment concentration 15000 ppm. These results suggested that these three polymorphic bands could be considered as a biochemical marker for the identification high salt tolerant genotypes. Both qualitative and quantitative changes over Peroxidase isozymes clearly indicated that the most cathodal isoperoxidase faint band, which had the relative mobility of 0.364 cathodal to the origin, was only stimulated with 15000 ppm salt treatment in all genotypes except *L. esculentum*. It is noteworthy that at 15000 ppm salt treatment, the highest qualitative and quantitative changes in peroxidase isozymes were associated with, the highest proline contents; 9.4, 5.3, 6.5 and 12.1 ug/gm for the four tomato genotypes *L. esculentum*, *L. pennellii*, *L. esculentum* strain B and *L. esculentum* Floradid, respectively. Moreover, for each tomato genotype, the total Peroxidase isozymes score over the four salt treatments; 72, 68, 71 and 77 were found to be parallel to the proline content. 5.9, 3.5, 4.2 and 8.42 (Table 1) which were observed for *L. esculentum*, *L. pennellii*, *L. esculentum* strain B and *L. esculentum* Floradid, respectively.

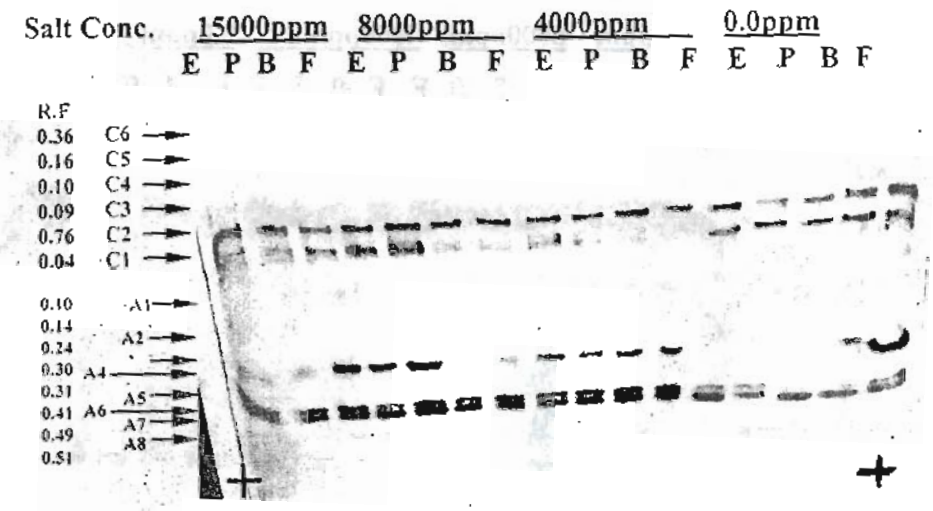

Figure 3: Zymograms of Peroxidase isozymes patterns of four Tomato genotypes under four salt concentration stresses.
E: *L. esculentum*, wild type, P: *L. Pennell*
B: Strain B , F: Floradid.

This result Indicated that the tomato genotype *L. esculentum* Floradid was characterized with the highest value for both the isoperoxidase score and the proline content. In addition, the increase in isoperoxidase scores over all genotypes at 15000 ppm treatment concentration is mainly due to the increments in both number and intensity of Peroxidase bands. Increments in peroxidase as a result of salt stress was foun to be associated with lignification (Peyrano *et al.*, 1997).

4.2.2. Acid Phosphatase Isozymes.

A total number of three anodal (A) bands were observed. However no distinct cathodal (C) Acid Phosphatase isozymes could be detected except the unique isozyme A_1. which was appeared at 4000ppm salt treatment. In the profile of the wild type *L. esculentum*, no qualitative changes could be detected as a result to salt treatments over all genotypes. However, quantitati e changes of intensity clearly suggest that salt treatment at 15000 ppm caused a notable reduction in the number of transcripts expressed as acid Phosphatase isozymes A_2 and A_3 (Fig 4). It is noteworthy that at 15000 ppm salt treatment the lowest acid phosphatase isozymes were associated with the highest proline contents; 9.4,5.3,6.5 and 12.1 ug /gm for the four tomato genotypes *L. esculentum L. pennellii, L. esculentum* strain B and *L. esculentum* Floradid, respectively. Moreover, for each tomato genotype, the trend of intensity and number of bands over the four salt treatments were found to be opposite to the proline content trend 5.9,3.5,4.2 and 8.42 which were observed for *L. esculentum L. pennellii, L. esculentum* strain B and *L. esculentum* Floradid, respectively.

