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IDENTIFICATION OF DIFFERENTIAL DISPLAYED MOLECULES INVOLVED IN EARLINESS AND GROWTH ENHANCEMENT IN TOMATO PLANTS

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ABSTRACT

Three tomato genotypes; late flowering, Lycopersicon lycopersicum "Brandywine", early flowering Lycopersicon lycopersicum "Sweetie" and the wild type Lycopersicon esculentum "PI 176631", were used to study the effects of both indole acetic acid (IAA) and drought treatments on some morphological and biochemical characters. The results showed that, IAA treatment increased both the growth rate and protein concentration in the three tomato genotypes and stimulated a fragment of cDNA isolated from tomato leaf and stem tissues with a molecular weight of about 740 bp which might be related to auxin regulated gene "LeExp2". PCR amplified genomic DNA fragment of 1000bp suggested that the tomato varieties Castle Rock and Super Strain B could be considered as growth nimiety varieties, while the varieties Peto 86, Floradid and Edcawy could be considered as growth unanimity varieties.

Drought treatment caused an increment in protein concentration promoted the flowering in the three tomato genotypes and stimulated the induction of mRNA isolated from tomato flowers on which a cDNA fragment with of about 750bp was PCR hunted with specific primers for the flower gene "Tomf 216". Fragment with molecular weight of about 850bp which may related to Tomf216 gene was PCR amplified form the three tomato varieties, Castle Rock , peto 86 and Floradid compared with the same fragment which was isolated form tomato Lycopersicon esculentum "PI176631". These results suggested that these tomato varieties could considered as an early flowering varieties, while the tomato varieties Super Strain B and Edcawy could be considered as late flowering varieties.

INTRODUCTION

Plant growth is controlled by numerous hormonal and environmental stimuli that interact to regulate cell division and both the direction and rate of cell expansion. Auxin has been implicated in the control of cell elongation (Catala *et al.*, 2000) and auxin induced elongation has been extensively studied in excised stem segments. There is also recent evidence to suggest that auxin is capable of promoting cell elongation in intact plant (Gray *et al.*, 1998). Exogenous auxin is thought to induce rapid elongation in plant tissue throught an increase in mechanical extensibility of cell wall. Several changes in the architecture of the primary wall and in the apoplectic environment have been associated with the action of auxin, including cell wall acidification and modification of specific cell wall polymers. The plant growth hormone auxin

typified by indole acetic acid (IAA) transcriptionally activates many genes such as Ps-IAA4 in pea (Wong *et al.*, 1996) and LeExp2 in tomato encoding to the expansin protein which plays an important role in cell elongation and cell wall modification (Catala *et al.*, 1997). On the other hand, auxin hormone exhibited a great effect on fruit repining and reduces the plant life cycle.

Water deficit is an important factor limiting plant productivity and geographical distribution. Water deficit leads to dehydration of plant tissue. Not only water supply but also low temperature and salt condition cause cellular water loss. Water deficit arisen when the rate of transpiration was higher than water up take. Stomatal transpiration was the main cause of water loss and varied widely between plant species. The highest level of water loss into the plant cell could get by drought treatment imposed by withholding irrigation. This condition can put the plant under environmental stress known as osmotic stress (Bhatt *et al.*, 2002). Drought is considered as an osmotic stress which produces an excess in gene expression for many genes when it used. From those genes there is a promote flowering process which occurred because the plant will inter the reproductive stage which begins with transition to flowering ending with seed production to maintain the kind and protect the plant from that environmental stress (Levy and Dean, 1998). The flower-specific cDNA Tomf216 encoding an osmotin like protein is considered as an important gene which was stimulated with drought treatment in tomato flowers (Ridong *et al.*, 1996). There are another genes which were stimulated and encode to important proteins. These proteins played an important role in the transition to flowering when drought was applied, this matter causes an early flowering and reduced the reproductive stage in the tomato plant.

The aim of this investigation was to study the suitable way to use the plant growth hormone and the LeEXP2 gene which is considered as an important gene in the plant development which might be induced after auxin treatment. Drought imposed by withholding irrigation was ,also, used to study the biochemical response in some tomato genotypes in the purpose to identify the suitable environmental conditions to stimulate flowering and to study the flower specific gene Tomf216 which is one of the important genes in the plant development and might be induced after drought treatment. Screening the presence of both genes on some tomato genotypes, was also achieved in the purpose of identifying how far these genotypes varied in either the growth rate or concerning drought tolerance.

MATERIALS AND METHODS

The gentic materials used in this study were three tomato genotypes, which were kindly, provided by Prof. Dr. Robert W.Thornberg, Molecular Biology Department, Iowa State University, Iowa, USA. These genotypes comprised two *Lycopersicon lycopersicum* commercial cultivars; the late mature "Brandywine" (85 days) and the early mature "Sweetie" (60 days). The third was the medium mature (75 days) tomato *Lycopersicon esculentum* "P.I.176631" CI (Turkey) from the USDA, ARS, Plant Genetic Resources Unit, Cornell University, Geneva, NY. These materials were used

to study the effect of the auxin treatment on the gene complexes of both late and early flowering tomato plants and the medium one as well. The effect of water deficit on the transition to flowering was also investigated using the three genotypes.

Tomato varieties; Castle Rock, Super Strain B, Peto 86, Floradid and Edcawy, recommended by Bejo company by personal communication, were screened for the presence of both the auxin inducible gene and the early flowering gene to classify these varieties for growth indeterminate and early flowering.

Conditions of Seed Germination:

Seeds of the three tomato genotypes were surface sterilized for five minutes with 3% (w/v) sodium hypochlorite containing 1% (v/v) tween 20, and washed 4 times with sterile distilled water, as described by Dielen *et al.* (2001).

