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البصمة الوراثية للRNA الخاص بجينات بروتينات الصدمة الحرارية في القمح

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الملخص العربي

يؤثر تغير المناخ والاجهاد غير الحيوى سلبا على الزراعة وإنتاج المحاصيل. في هذه الدراسة استخدمت ثلاثة بادئات عشوائية (هي OPC07, OPV03, OPW03) لعمل البصمة الوراثية للRNA من التراكيب الوراثيه للقمح تحت الإجهاد الحراري و ثلاثة بادئات خاصه بجينات الصدمة الحرارية (هي DD16.9, DD70, DD101) باستخدام تقنية الDifferential Display. تم استخدام أربعة أصناف مختلفة من القمح السداسي صنفان من الاقماح المصريه (جيزة ١٦٨ وجميزة ٧) وصنف مكسيكي متحمل للحرارة SERI 82 بالإضافة إلي صنف صيني حساس للحرارة هو Chinese Spring. تم تعريض البادرات (عمر عشره ايام) للصدمه الحراريه على ٣٧ درجة مئوية لمدة ساعتين .وتم عزل ال RNA وكذلك عملRT-PCR بطريقة ال Differential Display لتخليق جزيئات ال cDNA ثم البوادئ سابقة الذكر. تم فصل النواتج علي جل من الاجاروز حيث تم إجراء التحاليل اللازمة. تراوح عدد الحزم (الجينات التي حدث لها تعبير) المتحصل علبها نتيجه لاستخدام البادئات العشوائية من خمسة حزم لمجموعة البادئات OPW03 + DDPT إلى ١٥ حزمة لمجموعة البادئات OPC07 + DDPC. وقد تم الحصول على عدد مقارب كذلك من جينات تم التعبير عنها وتضاعفت بإستخدام البؤادى الخاصه بجينات الصدمة الحرارية (hsp16.9، hsp70 وhsp101) .وكانت كل من البادئات العشوائية والخاصه قادرة على تمييزالنباتات المعرضه للصدمه الحراريه عن مثيلاتها غير المعرضه. إستطاع التحليل العنقودي لبيانات الDifferential Display فصل التراكيب الوراثية المستخدمة في مجموعتين رئيسيتين. تضمنت المجموعة الأولى كلا من الطرز المعاملة حراريا والغير معاملة لأصناف جيزة ١٦٨ و82 SERI جنبا إلى جنب مع الطراز الغير معامل من الصنف جميزة ٧. وشملت المجموعة الثانية كلا الطرازين من الصنف Chinese Spring (المعامل حراريا والغير معامل) بالإضافة إلي الطراز المعامل حراريا للصنف جميزة ٧. و تشير هذه النتائج الى ان كلا الصنفين جميزة ٧ وChinese Spring هي أصناف حساسه للحرارة بينما الأصناف جيزة ١٦٨ و82 SERI هي أصناف متحملة للحرارة. وعليه يمكن القول بأن استخدام الDifferential Display كان ناجحا في دراسة التعبير الجيني لجينات بروتينات الصدمة الحرارية في أصناف قمح ذات درجات مجتلفة من التحمل الحراري.ويمكن إدخال هذه الأصناف المتحملة للحرارة في برامج تربية القمح لتحمل الحرارة.

RNA DIFFERENTIAL DISPLAY OF HEAT SHOCK PROTEIN GENES IN WHEAT

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ABSTRACT: *Climate change and abiotic stress affect agriculture and crop production adversely. Three primers of 10-mer arbitrarily random primers were used to produce RNA fingerprinting of wheat genotypes under heat stress as well as three specific heat shock protein gene primer pairs using differential display technique. Four different hexaploid wheat varieties were used in this study; two of them were Egyptian ('Giza 168' and 'Gemmeiza 7'); one Mexican variety ('SERI 82') as heat tolerant variety (and one Chinese ('Chinese Spring') as heat susceptible variety. Ten-days-old seedlings were exposed to heat stress at 37 °C for 2 h and the leaf tissues were used for cDNA synthesis. The random primers generated a number of amplified expressed genes ranged from five for the primers combination OPW03+DDPT to 15 for the primers combination OPC07+DDPC. Comparable number of amplified fragments (amplified expressed genes) had been obtained for the different genes primers pairs (e.g. hsp16.9, hsp70 and hsp101 primers pairs). Both random and specific primers were amenable to differentiate between the heat treated and the untreated samples by amplifying specific fragments. The cluster analysis separated the genotypes into two main clusters. The first cluster contained the heat treated and the untreated types of the varieties 'Giza168' and 'SERI 82' along with the untreated type of the variety 'Gemmeiza 7'. The second cluster included both types of the variety Chinese Spring (heat treated and untreated) along with the heat treated variety 'Gemmeiza 7'. These results could suggest that both 'Gemmeiza 7' and Chinese Spring varieties are heat susceptible varieties while 'Giza 168' and 'SERI 82' are heat tolerant varieties. Our results revealed that differential display is successful to compare gene expression profile in different tested thermo tolerant wheat varieties under heat shock condition. So that, these wheat varieties could be used in the wheat breeding programs for heat stress breeding.*