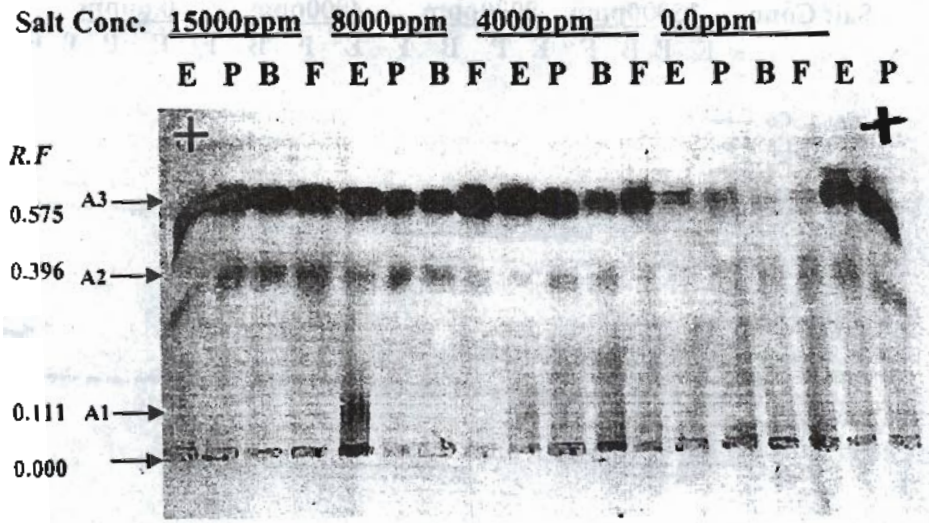

Figure 4 : Zymograms of Acid phosphatase isozymes patterns of four tomato genotypes under four salt concentration stresses.
E: *L. esculentum*, wild type, **P:** L. pennellii,
B: Strain B, **F:** Floradid.

These results indicated that the tomato genotype *L. esculentum* Floradid which was shown to be most tolerable genotype among the four ones characterized with the lowest number and intensity of the acid phosphatase bands and the highest in proline content. It is worthy to mention that the four genotypes higher significant proline values than their respective average at the 8000 ppm of salt treatment. However, *L. pennellii* showed the least response as proline content increment while *L. esculentum* Floradid showed the highest increment proline content at all salt treatments.

tomPRO2 Gene in Relation to Salt Stress in Tomato Genotypes: Detecting and Isolating a tomPRO2 cDNA from Tomato:

The total RNA was used as a template with specific primer and the superscript reverse transcription kit were used to amplify the product. The amplified product was approximately 2145 pb in length (Figure 5) as expected according to analysis by phoretix program 1D gel analysis software version 4.01.

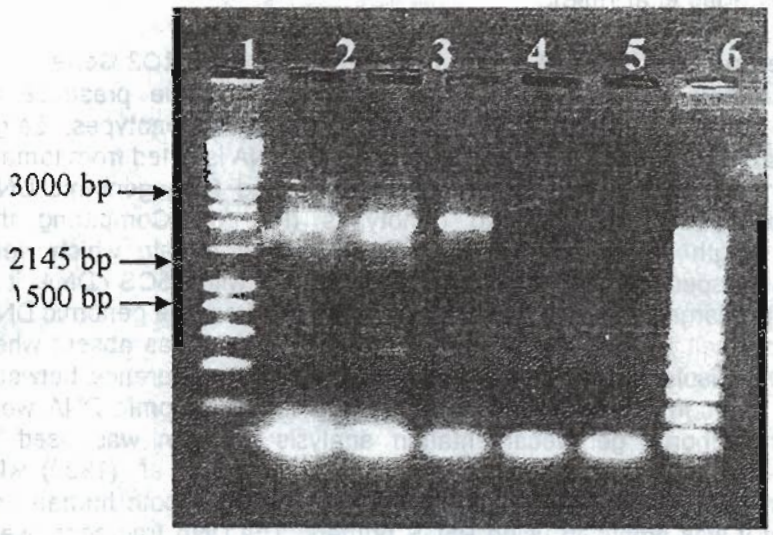

Figure 5 : RT-PCR amplification products.
Lane1: 1Kb DNA marker, **Lane 2**: amplification product of tomPRO2 cDNA from salt stressed *L. pennellii*, **Lane 3and Lane 4**: amplification product of tomPRO2 cDNA from salt stressed Floradid, **Lane 5 Lane 6**: Negative Control, **Lane7**: 100pbDNA marker.