The seeds were germinated in Petri-dishes, which were previously sterilized at 25oC for two hours. Filter papers inside each dish were moistened with tap water and the seeds were laid on it, and incubated at 37oC in the dark for three days. Then the seeds were transferred to light at room temperature for 4 days. After one week the seed were transferred to peat moss media in foam tray for 10 days, then the seedlings were transferred to the greenhouse in media mixture contained peat moss: vermiculite: sand in ratio (1:1:1) which were previously sterilized on autoclave at 200oC for 1 hours.

Indole Acetic Acid Treatment:

The seedlings of each of the three tomato genotypes; "late flowering", "early flowering", and the "PI176631" were divided into five replications. Each replication was divided into three subordinate replications included five seedlings for each one. The first one was treated with 0.1m M of IAA and the second one was treated with 0.3m M of IAA and the last one was treated with 0.0m M of IAA and used as a control subordinate replication. The treatment with each of the three concentrations of IAA was applied for 10 daily successive times for tomato seedling by spraying the meristemic shoot apex. The data were recorded on plant height, number of leaves and number of auxiliary buds after the whole 10 sprays treatments at 27 days of sowing. The method of preparing spraying and applying the different IAA concentrations were achieved according to Reinhardt *et al.* (2000).

Drought Treatment:

The drought treatment was applied to tomato seedlings after 27 days of sowing. Drought imposed by withholding irrigation for 10 days in seedlings of all the tomato genotypes.. The plants were divided into five replications. Each replication was divided into two subordinate replications included five plants for each one. The first one was treated with drought and the second one was used as a control plant (normal irrigation regime). (*Bhatt et al., 2002*). Both flowering time and the number of flowers was recorded for either drought treated and untreated plants.

Protein Assays:

Total protein concentration was measured after the treatments in tissue samples of tomato leaves, stems, roots, and flowers according to Bradford protein assay modified method (Bradford, 1976; and Stoscheck., 1990).

Protein Electrophoresis:

Total protein was extracted from tissue samples of stems, leaves and flowers of the treated and untreated genotypes according to the method of Laemmli (1970). The weight of 0.5 grams of each tissue sample was frozen with liquid nitrogen, and ground in a mortar and pestle then the ground tissue was transferred to clean tubes and 500uls of sample extraction buffer (6ml tris1M, pH 8.8+800uls EDTA 0.25M +dd H2O up to 100ml) were added to each sample. The tubes were centrifuged for 20 minuets at 12000 rpm at 4oC. The supernatant containing water soluble protein fraction was transferred to clean tubes and stored at -20oC until electrophoresis. De-staining was repeated several times until gel background was cleared, then the gels were photographed.

Total RNA Isolation and Detection of Gene Expression:

Total RNA was isolated from tomato leaves to amplify cDNA for the LeEXP2 and from flower tissues to amplify the Tomf216 cDNA using the SV Total RNA isolation kit according to the technical manual-TMO 48. Detection of either the LeEXP2; the auxin regulated gene after IAA treatments or the flower specific gene (Tom216) after drought treatment was achieved to study the expression of these genes in leaves and flower tissues, respectively.

RT-PCR Amplification:

Total RNA was next analyzed by reverse transcriptase polymerase chain reaction RT-PCR using specific primers for either the LeEXP2 gene to detect about 740bp fragment (Cardearas *et al.*, 2000) or to detect about 750bp fragment related to Tomf216 gene (Ridong *et al.*, 1996). The specific primers were used in the PCR reactions with total RNAs which were isolated from the three tomato genotypes to study the expression of these genes after the treatments. RT-PCR Ready To Go kit was used to isolate the cDNA fragments according to the manufacture system.

Screening of Genes:

Five tomato genotypes were used to screen the presence of either the auxin regulated gene LeExp2 to classify these varieties for growth nimiety or for the flower specific gene (Tomf216) to classify these varieties for flowering early. Genomic DNA was isolated from five tomato genotypes; Castle Rock, Super Strain B, Peto-86, Edkawy and Floradid using CTAB modified method according to Saghai-Maroof *et al.*, (1984).

PCR Amplification:

The amplification mixture of 100ul total reaction volume was prepared by adding the following reagent to a sterile microfuge tube for each sample: 6ul

of MgCl2 (25mM),10ul of 10x buffer (100mM tris, 500m M KCl, pH8.3), 2ul of PCR nucleotides mix (10mM), 1ul of forward primer (10uM),1ul of reverse primer (10uM), 0.5ul of Taq DNA polymerase (5u/ul), 1ul genomic DNA from each sample (100ng/ul) and 78ul distilled water.

Each of the mixtures were vortexed and centrifuged for 30 second at 2500 rpm to collect the samples at the bottom of tubes. The amplification was performed by heating the samples for five minutes at 95oC for one cycles, then 35 cycles of one minute at 94oC, one minute at 55oC. Final extension step for 10 min at 72oC was achieved and then hold at 4oC. After amplification the samples were kept at –20oC until electrophoresed.

Statistical Analyses:

All data presented for the quantitative characters of the three tomato genotypes were analyzed by analysis of variance and significance was determined using the mean square values at 1% and 5% levels of significance. Protein banding patterns from the SDS-PAGE in flowers of the different tomato genotypes after drought treatment were analyzed using the Phortix gel electrophoresis program.