Key words: *Wheat; Differential display; HSP genes; Heat shock response*

INTRODUCTION

Climate change and abiotic stress affect agriculture and crop production adversely. Temperature is one of the most important as higher temperatures adversely affect plant growth and yield. Global mean surface temperatures have risen by 0.74°C ± 0.18°C when estimated by a linear trend over the last 100 years (1906–2005). The rate of warming over the last 50 years is almost doubled over the last 100 years (0.13°C ± 0.03°C vs. 0.07°C ± 0.02°C per decade (IPCC 2007). An in depth analysis carried out by Lobell and Field (2007), involving effect of global warming on six major food crops from the year 1982–2002, revealed combined yield loss of around 40 million tones for wheat, corn and barley per year, where wheat alone accounts for almost half of the yield loss. Almost all stages of wheat growth and development are affected by heat stress. It has long been known that high temperature drastically reduces both yield (as much as 27%) and quality of wheat in Egypt as well as in many wheat-growing regions of the world (Saadalla, 1993; Maestri *et al.,* 2002; and Balla *et al.,* 2009).

Thermo tolerance of plants is correlated with stress-elicited biosynthesis of numerous Heat Shock Proteins (HSPs). Heat shock proteins are a class of ubiquitous and highly conserved proteins which show increased expression in response to an elevated temperature or other forms of environmental stresses (De Maio 1999). HSPs can be produced at particular stage of the cell cycle

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or during development in the absence of stress or constitutively present in both normal and stressed cells (Lindquist and Craig 1988). HSPs protect the structural and functional cell proteins from heat denaturation and aggregation by acting as molecular chaperones (Waters *et al.*, 1996). HSP was used as biochemical selection criterion in improving plant germplasm for hot environments (Krishnan *et al.*, 1989). Both low and high molecular weight HSPs are synthesized in wheat as a response to heat stress when leaf temperature increase ≈10ºC above the optimal growth temperature (18 to 23ºC) (Hendershot *et al.,* 1992).

The Differential-Display Reverse Transcription-PCR (DDRT-PCR) technique is a fingerprinting technology, PCR based method, a very sensitive technique developed by Liang and Pardee (1992) and Bauer *et al.,* (1993). In fact it has been widely used in plants to isolate and identify genes that are differentially expressed in response to various stresses (Malatrasi *et al.,* 2002; Yamazaki and Saito, 2002; Liu *et al.,* 2003; and Zhang *et al.,* 2007). This technique is a suitable and low-cost to identify differentially displayed genes. In this study, we used 10-mer arbitrarily primers to produce RNA finger-printing under heat stress in wheat used specific heat shock protein gene family degenerate primer to identify and analyze heat shock protein gene expression under heat stress.

MATERIAL AND METHODS

Plant material

Four different hexaploid wheat varieties (Table 1) were used in this study; two of them are Egyptians ('Giza 168' and 'Gemmeiza 7') and were kindly provided by Agricultural Research Center (ARC), Giza, Egypt; one Mexican variety ('SERI 82') as heat tolerant variety (Ibrahim and Quick 2001) and one Chinese ('Chinese Spring') as heat susceptible variety (Qin *et al.,* 2008). The last two varieties were kindly obtained from International Maize and Wheat Center (CIMMYT). Ten-day-old seedlings were exposed to heat stress at 37 °C for 2 h in a controlled incubator with 100% relative humidity. After treatment, leaf tissue was excised and immediately frozen in liquid N_2 and held at -75 °C for total RNA isolation. Untreated leaf tissues of 10 days old seedlings (grown as described above) were used as control samples.

Total RNA isolation

Total RNA was isolated from five gram control and heat shocked leaf tissues according to Gene Jet ^{TM} RNA purification kit (Fermentas). Genomic DNA contamination of the total RNA samples was removed by a treatment of 50 µg RNA with 10 U of DNase I purchased from (Promga Corporation).

Differential display method:

Differentially expressed cDNAs from control and heat stressed RNA samples were visualized on agarose gels. The entire procedure used is briefly described below.