In this concern, it is worthy to mention that two tomato (*Lycopersicon esculentum*) cDNA clones, tomPRO1 and tomPRO2, were isolated and specified as delta 1- Pyrroline– 5-Carboxylate Synthetase (P5CS), the first enzyme of proline (pro) biosynthesis. tomPRO1 is unusual because it resembles prokaryotic polycistronic operons, whereas tomPRO2 encodes a full-length P5CS.Treatment with 200 mM NaCl resulted in a >60-fold increase in pro levels in roots and leaves. However, there was a < 3-fold increase in the accumulation of the tomPRo2 message and no detectable induction in the level of the tomPRO1 message and no detectable induction in the level of the tomPRO1 message in response to NaCl stress, (Fujita *et al* 1998). Nucleotide and deduced amino acid sequences are presented for a PCR amplified osmotin cDNA from salt adapted suspension cultures of tobacco cv. white burley using primers derived from previously published sequence. The coding sequence was 738 pb long and encodes a pre protein of 26 697 Da and a mature protein of 24 283 Da (Kumar and Spencer 1992). The cDNA clone (PNP24) coding for a protein induced by exogenous NaCl was isolated from a tomato root cDNA library with the use of on inosine containing synthetic oligomer, the nucleotides sequence of PNP24 revealed a 5'signal sequences of an open reading frame of 718 nucleotides (King *et al* 1998).

PCR Screening of Different Tomato Genotypes for tomPRO2 Gene.

Five tomato cultivars were used for screening the presence of tomPRO2 gene in genomic DNA isolated from these genotypes. Large fragments at 4300 pb were amplified from genomic DNA isolated from tomato salt tolerant genotypes while no fragments amplified from genomic DNA isolated from susceptible tomato genotypes (Fig. 6). Comparing the fragments length of the various wild and cultivated tomato which were isolated using specific primers for tomPRO2 (P5CS), with P5CS cDNA, it is noted that the larger fragment 4.3 Kbp was amplified from the genomic DNA isolated from salt tolerant genotypes while this fragment was absent when genomic DNA isolated from salt susceptible ones. The difference between the length of fragments amplified on either sources of genomic DNA were used in the Phortix gel documentation analysis program was used to determine the MW. These results agreed with Chien *et al.* (1999) who isolated a PCR fragment of genomic DNA template from both human and murine and it was amplified using P5CS primers. The DNA fragments were found to be differed in length by 6 bp.

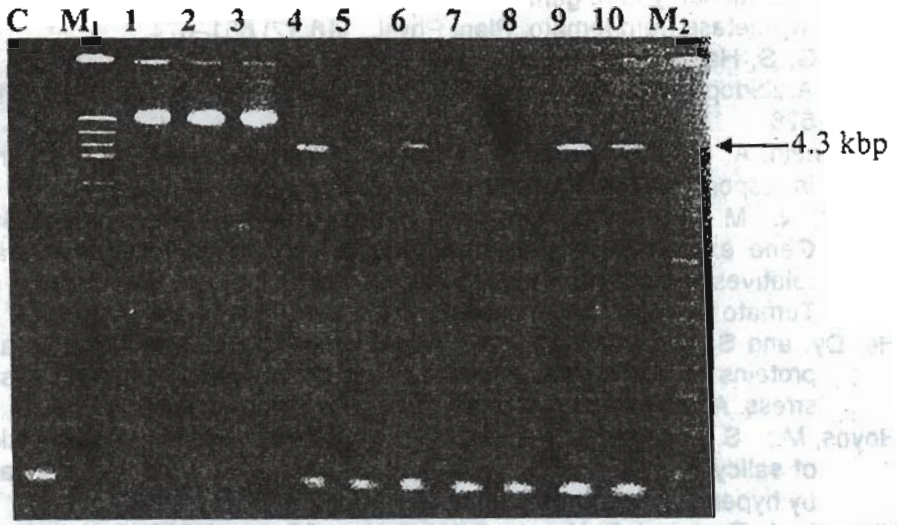

Figure 6 : Screening of the tomPRO2 gene amplified from different tomato genotypes.