RESULTS AND DISCUSSION

Indole Acetic Acid Effect on Protein Concentration:

The concentrations 0.0 mM, 0.1mM and 0.3 mM of IAA caused variations in protein concentrations in root, stem and leaf tissues. These variations were presented in Table,1.The results revealed that the highest concentration of protein was obtained as a result to the treatment with the concentration 0.3mM of IAA treatment comparing with the control treatment (0.0mM IAA). In the same time, the 0.3mM IAA treatment showed average increments, over all the three tomato genotypes, of 7.8ug/g, 9.6ug/g and 7.2ug/g protein in root, stem and leaf, respectively. Meanwhile, the concentration 0.1mM IAA showed an average increments of 3.6 ug/g, 5.4ug/g and 4.4ug/g in root, stem and leaf, respectively over the three tomato genotypes compared with the control plants Table 1. Highly significant MS values for both treatment and genotypes were obtained for the protein concentration in the three tissues Table 2. Mean square values of the interaction of genotype x treatment showed a highly significant value for protein concentration in only the stem tissue. These results clearly suggested that IAA more likely involved in enhancing the protein coding genes especially the auxin responsive factors in root, stem, and leaf tissue during the vegetative stage in all the three tomato genotypes. These results were in concordance with those obtained by Tao *et al.*,(2002). The high concentrations of protein in treated tomato were due to the interaction between two protein families included a short- lived inhibitory proteins known as auxin/indole acetic acid (Aux/IAAs) protein and auxin response factors (ARF); transcription factors binding to auxin-responsive element (Hamann *et al.*, 2002).

Table 1 : Protein concentration (ug/g dry tissue) of different dried-tissues of the three tomato genotypes after treatment with either different concentrations of indole acetic acid (IAA) or with drought and normal irrigation regimes.

Treatment	Indole Acetic Acid Concentrations(mM IAA)												Drought and Normal Regime					
Genotype	Root Tissue				Stem Tissue				Leaf Tissue				Root Tissue			Flower Tissue		
	0.0 mM IAA	0.1 mM IAA	0.3 mM IAA	Avg.	0.0 mM IAA	0.1 mM IAA	0.3 mM IAA	Avg.	0.0 mM IAA	0.1 mM IAA	0.3 mM IAA	Avg.	Normal	Drought	Differen-ce	Normal	Drought	Difference
L.esculentum "PI 176631"	19.3	23.3	27.3	23.3	29.3	33.6	39	34	21	26.3	29.34	25.6	18.6	20	1.4	15.3	18.6	3.3
L.lycopersicum "Brandywine"	17.3	21.3	25.7	21.43	27	35	37.3	33.1	23	27.7	30.7	27.1	16.3	20.3	4	16	17.7	1.7
L.lycopersicum Sweetie" "	20	23	27	23.33	34.3	38.3	43	38.5	26	29.3	31	28.8	19	22.3	3.4	16	19.6	3.3
Average	18.9	22.5	26.7	22.7	30.2	35.6	39.8	35.2	23.3	27.8	30.5	27.2	17.9	20.9	3	15.9	18.6	2.7

Aux/IAAs protein contained many types of protein such as Aux/IAA5 and Aux/IAA6 like protein (Abel and Theologis, 1995). Auxin response factors (ARFs) are short peptides of protein susceptible to auxin treatment. The increment of auxin levels in the plant caused an increasing proportion of Aux/IAAs protein families to be degraded, allowing the formation of a great number of ARF-ARF dimers on auxin response element (AuxREs) (Kepiniski and Lyser, 2002). Indole acetic acid treatment also caused an increasing of components of the ubiquitin mediated proteolytic pathway; it seems that regulated proteins degradation is central to most aspects of the response to auxin treatment (Gray *et al.*, 2003).

Table (2): Mean square values of protein concentration in different tissues of the three tomato genotypes after IAA and drought treatments.

Source of variance	DF	Mean squares (Ms)					
		IAA treatment			Drought Treament		
		Protein conc. in root	Protein conc. In stem	Protein conc. in leaves	DF	Protein conc. in root	Protein conc. in flower
Measurements	2	0.7	0.1	11.15 **	2	0.05	0.05
Treatments (T)	2	136.8 **	20.6.3**	116.15 **	1	37.6 **	33.7 **
Genotypes (G)	2	10.7 **	76.8 **	21.8 **	2	8.3 **	2.4 **
G X T	4	0.4	4.05 **	0.27	2	2.9 **	1.9 *
Error	16	0.16	0.25	1.44	10	0.26	0.32

*, **: Significant at 5% and 1% levels, respectively.

Drought effect on protein concentration:

For the drought treatment imposed by withholding irrigation for 10 days, the results suggested that it had considerable effects on both the flowering time and number of flowers over all the tomato genotypes especially for the late flowering genotype "Brandywine". However, the early flowering genotype "Sweetie" showed the least response to drought treatment on both characters (Guirgis *et al.* unpublished data).

Data in Table 1 showed that the average concentrations of total protein over the three tomato genotypes showed that the drought treatment caused an increment of 3ug/g and 2.7 ug/g compared with normal treatment in roots and flowers, respectively. These results suggested that the drought imposed by water deficit increased the protein concentration in roots and flowers in the treated plants. Moreover, the late flowering "Brandywine" showed the highest response to the drought treatment since it showed an increment of 4 ug/g in roots. Meanwhile, both the wild type "PI 176631" and the "Sweetie" variety showed equal increments in response to drought in increasing the protein concentration in flowers, since each of them showed an increment of 3.3ug/g protein over that of their respective normal treatments.

Highly significant mean square due to the genotypes, treatments and the interaction between treatments and genotypes were observed for protein concentration in both root and flowers tissues Table 2. The increment of protein concentrations in roots and flowers after drought treatment was due to the expression of many genes which were stimulated by the osmotic

stress. Some of these genes encoded to an osmotin like protein (Ridong *et al.*, 1996), and others encoded to myrosin like protein (Takechi *et al.*, 1999). In addition, the osmotic stress caused higher photosynthesis rate and higher biological yield (Bhatt *et al.*, 2002).