Reverse transcription

Total DNA-free RNA was used for first-strand cDNA synthesis. Reverse transcription (RT) reactions were set per RNA sample using 2µg of total RNA with 1 µM of either of the T_{12}VN oligonucleotide (where V is the 3-fold degenerate for A, G, and C; N could be either A, C, G or T). RT reaction samples were incubated in the water bath at 65 °C for 5min, according to H^- Revert aid first strand cDNA synthesis kit (Fermentas). After incubation 4 µ1of 5x reaction buffer, 1 µ1of RNase inhibitor (20 µ/ µ1), 10mM dNTPs, and 100U of M-MuLV Reverse Transcriptase were added. RT reaction samples were incubated in a PCR thermocycler programmed for one cycle of 45 ° C for 60

min, 70 °C for 5 min and storage of samples at 4 °C. The first-strand cDNA synthesis samples were stored at -20 °C until use. Control reactions were performed in the absence of the enzyme reverse transcriptase.

PCR amplification of cDNAs of interest

Four PCR reactions were set per sample using four anchored T12VN primers (DDPA, DDPC, DDPG, and DDPT) as 3′primer and three 10-mer arbitrarily primers as 5′ primer to compare gene expression under heat shock stress. Meanwhile, three heat shock protein genes family specific primers were used as 5′ primer to identification of different members of heat shock protein multi gene families in wheat (Table 2). Each reaction mixture of 25 µ1 contained, 2µl of the first-strand synthesis of cDNAs containing 5 µl of 5x PCR buffer, 1.5 mM of MgCl2, 0.4 mM of dNTPs, 0.35 µl of each primer , and 1 U GoTaq® DNA Polymerase (Promega, Madison ,USA). The PCR program consisted of 45 cycles of 94 °C for 30 s, 37 °C for 2 min and 72 °C for 30 s followed by a final extension step at 72 °C for 10 min. Products were separated on 2 % agarose gel electrophoresis using 1Kbp DNA as a marker. After staining with ethidium bromide, the products were visualized under UV light and photographed with digital camera.

Table (1): Heat shock protein genes specific primer sequences used as forward primer.

Primer name	Primer type	Sequence (5′–3′)
DD16.9	Family-specific degenerate primer	ACCGTCACCGTGCCC Joshi and Nguyen (1996)
DD70	Family-specific degenerate primer	GGNCCNAA[G/A]-ATT-GA*Joshi *et al.,* (1996)
DD101	Family-specific degenerate primer	TTCACGCACAAGACCAA designed#

** N could be A, C, G, and T, # based on heat shock protein 101 genes conserved sequences*

Table (2): Random primers for differential display in wheat heat tolerance

Genotypes	TN	P	D	R	NR	TN	P	D	R	NR	TN	P	D	R	NR
	OPC07+DDPA					OPV03+DDPA					OPW03+DDPA				
G168_Control	10	0	0	0	0	7	1	0	1	0	9	2	2	2	0
G168_Treated	13	3	3	3	0	7	1	0	1	0	9	2	2	1	1
GEM_ Control	13	3	0	3	0	8	2	2	2	0	9	2	1	2	0
GEM_ Treated	13	3	0	3	0	6	0	0	0	0	8	1	0	1	0
SERI _ Control	13	3	2	2	1	9	3	2	3	0	9	2	1	2	0
SERI _ Treated	11	1	0	1	0	7	1	1	1	0	9	2	1	2	0
CS_ Control	11	1	0	1	0	7	1	0	1	0	7	0	0	0	0
CS_ Treated	12	2	1	2	0	8	2	1	2	0	9	2	2	2	0
	OPC07+DDPC					OPV03+DDPC					OPW03+DDPC				
G168_ Control	14	5	0	5	0	4	0	0	0	0	9	3	2	3	0
G168_ Treated	15	6	1	6	0	6	2	2	2	0	9	3	2	3	0
GEM_ Control	13	4	0	4	0	6	2	1	2	0	9	3	3	3	0
GEM_ Treated	14	5	1	5	0	5	1	0	1	0	7	1	1	1	0
SERI _ Control	11	2	1	2	0	8	4	2	4	0	7	1	0	1	0
SERI _ Treated	12	3	2	3	0	6	2	0	2	0	8	2	1	2	0
CS_ Control	13	4	3	4	0	8	4	1	4	0	7	1	1	1	0
CS_ Treated	13	4	3	4	0	7	3	0	3	0	12	6	6	5	1
	OPC07+DDPG					OPV03+DDPG					OPW03+DDPG				
G168_ Control	9	4	1	3	1	10	8	0	8	0	5	0	0	0	0
G168_ Treated	8	3	0	3	0	10	8	0	8	0	5	0	0	0	0
GEM_ Control	7	2	1	2	0	4	2	2	1	1	5	0	0	0	0
GEM_ Treated	7	2	1	2	0	8	6	6	6	0	6	1	1	1	0
SERI _ Control	8	3	1	3	0	8	6	0	6	0	5	0	0	0	0
SERI _ Treated	8	3	1	3	0	9	7	1	7	0	5	0	0	0	0
CS_ Control	6	1	1	1	0	9	7	0	7	0	6	1	0	1	0
CS_ Treated	6	1	1	1	0	10	8	1	8	0	6	1	0	1	0
	OPC07+DDPT					OPV03+DDPT					OPW03+DDPT				
G168_ Control	10	6	4	3	3	7	1	1	1	0	3	2	1	2	0