Lane C : Negative control, **Lane M_1** :DNA ladder (Lambda DNA/*Hind* III), **Lane 1-3**:Total genomic DNA of tomato, **Lane 4-10** : *Lycopersicon pennellii* wild type, *Lycopersicon esculentum* wild type, Edkawy (variety), Super Strain B, Castle Rock, Beto 86 Variety and Floradid Variety and **Lane M_2**: DNA Ladder pb).

REFERENCES

Bates, L. S; N, Barnett and A. Naylor(1973): Rabid determination of free proline for water stress studies. Plant and Soil 39: 205-207.

Boyer, J. S. (1982): Plant productivity and environment. Sci., 218:443- 448.

Chien-an A. Hu, Wei-Wen Lin, Cassandra Obie, and David Valle.(1999): Molecular Enzymology of Mammalian Δ^1-Pyrroline-5-carboxylate Synthase. J. Biol. Chem., Vol. 274, 10, 6754-6762.

Claes, B; R. Dekeyser; R. Villorroel; V. Bulckem; B. Bauw Van M Caplan (1990): Characterization of rice gene showing organ-specific expression in response to salt stress and drought. Plant Cell, 2:19-27.

Csonka, L. N. and A. D. Hanson (1999): Prokaryotic osmoregulation: Genetics and Physiology. Annu. Rev. Microbial 45: 569-606.

Cushman, J.C.; E. J. Derocher and H. J. Bohnert (1990): Gene expression during adaptation to salt stress. In Environmental Injury to plants, F. J. Kater-man, ed. New York, Academic press. pp. 173-203.

Douglas, E. S and S. S. Pamela (1990) Isozymes in Plant Biology. Timber Press, Portland, Oregon, publishers, USA. 2: 134-136.

El–Enany, A. (1997): Shoot regeneration and protein synthesis in tomato tissue cultures. Biologia–Plantarum, 39(2) 303 – 308.

Fujita, T. A; Maggio, M. R; R. Bressan and L. Csonka (1998): Comparative analysis of the regulation of expression and structures of two evolutionarily divergent genes for DELTAL 1-pyrroline–5– carboylate synthetase from tomato. Plant Physi., 118: (2) 661–674.

Gale, G; S, Haran; M. Bratanova and I. Raskin (2002): Characterization of Arabidopsis acid phosphatase expression. Plant Physiol., 124: 615-626.

Hassanein, A. (1999): Alterations in protein and esterase patterns of peanut in response to salinity stress. Biologia–Plantarum, 42 (2) 241–248.

Hazak, N. M. Tal; J. Cuartero;G. M. Gomez and M. R. Fernande (1990): Gene expression in the cultivated tomato and its wild salt tolerant relatives in response to salt stress. Proceed. Eucarpia Meeting on Tomato Genetics and Breeding, 117.

He, Dy. and S. YU (1995) : Changes in composition of amino acids and proteins of high proline producing variant from rice callus under salt stress. Acta phytophysiologica sinica, 21 (2) 123–130.

Hoyos, M.; S. Zhang and Q. Zhang (2000): Calcium–independent activation of salicylic acid–induced protein kinase and a 40 KDa protein kinase by hyperosomotic stress. Plant Physiol, 122(4) 1355- 1363.

King, G.; A. Turner; J. C. Hussey;S. Wurtele and S. Lee (1998): Isolation and characterization of a tomato cDNA clone which codes for a salt–induced protein Plant Mol. Biol., 18(3) 621 – 622.

Kumar,V. and M. Spencer (1992):Nucleotide sequence of an osmotin cDNA from the *Nicotina tabacum* cv. White barley generated by the Polymerase chain reaction. Plant Mol. Biol., 18(3)621-622.

Laemmli, U. K. (1970): Cleavage of structure proteins during assembly of head bacteriophage T_4. Nature Vol., 227.

Peyrano, G.; E. Taleisnik; M. Quiroga; D. S. Forchetti; H. Tigier and S. Forchetti (1997): Salinity effects of hydraulic tomato roots. Plant Physiol. and Biochem, Paris., 35(5) 387- 393.

Rajasekaran, I.; D. Aspinall and L. Paleg (2000): Physiological mechanism of tolerance of *Lycopersicon* spp. Exposed to salt stress. Canad. J. Plant Sci., 80(1) 151 – 159.

Saghai-Maroof, M. A. ; K. M. Soliman ; R. A . Jorgenson and R. W. Allard, (1984): Ribosomal DNA spacer length polymorphism in barley Mendelian inheritance , chromosomal location and population dynamics. Proc. Natl., Acad. Sci. USA, 81: 8014 – 8018.