SDS- PAGE, from leaf tissues after IAA treatment:

Electrophoretic analyses were carried out using SDS-PAGE for water soluble protein fraction and were stained to detect the whole protein banding pattern in tomato leaves after treatment with different IAA concentrations in the three tomato genotypes Figure 1. Analyses of SDS-PAGE showed that a total of 13 bands with molecular weights (MW) ranged from 107.5-21.9 KDa were found in tomato leaves. All IAA treated genotypes were characterized by the accumulation of two unique bands with molecular weights about 52.9 and 25.3 KDa. The highest number of bands (13) was observed in lane 2 with MW ranged from 107.53-21.9 KDa which is represented in the treatment of 0.3mM IAA in tomato "Brandywine", and the lowest number of bands was observed in lanes 4 and 10 which both had 7 bands relative to the untreated tomato "Brandywine" and "Sweetie" varieties, respectively Table 3.

In addition, two bands with a MW of 52.9 and 25.3 KDa can be considered as markers associated with IAA treatment, where both was highly expressed under treatment with 0.3mM of IAA in leaves over all the three tomato genotypes. Moreover, these two bands might be a new type of Expansin protein which was isolated from leaves after IAA treatment Llop-Tous *et al.*, (1999) and Catala *et al.*, (2000).

SDS-PAGE analysis in stem tissues after IAA treatment:

The whole protein banding pattern was analyzed by SDS-PAGE in stem tissues of three tomato genotypes after treatment with different concentrations of IAA Figure 2.

Analysis of SDS-PAGE revealed that a total of 20 bands with molecular weights ranged from 122.8 – 14.9 KDa Table 3. All IAA treated genotypes were characterized by the accumulation of three unique bands with molecular weights of about 107.3, 51.2, and 25.3 KDa (Table 3; lanes 2,3,5,6,8 and 9). Moreover, eight bands related to the treatment with IAA in tomato "Brandywine" variety were found to have, MW between 122.8 – 14.9 KDa. While, there only 3,6 and 8 bands were observed as a result to the treatment with 0.1mM compared with 0.0mM IAA in "Sweetie", "PI176631" and "Brandywine" tomato genotypes, respectively.

The increments in the number of bands due 0.3mM IAA treatment comparing to the untreated controls were, 11, 9 and 3 bands in "Brandywine", "PI 176631" and "Sweetie", respectively. These results indicated that the late flowering "Brandywine" variety was the most sensitive genotype to IAA, while the early flowering "Sweetie" variety was the least sensitive one. Since, the increment in the number of genes which are switched on as a result to 0.3m M IAA treatment on "Brandywine" tomato plants were considerably higher than those expressed in similar tissue of the "Sweetie" variety when treated with the same IAA concentration Table 3.

Table 3 : Protein banding patterns of SDS-PAGE represented as presence (1) versus absence (0) of either leaf (L) or stem (S) tissue after IAA treatments and in flower tissue after drought treated (T) and untreated (U) tomato plants of the three tomato genotypes .

IAA Treatment																				*Drought Regimes*					
Genotypes		"Brandywine"						"PI176631"						"Sweetie"						"Brandywin		"PI176631"		"Sweetie"	
IAA treatment		0.3mM		0.1mM		0.0mM		0.3mM		0.1mM		0.0mM		0.3mM		0.1mM		0.0mM		T	U	T	U	T	U
Lane No.		Lane 2		Lane 3		Lane 4		Lane 5		Lane 6		Lane 7		Lane 8		Lane 9		Lane 10		Lane 2	Lane 3	Lane 4	Lane 5	Lane 6	Lane 7
Band No.	MW (KD)	L	S	L	S	L	S	L	S	L	S	L	S	L	S	L	S	L	S						
1	122.81		0		0		0		0		0		0		1		1		0						
2	110.81																			1	1	1	1	1	1
3	107.53	1	1	0	1	1	0	1	1	0	1	1	0	1	1	0	1	0	0	1	1	1	1	1	1
4	101.77	1		0		0		0		0		0		1		1		0							
5	95.08		0		0		0		1		1		0		1		0		0						
6	76.90		1		1		1		1		0		0		0		0		0						
7	71.23	1		1		0		0		0		0		0		0		0							
8	68.96		1		1		1		1		1		1		1		1		1						
9	66.78																			1	0	0	0	0	0
10	64.56		0		0		0		1		1		0		1		0		0	1	1	1	1	1	1
11	61.03		0		0		0		0		0		0		1		1		0						
12	56.26		1		1		1		1		1		1		1		1		1						
13	55.61																								
14	54.92	1		1		0		1		0		1		1		1		1		1	1	1	1	1	1
15	52.89	1		1		0		1		1		0		1		1		0							
16	51.19		1		1		0		1		1		0		1		1		0						
17	45.12	1		1		1		1		1		1		1		1		1							
18	44.48		1		1		1		1		1		1		1		1		1						
19	43.51	1		1		1		1		1		1		1		1		1							
20	40.33		1		1		1		1		1		1		1		1		1						
21	35.54		1		1		1		1		1		1		1		1		1						
22	33.58	1		1		1		1		1		1		1		1		1							
23	31.92	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1						
24	29.35		0		0		0		1		0		0		1		0		0						
25	26.87																			1	0	1	0	1	0
26	25.33	1	1	1	1	0	0	1	1	1	1	0	0	1	1	1	1	0	0						
27	24.31	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1						
28	22.92	1	0	0	0	0	0	0	1	1	1	1	0	1	1	0	1	0	0						
29	21.92	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1						
30	16.32		0		0		0		1		0		0		1		1		0						
31	14.95		1		1		1		1		1		1		1		1		0						