G168_ Treated	7	3	1	3	0	7	1	1	1	0	2	1	0	1	0
GEM_ Control	8	4	3	3	1	7	1	0	1	0	2	1	0	1	0
GEM_ Treated	5	2	0	2	0	10	4	3	4	0	2	1	0	1	0
SERI _ Control	9	5	4	4	1	10	4	1	4	0	5	4	3	2	2
SERI _ Treated	5	2	0	2	0	9	3	0	3	0	2	1	0	1	0
CS_ Control	7	3	2	3	0	6	0	0	0	0	2	1	0	1	0
CS_ Treated	5	1	0	1	0	9	3	3	3	0	2	1	0	1	0

TN: Total bands number; P: Polymorphic bands number; D: Differentially bands number; R: Redundant bands number; NR: Non redundant bands number.

RESULTS AND DISCUSSION

Random RNA differential display amplification:

Three random decamer primers have been selected to create the differential display reaction as forward primers in combination with the four anchored differential display primers as reverse primers (with A; C; G or T anchored base) as presented in Table (2) to compare gene expression under heat shock stress. Another three specific primers for different genes of heat shock proteins have been used as forward primers in combination also with the four anchored differential display primers to identification of different members of heat shock protein multi gene families in wheat (Table 3). The total number (TN) of generated bands from each primer combination was calculated (total number of expressed genes) as well as the number of polymorphic bands (P). The number of differentially bands (D) was calculated when the band fragment (representing gene expression) was present or absent in specific treatment (control or heat treatment) for the same genotype. When the polymorphic fragment appeared in more than one genotype, it was considered as redundant gene expression (R) and it was considered as non redundant gene (NR) when it was appeared in only one genotype at any given treatment (Tables 2 and 3).

For the random primers, the total expressed genes number generated from the primer combinations ranged from five for the primers combination OPW03+DDPT to 15 for the primers combination OPC07+DDPC (Table 2 and Figure 1). Most expressed genes were monomorphic and appeared in all of the genotypes (Figure 1). The low percentage of polymorphism has been observed in all primers combinations and represents the genes that are affected by the heat treatments. Some primers combinations produced differential genes in the control rather than the treatments (such as the primer combinations of OPV03+DDPC and OPC07 + DDPT, Table 2). While others showed differential genes that have been expressed in the treatments (heat treatment) but not in the control. As examples for the later are the primers combinations OPC07+DDPC and OPV03+DDPG (Table 2). The highest number of differentially expressed genes was obtained for the heat treated variety ‘Chinese Spring’, hence six primers combinations produced differentially expressed genes over the control (i.e. OPC07+DDPA, OPV03+DDPA, OPW03+DDPA, OPW03+DDPC, OPV03+DDPG and OPV03+DDPT, Table 2). While the other varieties produced differentially expressed genes only with three primers combinations. Most of the polymorphic amplified fragments (genes) were redundant except for four genes in the untreated (control) variety ‘SERI 82’ (with the primers combinations OPC07+DDPA, OPC07+DDPT and OPW03+DDPT, Table 2), four genes in the untreated (control) variety Giza 168 (with the primers combinations OPC07+DDPG and OPC07+DDPT, Table 2), two genes in the non treated (control) variety Gemmeiza 7 (with the primers combinations OPV03+DDPG and OPC07+DDPT); and one for each the heat treated varieties Giza 168 (with the primers combinations OPW03+DDPA) and Chinese Spring (with the primers combinations OPW03+DDPC, Table 2). Previous studies have suggested the efficiency and the suitability of differential display system by using single random 5' primers and four T12VN 3' primers for visualizing and delineating the differences between control and heat-shocked mRNAs wheat seedling (Joshi *et al.,* 1996). Other studies have applied the mRNA differential

display technique by using different one-base anchored oligo dT primers, T_{11}PA, T_{11}PC, T_{11}PG, and 13-mer arbitrary primers to isolate and characterize genes specifically expressed in ethylene treated watermelon fruit (Karakurt and Huber 2008). Additionally, several studies indicated that differential display becomes an important technique to look into plant genetic similarity and differences in disease response (Manoj *et al.,* 2008 and You-Xiong *et al.,* 2009).