Snedecor,G.W. and W.G.Cochran (1972): Statistical Methods, 6th Ed. Iowa State Univ., Ames, Iowa.

Tabaeizada, Z.; H. chamberland;R. Chen; L. Yu;G. Bellemare and J. Lafontain (1995): Identification and immunolocalization of a 56 KDa drought induced protein in cultivated tomato *Lycopersicon esculentum*. Protoplasma, 186(3–4) 208 – 219.

Tanksley, S. D. and T. J. Orton (1983) : Isozymes in Plant Genetics and Breeding. Part, B., Elsevier, Amsterdam.

Topculoglu, B; C. kutuk and U. Akslyk (2002): Dry matter fruit yield and the distribution of calcium and oxalic acid contents in the upper and lower tissues of tomato plant in salinity stress. Acta, Horti, 573; 449- 454.

تعريف وفحص وجود cDNA لجين tomPRO2 والاستجابة المورفو-فسيولوجية والمحصول لضغوط الملوحة على بعض التراكيب الوراثية في الطماطم

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استخدم في هذه الدراسة أربعة تراكيب وراثية من الطماطم، هي التركيب الوراثي المتحمل للملوحة *L. pennellii* والتركيب الوراثي الحساس *L. esculentum* واثنين من أصناف الطماطم المصرية المنزرعة وهما "السلالة B" و"فلورايد"، وتم تعريضهم إلى أربعة تركيزات من الملح (صفر، ٤٠٠٠، ٨٠٠٠، ١٥٠٠٠ جزء في المليون) للتعرف على الاستجابات المورفولوجية والفسيولوجية والمحصول والتحليل الكيميائي الحيوي . كما فحصت تلك التراكيب الوراثية الأربعة لوجود الجين tomPRO2. وقد أظهرت النتائج استجابة عالية لتأثير الملح على صفات طول النبات، الوزن الطازج للنبات وعلى المادة الجافة في جذور النبات بينما كان التركيب الوراثي *L. pennellii* هو الأكثر احتمالا للملوحة بينما كان التركيب الوراثي *L. esculentum* هو الأكثر حساسية للملوحة.

وبينما كان محتوى الحمض الأميني (برولين) في التركيب الوراثي "فلورايد" قد أظهر أعلى زيادة بالمقارنة بين كل التراكيب الوراثية الأربع الأخرى، وقد أظهر الطراز البري *L. esculentum* أقل زيادة في محتوى البرولين.

وقد أظهر التركيب الوراثي البري *L. pennellii* أنه أكثر احتمالا للملوحة فيما يخص المحصول بينما كان التركيب الوراثي البري *L. esculentum* هو الأكثر حساسية.

وقد أشار نموذج حزم البروتين بعد ٣ أيام من المعاملة بالملوحة إلى وجود حزمة كثيفة الصبغ وفريدة في السلالة B فقط، وبعد ٣٣ يوما من المعاملة بالملح ظهر أنه تم تخليق حزمة بروتينية جديدة بتأثير تركيزات الملح ٤٠٠٠، ٨٠٠٠ جزء في المليون.

وتقترح الدراسة أن حزمة البيروكسيديز (C_6) التي تنفصل على مسافة ٠٫٣٦ سم في اتجاه الكاثود تعتبر معلم كيميائي حيوي لأصناف الطماطم التي تحتمل الملوحة.

كما لوحظت حزمة فريدة لأيسوزيم أسيد فوسفاتيز (A_1) نتيجة للمعاملة بالملح بتركيز ٤٠٠٠ جزء في المليون.

في هذه الدراسة تم تعريف وإكثار cDNA كامل الطول لجين الـ tomPRO2 (٢١٤٥ زوج من القواعد) وذلك باستخدام تقنية RT-PCR للحمض النووي الريبوزي الكامل المعزول من النباتات المعاملة، وقد تم فحص وجود جين tomPRO2 وإكثاره من التراكيب الوراثية المختلفة لاختبار احتمالها للملوحة حيث وجد أن هذا الجين موجود في بعض التراكيب الوراثية ويغيب عن البعض الأخر.

هذه النتائج قد تؤدي إلى مساعدة عظيمة القيمة لمربي الطماطم في مصر حيث تمكنهم من اختيار أفضل التراكيب الوراثية والأكثر احتمالا للملوحة وضمها إلى برامج التربية والتحسين التي يقومون بإجرائها.