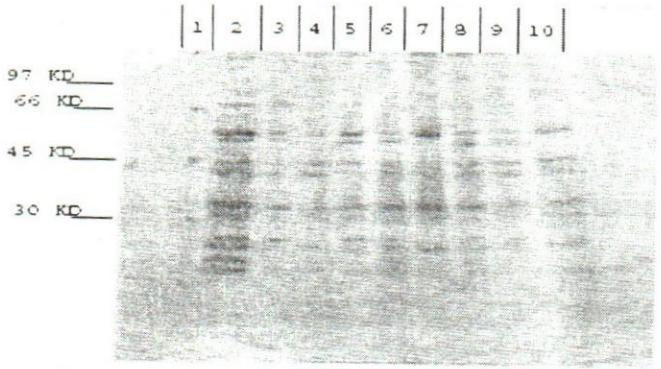

Figure 1: SDS-polyacrylamide gel electrophoresis for soluble proteins of leaf tissues in three tomato genotypes after indole acetic acid treatments.

Lane 1: Standard protein molecular weight marker.

Lanes 2, 3, and 4: Banding pattern of leaf samples of "Brandywine" treated with the 0.3, 0.1, and 0.0 mM of IAA, respectively.

Lanes 5, 6, and 7: Banding pattern of leaf samples of "PI 176631" treated with 0.3, 0.1, 0.0 mM of IAA, respectively.

Lanes 8, 9, and 10: Banding pattern of leaf samples of "Sweetie" treated with 0.3, 0.1, 0.0 mM of IAA, respectively.

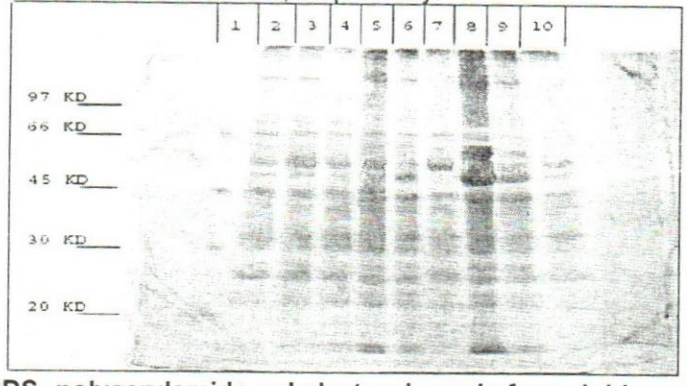

Figure 2: SDS- polyacrylamide gel electrophoresis for soluble protein of stem tissues in three tomato genotypes after indole acetic acid treatments.

Lane 1: Standard protein molecular weight marker.

Lanes 2, 3, and 4: Banding pattern of stem samples of "Sweetie" treated with the 0.3, 0.1, and 0.0 mM of IAA, respectively.

Lanes 5, 6, and 7: Banding pattern of stem samples of "PI 176631" treated with 0.3, 0.1, 0.0 mM of IAA, respectively.

Lanes 8, 9, and 10: Banding pattern of stem samples of "Brandywine" treated with 0.3, 0.1, 0.0 mM of IAA, respectively.

The higher intensity bands were found to be common in all IAA treated tomato and have a molecular size of 51.2 and 25.3KDa which were found in lanes 2, 3, 5, 6, 8 and 9. These bands might be related to the genes which were encoding xyloglucan protein or those proteins involved in cell

elongation and considered as a marker with IAA treatment (Llop-Tous *et al.*, 1999; Catala *et al.*, 2000). These proteins were encoded by IAA inducible genes which control the development (Nagpal *et al.*,2000).

Indole acetic acid treatment induced a type of protein known as Expansin 5 protein which had the ability to induce cell wall extension and were encoded by large gene family. This protein contained different types of small and large size of protein (Carderas *et al.*, 2000). One of these types of protein has a molecular size of about 25 KDa which was encoded by auxin regulated gene (LeExp2) and contained 247 amino acid. (Catala *et al.*, 2000). Another types of Expansin protein have a molecular size about 52 KDa and 60 KDa related to xyloglucan enzymes such as endo-1,4-beta glucanase which had a molecular size of 52 KDa. This protein promoted the plant to increase the cells expansion and the rate of growth Llop-Tous *et al.*, (1999). In addition, the bands with a MW of about 52 and 25 KDa can be considered as a marker associated with IAA treatment in tomato stems over all the three tomato genotypes. These two proteins might be considered as a type of Expansin protein which was found in tomato stem after IAA treatment Llop-Tous *et al.*, (1999) and Catala *et al.*, (2000).

The treated " Brandywine" tomato genotypes showed to have a group of eight IAA induced proteins in their stem tissues which had the molecular weight of 122.8, 107.3, 51.2, 25.3, 22.9, 16.3 and 14.9 KDa (Table 3). In this concern, it is worthy to mention that xyloglucan-specific endo-1,4-beta glucanase (EGase) was isolated from auxin treated pea stems and leaves and the molecular size was determined to be 77 KD by SDS-PAGE, and 70 KD by gel filtration Matsumoto *et al.*, (1997). Harpster *et al.*, (1998) found a cDNA (Cell) encoded an endo-1,4- beta glucanase (EGase) isolated from strawberry by using SDS-PAGE and showed a molecular size of 62 KDa in repining fruit. This protein was related to Expansin protein. Llop-Tous *et al.*, (1999) found another type of endo-1,4- beta glucanase which was isolated from ripe strawberry by *in vitro* translation. They showed that the two cDNA clones Ce11 and Ce12 encoded to mature protein of 52 and 60 KDa, respectively.