Table (3): Specific primers for differential display of different genes in wheat heat tolerance

Genotypes	TN	P	D	R	NR	TN	P	D	R	NR	TN	P	D	R	NR
	DD16.9+DDPA					DD70+DDPA					DD101+DDPA				
G168_Control	6	5	0	5	0	12	6	1	6	0	5	1	1	1	0
G168_Treated	6	5	0	5	0	13	7	2	7	0	4	0	0	0	0
GEM_Control	4	3	0	3	0	12	6	1	6	0	5	1	1	1	0
GEM_Treated	6	5	2	5	0	12	6	1	6	0	4	0	0	0	0
SERI_Control	4	3	0	3	0	13	7	2	7	0	5	1	1	1	0
SERI_Treated	5	4	1	4	0	13	7	2	7	0	4	0	0	0	0
CS_Control	6	5	5	5	0	9	3	2	3	0	5	1	1	1	0
CS_Treated	3	2	2	0	2	10	4	3	3	1	4	0	0	0	0
	DD16.9+DDPC					DD70+DDPC					DD101+DDPC				
G168_Control	6	0	0	0	0	12	4	1	4	0	13	6	5	2	4
G168_Treated	6	0	0	0	0	13	5	2	4	1	12	6	5	5	1
GEM_Control	6	0	0	0	0	12	4	1	4	0	13	6	1	6	0
GEM_Treated	6	0	0	0	0	13	5	2	4	1	12	5	0	5	0
SERI_Control	6	0	0	0	0	10	2	1	2	0	13	6	0	6	0
SERI_Treated	6	0	0	0	0	11	3	2	3	0	13	6	0	6	0
CS_Control	6	0	0	0	0	9	1	0	1	0	12	5	0	5	0
CS_Treated	6	0	0	0	0	11	3	2	2	1	12	5	0	5	0
	DD16.9+DDPG					DD70+DDPG					DD101+DDPG				
G168_Control	5	1	0	1	0	8	4	0	4	0	5	0	0	0	0
G168_Treated	5	1	0	1	0	14	10	6	10	0	5	0	0	0	0
GEM_Control	5	1	0	1	0	12	8	3	8	0	5	0	0	0	0
GEM_Treated	5	1	0	1	0	10	6	1	6	0	5	0	0	0	0
SERI_Control	5	1	0	1	0	13	9	2	9	0	5	0	0	0	0
SERI_Treated	5	1	0	1	0	11	7	0	7	0	5	0	0	0	0
CS_Control	5	1	1	1	0	11	7	1	7	0	5	0	0	0	0
CS_Treated	4	0	0	0	0	10	6	0	6	0	5	0	0	0	0
	DD16.9+DDPT					DD70+DDPT					DD101+DDPT				
G168_Control	5	1	0	1	0	7	1	1	0	1	9	2	1	2	0
G168_Treated	5	1	0	1	0	6	0	0	0	0	12	5	4	5	0
GEM_Control	5	1	1	1	0	6	0	0	0	0	12	5	1	5	0
GEM_Treated	4	0	0	0	0	6	0	0	0	0	14	7	3	7	0
SERI_Control	4	0	0	0	0	6	0	0	0	0	10	3	2	3	0
SERI_Treated	4	0	0	0	0	6	0	0	0	0	12	5	4	5	0
CS_Control	5	1	1	1	0	6	0	0	0	0	11	4	4	4	0
CS_Treated	4	0	0	0	0	6	0	0	0	0	10	3	3	2	1

TN: Total bands number; P: Polymorphic bands number; D: Differentially bands number; R: Redundant bands number; NR: Non redundant bands number.