SDS-PAGE analysis in flowers after drought treatment:

After 10 days of drought treatment imposed by withholding irrigation, soluble protein was isolated from flowers from the three different tomato genotypes by using SDS-PAGE. A total of 6 bands with molecular weight ranged from 110.8 KDa to 26.9 KDa was detected (Figure 3). Only, treated genotypes were characterized by a common protein band of 26.8 KDa due to the osmotic stress in tomato flowers Table 3. Meanwhile flower tissues of the three untreated tomato genotypes, Brandywine, PI 176631 and Sweetie showed no expression of such protein band Table 3. Meanwhile, this bands clearly expressed in the same tissue of corresponding treated plants Figure 3; lane 2, 4 and 6. Osmotin like protein which produced after the osmotic stress was found in the treated tomato with low water potential Singh *et al.*, (1987) and (1989).

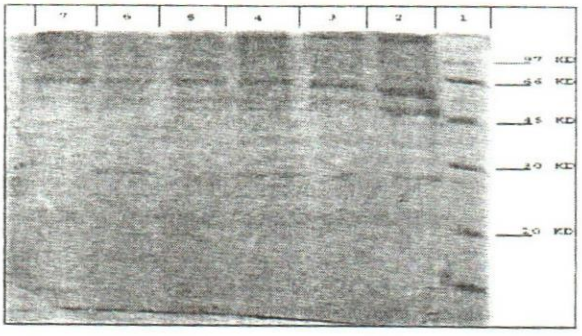

Figure 3: SDS- polyacrylamide gel electrophoresis for soluble proteins of flower tissues in three tomato genotypes after drought treatment.

Lane 1: Standard protein molecular weight marker.
Lanes 2 and 3: Banding pattern of flower samples of "Brandywine" treated with drought and normal irrigation regimes, respectively.
Lanes 4 and 5: Banding pattern of flower samples of "PI 176631" treated with drought and normal irrigation regimes, respectively.
Lanes 6 and 7: Banding pattern of flower samples of "Sweetie" treated with drought and normal irrigation regimes, respectively.

Generally, these results indicated that, the drought imposed by withholding irrigation is considered as an osmotic stress and produced the expression of gene which might be encoded to osmotin-like protein or other stress-related proteins with a molecular weight of about 26.8 KDa expressed in tomato flowers. However, osmotin was characterized by Singh *et al.*, (1987) who found that, the plant cell adapted to grow under osmotic stress like NaCl treatment synthesized and accumulated a 26KDa named osmotin which can constitute as much as 12% of total cellular protein. A low water potential environment appears to be required for accumulation of osmotin like protein of 26KDa Singh *et al.*, (1989). Moreover, the osmotin gene showed to be transcriptionally activated with abscisic acid (ABA) and ethylene Nelson *et al.*, (1992). Also, Ridong *et al.*, (1996) reported that, osmotin-like protein was identified by the differential screening of cDNA library produced from permeiotic tomato flowers. It has a molecular mass of 27.2 KDa contained about 30% amino acid sequence identity to pathogenesis related osmotin.

Auxin regulated gene isolation:

LeExp2 gene was isolated after IAA treatment from tomato apical meristem to investigate the effect of both IAA concentrations 0.1mM and 0.3mM on the stimulation of gene expression. LeExp2 gene encoded to Expansin protein expressed in response to auxin treatment. To study the general characters of this gene, LeExp2 gene was isolated from tomato Total RNA using specific primers based on the gene sequences. The oligoneucletides FHEXP and RHEXP were used as specific primers to detect LeExp2 gene. The fragment with a molecular weight about 740bp was isolated from shoot apical meristem by RT-PCR in the three tomato genotypes after treatment with 0.1m M and 0.3m M of IAA Figure 4.

The fragment of cDNA with a molecular weight about 740bp was isolated from tomato leaf tissues after treatment with 0.3mM IAA by using from tomato late flowering "Brandywine" and early flowering "Sweetie" varieties Figure 5. This result suggested that, the fragment of 740bp of cDNA may be related to LeExp2 gene which was stimulated after IAA treatment. LeExp2 gene has a full length of 1147bp encoding a predicted polypeptide of 247 amino acid, including a signal peptide of 20 amino acid Catala *et al.*, (2000). Cardearas *et al.*, (2000) found that a genomic fragment contained a proximately 2Kbp of non coding sequence including an open reading frame about 740bp related to "LeExp2" was isolated from tomato stem encoded an Expansin protein.

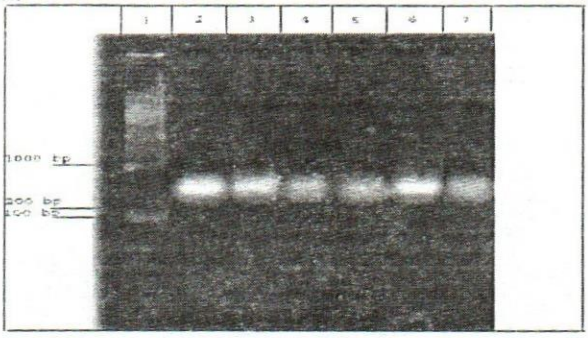

Figure 4: RT- PCR amplification products from tomato apical meristem after treatment with 0.1mM and 0.3mM of IAA.

Lane (1), 100bp DNA marker.
Lane (2) and (3), amplification of tomato "Brandywine" with the concentrations 0.1mM and 0.3mM of IAA, respectively.
Lane (4) and (5), amplification of tomato "PI 176631" with the concentrations 0.1mM and 0.3mM of IAA, respectively.
Lane (6) and (7), amplification of tomato "Sweetie" with the concentrations 0.1mM and 0.3mM of IAA, respectively.