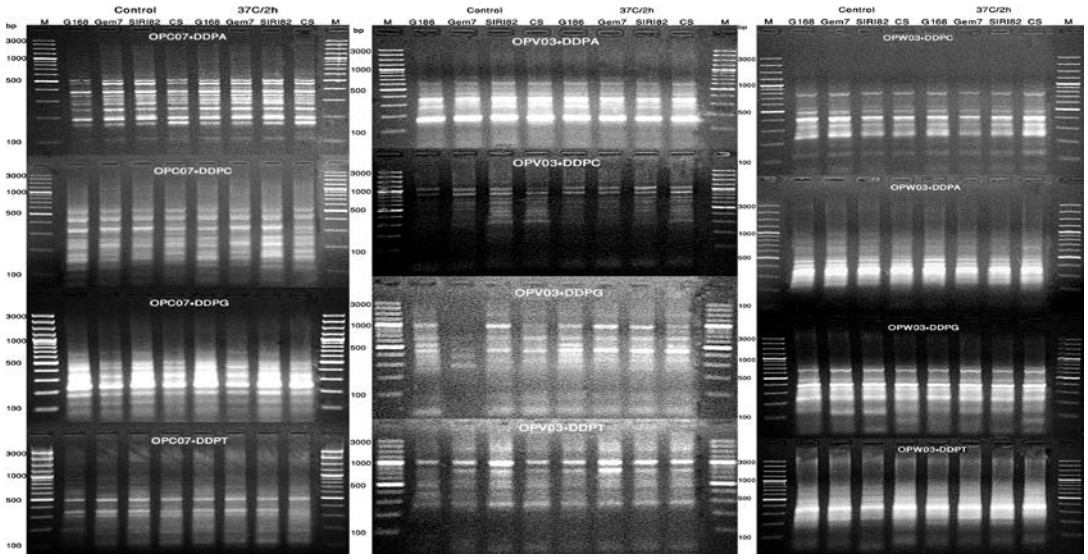

Figure (1): RNA differential display pattern of 12 random primer combinations of wheat genotypes with different backgrounds for heat tolerance.

Specific RNA differential display amplification:

Three specific differential display primers specific for heat shock protein genes have been selected to carry out the differential display reaction on the RNA samples of both heat treated and non-treated (control) wheat genotypes. The selected specific primers were for the heat shock proteins 16.9, 70 and 101 genes and were used as forward primers for the PCR amplification. The four anchored differential display primers had been used as reverse primers. Such amplification allows the forward primer to anneal with and amplify of all the expressed members of the specific genes (Figure 2).

In general, low number of amplified fragments had been obtained for the different genes in comparing to the random primers (Figure 2). The maximum number of amplified gene members for the HSP16.9 gene was six fragments (six gene members) and this number was increased to 14 for both HSP70 and HSP101 genes (Table 3). Low number of differential fragments (expressed gene members) was obtained for the gene HSP16.9 using the primer DD16.9. Two fragments differentiated the heat treated varieties Gemmeiza 7 and Chinese Spring for the HSP16.9 gene (using the primers combinations DD16.9+DDPA) and one fragment differentiated the treated variety SERI 82 using the same primers combinations (Table 3). Five amplified fragments differentiated the untreated variety Chinese Spring; two of them were non redundant using the primers combinations DD16.9+DDPA. Another one fragment differentiated the untreated variety Chinese Spring using the primers combinations DD16.9+DDPG and DD16.9+DDPT (Table 3). Use of wheat HSP16.9 family-specific primer (5' primer) in the differential display of control and heat-shocked mRNA samples drastically changed the pattern of cDNAs separated on agarose gels (Figure 2). Only 1-3 distinct bands of 200 to 600 bp were visible in each lane of heat shocked samples in varieties 'SERI 82' and 'Chinese Spring' but not visible in the untreated samples. Joshi and Nguyen (1996) have used differential display for characterization of 12 members of HSP 16.9 multigene family.

Many differentiated fragments had been assigned for the treated varieties using DD70 primer. The highest number of differentiated fragments (gene members that expressed when the tissues are heat stressed) was obtained from 'Giza 168' using the primers combinations DD70+DDPG. Non redundant fragments were observed for the treated 'Chinese Spring' variety using the primer combinations DD70+DDPA and

DD70+DDPC, ‘Giza 168’ and ‘Gemmeiza 7’ varieties using the primer combinations DD70+DDPC (Table 3). Comparison of amplification patterns between control and heat stressed mRNA sample lanes using the same HSP70 and T12VN primers indicated that many bands were common to both sample lanes and the remainder was specific to either control or heat-stressed samples. This observation was expected because HSP70 is a multigene family and several gene family members are expressed constitutively or induced upon heat stress. A similar observation has been noted by Joshi *et al.,* (1996). They modified the differential display technique to isolate 3' regions from different members of the wheat HSP70 gene family, and they stated that differential display could successfully be used to isolate 3' regions of different members of a multigene family in a relatively short period, even if the members had highly similar protein-coding regions.

The primer DD101 produced fragments differentiated all the untreated varieties (gene member that expressed in the normal state but not in the stressed state) using the primer combinations DD101+DDPA. The primers combinations DD101+DDPT produced differentiated fragments for both treated and untreated varieties while the primers combinations DD101+DDPG did not produced any differentiated fragments. Four non redundant fragments were obtained for treated variety ‘Giza 168’ and one fragment for the untreated variety using the primers combinations DD101+DDPC (Table 3). These observations clearly demonstrate that the use of specific 5' and 3' primers for differential display was successful in heat shock protein genes isolation in wheat. Furthermore, other studies Bozkurt *et al.,* (2007) used differential display reverse transcriptase-PCR method (DDRT-PCR) to evaluate the plant materials (resistant and/or susceptible) and analyze the expression level of 33 genes on two of the yellow rust differential lines of wheat.

Cluster analysis:

The molecular data of the RNA differential display analysis were put for cluster analysis in NTSYS pc software. Similarity matrix was calculated using the simple matching coefficient and the UPGMA method was used to construct the tree dendrogram. According to the RNA differential display data, the cluster analysis separated the genotypes into two main clusters. The first cluster contained the heat treated and the untreated types of the varieties ‘Giza168’ and ‘SERI 82’ along with the untreated type of the variety ‘Gemmeiza 7’ (Figur 3). The second cluster included both types of the variety ‘Chinese Spring’ (heat treated and untreated) along with the heat treated variety Gemmeiza 7 (Figur 3). These results could suggest that both ‘Gemmeiza 7’ and ‘Chinese Spring’ varieties are heat susceptible varieties while ‘Giza 168’ and ‘SERI 82’ are heat tolerant varieties. The results showed also that the varieties ‘Giza 168’ and ‘SERI 82’ are the most related varieties and that because they have common ancestor in their pedigree (Table 1). Although the untreated variety ‘Gemmeiza 7’ was grouped with ‘Giza 168’ variety (heat tolerant variety), its heat treated type was clustered with Chinese Spring variety (heat susceptible variety). This could be due to the same environment in which the variety ‘Giza 168’. However, it showed its origin when it was heat stressed. Our results revealed that differential display was successful for comparing gene expression profile in different thermo tolerant wheat varieties under heat shock condition.

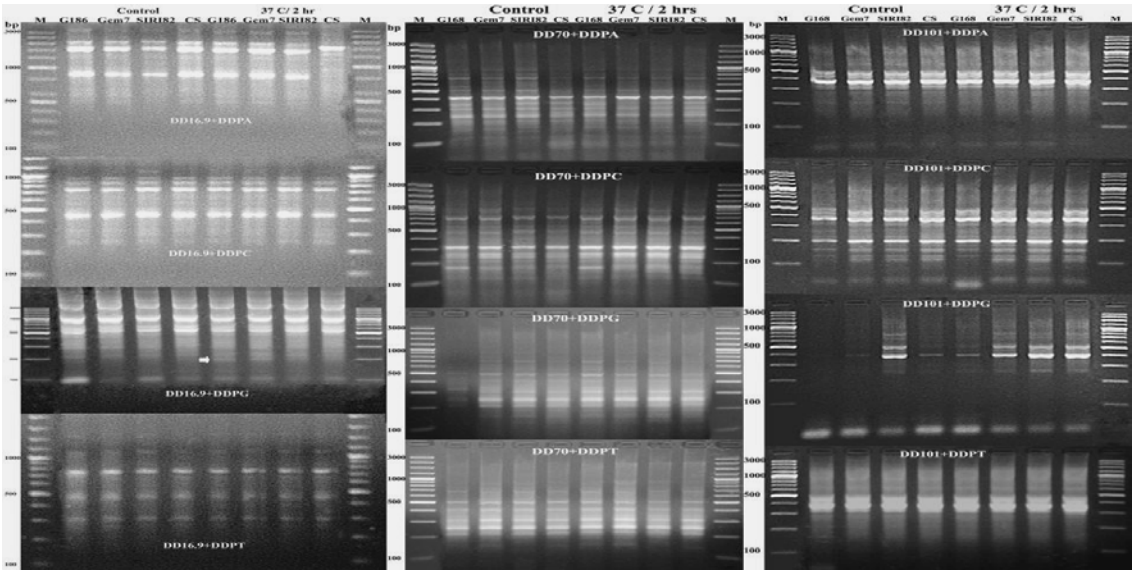

Figure (2): RNA differential display pattern of 12 random primer combinations of wheat genotypes with different backgrounds for heat tolerance.