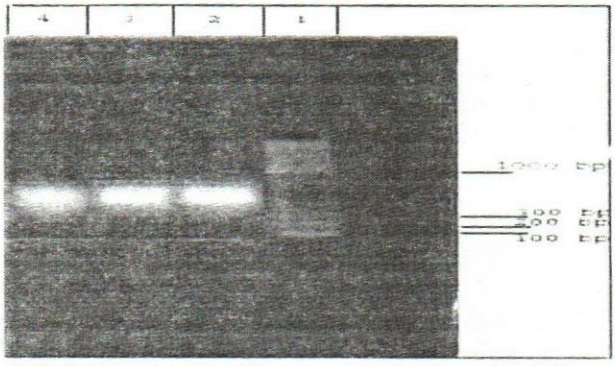

Figure 5: RT- PCR amplification products from leaf tissues in the three tomato genotypes after treatment with 0.3mM of IAA.

Lane (1), 100bp DNA marker.
Lane (2), amplification of tomato "Brandywine" variety.
Lane (3), amplification of tomato "PI 176631" variety.
Lane (4), amplification of tomato "Sweetie".

Screening of "LeExp2" gene:

Tomato varieties, Castle Rock, Super Strain B, Peto 86, Floradid and Edcawy were experimented to screen the presence of auxin regulated gene LeExp2 and classify these varieties for growth nimiety.

Fragment with molecular weight about 1000bp which may related to LeExp2 gene was observed in PCR amplification for two tomato varieties; Castle Rock and Super Strain B compared with the same fragment which was isolated form tomato *Lycopersicon esculentum* "PI176631" Figure 6. Meanwhile, a fragment with molecular weight about 500bp was observed of the other varieties. This result suggested that the two tomato varieties Castle Rock and Super Strain B can considered as growth nimiety varieties, while the tomato varieties Peto 86, Floradid and Edcawy could be considered as growth unanimity varieties. This result is in agreement with that obtained by Catala *et al.*, (2000).

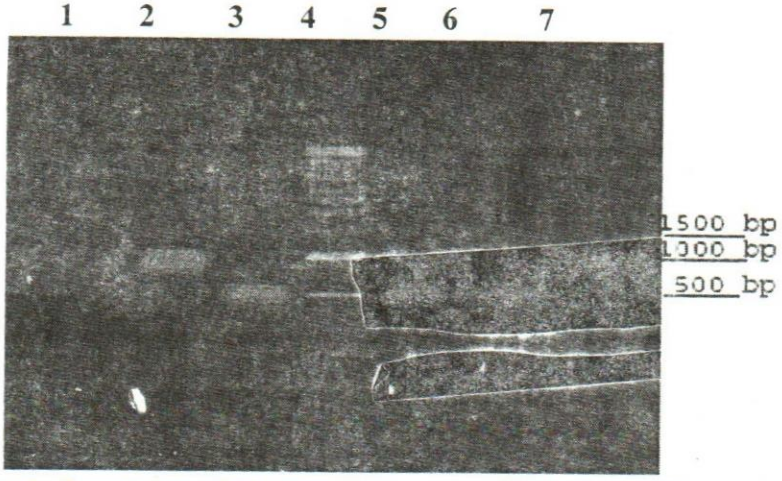

Figure 6: Screening of "LeExp2" gene amplified from different tomato varieties.

Lane (1), tomato *Lycopersicon esculentum*, lane (2), tomato Castle Rock lane (3) tomato, Peto 86 lane (4), 1Kbp DNA marker, lane (5), tomato Floradid, , lane (6) tomato Edcawy, lane (7), tomato Super Strain B.

Flower specific gene (Tomf216) isolation:

Tomf216 gene which encoding an osmotin like protein was isolated from tomato flowers after drought treatment by using the specific primers to detect a fragment with a molecular weight about 755bp from total RNA. RT- PCR was used to isolate the gene from tomato flower. The results showed that, a fragment with a molecular weight about 750bp was isolated from different tomato genotypes (Figure 7). According to Tomf216 gene sequence which was isolated by Ridong *et al.*, (1996), the cDNA was 894 nucleotides in length, contained a coding region of 756bp encoded to 252 amino acid to form the osmotin like protein as a response to osmotic stress. According to this fact, the fragment with a molecular weight about 745bp which was

isolated from different tomato flowers may be relative to the flower specific gene (Tomf216). The full length of Tomf216 gene is about 894 nucleotides in length contained a coding region 756bp encoding a protein of 252 amino acids. The deduced protein has a molecular mass about 27KDa Ridong *et al.*, (1996). These results suggest that, the drought treatment imposed by withholding irrigation caused an osmotic stress which stimulated the expression of flower specific gene (Tomf216) in both tomato roots and flowers. This gene has a coding region about 756bp encoding an osmotin like protein. This protein stimulated the flowering in the late flowering tomato genotypes. These results are in concordance with those obtained by Ridong *et al.*, (1996); Capelli *et al.*, (1997). Ridong *et al.*, (1996) found that, the flower specific gene "Tomf216" which was isolated from tomato flowers *Lycopersicon esculentum* had a full length about 894bp contained a coding region 756bp encoding a protein of 252 amino acids Capelli *et al.*, (1997) found a gene in tobacco had a 734bp open reading frame encoded to an osmotin like protein isolated from old leaves after osmotic stress. Takechi *et al.*, (1999) found another gene with coding region about 450bp response to drought treatment was isolated from Arabidopsis flower and encoded to 150 amino acids.

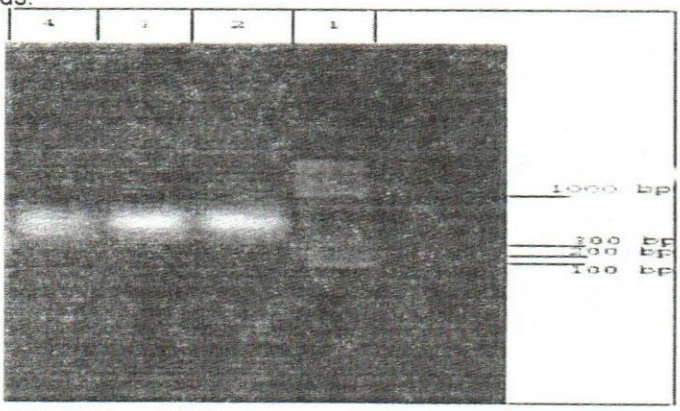

Figure 7 : RT- PCR amplification products.

Lane (1), 100bp DNA marker, lane (2), amplification of tomato "Brandywine" variety, lane (3), amplification of tomato "PI 176631" variety , and lane (4), amplification of tomato "Sweetie".

Screening of "Tomf216" gene:

Fragment with molecular weight of about 850bp which may related to Tomf216 gene was observed in PCR amplification for the three tomato varieties, Castle Rock , peto 86 and Floradid compared with the same fragment which was isolated form tomato *Lycopersicon esculentum* "PI176631" (Figure 8). Meanwhile, a fragment with molecular weight about 500bp was observed of the other varieties. These results suggested that the tomato varieties Castle Rock , peto 86 and Floradid can considered as flowering early varieties, while the tomato varieties Super Strain B and Edcawy can considered as flowering late varieties. This result is in agreement with that obtained by Ridong *et al.*, (1996).

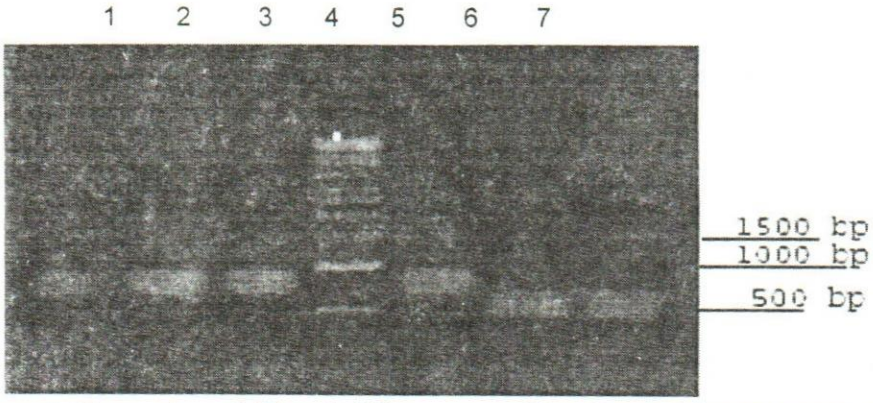

Figure 8: Screening of "Tomf216" gene amplified from different tomato varieties.

Lane (1), tomato *Lycopersicon esculentum*, lane (2), tomato Castle Rock lane (3), tomato Peto 86 lane (4), 1Kbp DNA marker, lane (5), tomato Floradid , lane (6) tomato Edcawy, lane (7), tomato Super Strain B.

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تعريف الجزئيات ذات العرض المتباين والمشتركه فى التبكير وزيادة النمو فى نباتات الطماطم

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استخدم فى هذا البحث ثلاثة تراكيب وراثية للطماطم منه صنفين يتبعان لنوع ليكوبيرسيكون ليكوبيرسكيوم وهما الصنف "براندى واين " المتأخر التزهير والصنف "سويتى " المبكر التزهير والتركيب الوراثى الثالث هو المدخل النباتى البرى رقم PI 176631 ويتبع النوع" ليكوبيرسيكون سكيولينتم " وذلك لدراسة تأثيرات المعاملة بالأندول أسيتك أسيد وكذلك تأثيرات المعاملة بالجفاف على بعض الصفات المورفولوجية والبيو كيميائية .

وقد أظهرت النتائج أن المعاملة بالاندول استيك أسيد أدت الى زيادة كل من معدل النمو وتركيز البروتين فى الثلاث تراكيب وراثية موضع الدراسة وحثت على تكوين شظية وراثية cDNA حجمها حوالى ٧٤٠ نيكليوتيدة والتى تم عزلها من انسجة اوراق وسوق الطماطم والتى قد تنتمى الى جين تنظيم الأوكسين "LeExp2" .

وتقترح الشظية التى تكونت بتفاعل البلمرة المتسلسل بطول ١٠٠٠ نيوكليوتيدة على DNA جينومى انه يمكن اعتبار أن اصناف الطماطم كاستل روك وسوبر إسترين بى انها أصناف سريعة النمو بينما الأصناف بيتو ٨٦ و فلوراديد والأدكاوى يمكن اعتبارهما أصناف بطيئة النمو .

وقد ادت معاملة الجفاف الى الزيادة فى تركيز البروتين الذى يحفز تكوين الأزهار فى التراكيب الوراثية الثلاث كما نشط انتاج الحمض النووى الريبوزى المعزول من ازهار الطماطم والذى امكن عليه تكوين شظية cDNA طولها حوالى ٧٥٠ نيكليوتيدة باستخدام تفاعل PCR وبادئ متخصص لجين Tomf 216 .

تم تكبير شظية DNA طولها حوالى ٨٥٠ نيكليوتيدة باستخدام ال PCR والبادئ المتخصص لجين Tomf 216 وذلك من اصناف الكاستل روك ، بيتو٨٦ و الفلوراديد مقارنة بنفس الشظية المعزولة مند المدخل النباتى PI176631 .

وتقترح هذه النتائج أن اصناف الطماطم الثلاث تعتبر مبكرة التزهير فى حين يعتبر الصنفين سترين بى وإدكاوى اصناف متأخرة التزهير .