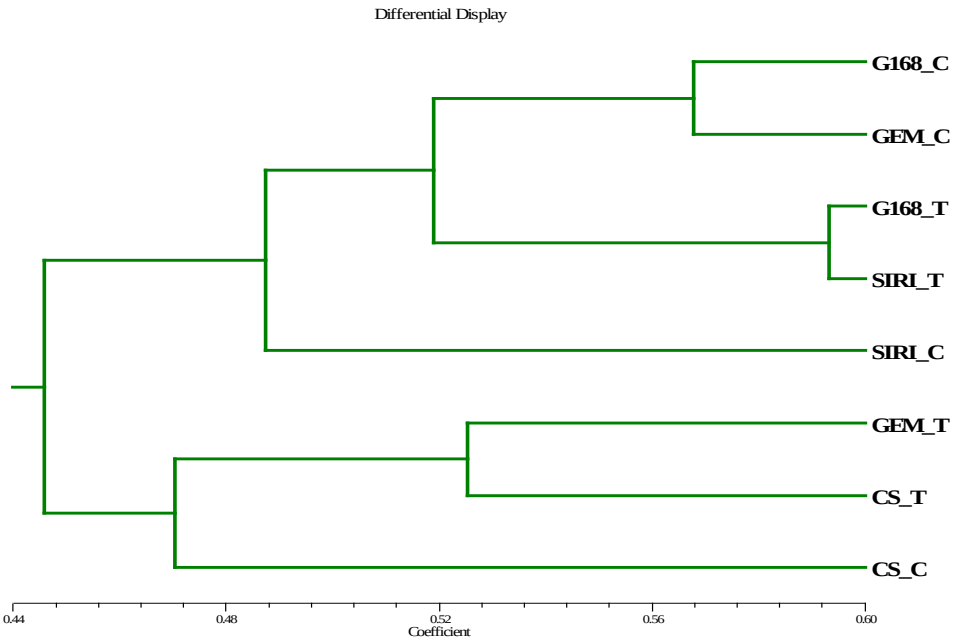

Figure (3): Dendrogram of Differentially display patterns of the total RNA from control and heat shocked wheat seedlings

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البصمة الوراثية للRNA الخاص بجينات بروتينات الصدمة الحرارية في القمح

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الملخص العربي

يؤثر تغير المناخ والاجهاد غير الحيوى سلبا على الزراعة وإنتاج المحاصيل. في هذه الدراسة استخدمت ثلاثة بادئات عشوائية (هي OPC07, OPV03, OPW03) لعمل البصمة الوراثية للRNA من التراكيب الوراثيه للقمح تحت الإجهاد الحراري و ثلاثة بادئات خاصه بجينات الصدمة الحرارية (هي DD16.9, DD70, DD101) باستخدام تقنية الDifferential Display. تم استخدام أربعة أصناف مختلفة من القمح السداسي صنفان من الاقماح المصريه (جيزة ١٦٨ وجميزة ٧) وصنف مكسيكي متحمل للحرارة SERI 82 بالإضافة إلي صنف صيني حساس للحرارة هو Chinese Spring. تم تعريض البادرات (عمر عشره ايام) للصدمه الحراريه على ٣٧ درجة مئوية لمدة ساعتين .وتم عزل ال RNA وكذلك عملRT-PCR بطريقة ال Differential Display لتخليق جزيئات ال cDNA ثم البوادئ سابقة الذكر. تم فصل النواتج علي جل من الاجاروز حيث تم إجراء التحاليل اللازمة. تراوح عدد الحزم (الجينات التي حدث لها تعبير) المتحصل علبها نتيجه لاستخدام البادئات العشوائية من خمسة حزم لمجموعة البادئات DDPT + OPW03 إلى ١٥ حزمة لمجموعة البادئات DDPC + OPC07. وقد تم الحصول على عدد مقارب كذلك من جينات تم التعبير عنها وتضاعفت بإستخدام البؤادى الخاصه بجينات الصدمة الحرارية (hsp16.9، hsp70 وhsp101) .وكانت كل من البادئات العشوائية والخاصه قادرة على تمييزالنباتات المعرضه للصدمه الحراريه عن مثيلاتها غير المعرضه. إستطاع التحليل العنقودي لبيانات الDifferential Display فصل التراكيب الوراثية المستخدمة في مجموعتين رئيسيتين. تضمنت المجموعة الأولى كلا من الطرز المعاملة حراريا والغير معاملة لأصناف جيزة ١٦٨ و82 SERI جنبا إلى جنب مع الطراز الغير معامل من الصنف جميزة ٧. وشملت المجموعة الثانية كلا الطرازين من الصنف Chinese Spring (المعامل حراريا والغير معامل) بالإضافة إلي الطراز المعامل حراريا للصنف جميزة ٧. و تشير هذه النتائج الى ان كلا الصنفين جميزة ٧ وChinese Spring هي أصناف حساسه للحرارة بينما الأصناف جيزة ١٦٨ و82 SERI هي أصناف متحملة للحرارة. وعليه يمكن القول بأن استخدام الDifferential Display كان ناجحا في دراسة التعبير الجيني لجينات بروتينات الصدمة الحرارية في أصناف قمح ذات درجات مجتلفة من التحمل الحراري.ويمكن إدخال هذه الأصناف المتحملة للحرارة في برامج تربية القمح لتحمل الحرارة.