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HUNTING OF SOME DIFFERENTIALLY EXPRESSED GENES UNDER SALT STRESS IN WHEAT

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ABSTRACT

Ten wheat cultivars were assessed for salt tolerance on the basis of some yield-related traits namely; plant height, youngest elongate blade leaf length, shoot fresh weight, shoot dry weight, main spike length and main spike weight. The plants were subjected to salt stress at two concentrations (0 and 6000 ppm NaCl), in a greenhouse experiment at the ARC (in winter season, 2006-2007). The two most contrasting cultivars in salt tolerance were Sakha 93 and Sakha 61 as the most salt tolerant and the most salt sensitive ones, respectively, according to their performance under salt stress. RNA of the two selected cultivars were extracted, then reverse transcribed using poly T primer as anchor and RT enzyme to produce 1st strand of cDNAs. The produced cDNAs were used as templates for PCR reaction mix to perform differential display. The results of the differential display showed several modifications in gene expression under salt stress either newly synthesized fragments or up or down regulated as response for salt stress.

Key words: ***Wheat, Triticum aestivum. Salt tolerance, Differentially expressed genes, RT-PCR***

INTRODUCTION

The need for techniques designed to identify genes that are differentially expressed by a specific cell type or cells under various physiological or experimental conditions has become critical to modern biological research. Differential display (DD) is one of the new additions to the repertoire of such techniques. Prior to DD, differential cDNA library screenings of two mRNA populations was a popular approach. Differential screening detects mostly high to moderately abundant transcripts and is labor intensive (Liang *et al* 1995).

A novel efficient approach, termed differential subtraction display, was developed by Pardinas *et al* (1998) to identify the differentially expressed genes. Several critical parameters for the reproducibility and enhanced sensitivity of display, as well as steps to reduce the number of false positive cDNA species, have been defined. These steps include, (a) use of standardized oligo(dT)- primed cDNA pools rather than total RNA as the starting material for differential display, (b) critical role of optimal cDNA input for each distinct class of primers, (c) phenomena of primer dominance and interference, and (d) design of a novel set of enhanced specificity anchor primers. Introduction of an efficient subtractive hybridization step prior to cloning of cDNA is either exclusively present in one sample or

show altered expression (up-/down-regulation) in RNA samples from two different tissues or cell types.

Four early salt-stress responding genes in common wheat *Triticum aestivum* L. were analyzed for their temporal accumulation of mRNA during salt stress by Nemoto and Sasakuma (2002). They reported that all genes showed transient stimulation by 0.15 M NaCl treatment. Some of these genes were induced by both osmotic stress and exogenous ABA treatment, while another gene responded to exogenous ABA, but not to osmotic stress. These results suggested that wheat has at least two salt stress signal transduction pathways, an ABA-dependent and ABA-independent pathway.

Differences in gene expression between salinity stressed and normally grown wheat seedlings were compared by Wang *et al* (2005) using the differential display technique. They reported that one derived cDNA clone was characterized as a partial sequence of the wheat asparagine synthetase (AS) gene by sequence analysis and homology search of Gene Bank databases. Two AS genes of wheat, *TaASN1* and *TaASN2*, were isolated by the RT-PCR (Reverse-Transcription Polymerase Chain Reaction) approach. *TaASN1* was dramatically induced by salinity, osmotic stress and exogenous abscisic acid (ABA) in wheat seedlings. *TaASN2* transcripts were very low in all detected tissues and conditions and were only slightly induced by ABA in roots.

Buchanan *et al* (2005) studied the genome wide changes in gene expression and monitored the changes in *Sorghum bicolor* seedlings responses to high salinity (150 mM NaCl). They reported that expression of approximately 2200 genes, including 174 genes with currently unknown functions, of which a subset appear unique to monocots and/or sorghum, was altered in response to dehydration, high salinity or ABA. Real-time PCR was used to quantify changes in relative mRNA abundance for 333 genes that responded to ABA, NaCl or osmotic stress. Osmotic stress inducible sorghum genes, which was identified for the first time, included a beta-expansin expressed in shoots.

The identification of genes associated with abiotic stress was reported by Xue *et al* (2006). They performed an expression profiling (microarray) analysis of approximately 16,000 unique wheat ESTs (Expressed Sequence Tag) to identify genes that were differentially expressed between wheat progeny lines with their contrasting levels. They also conducted a second microarray analysis to identify genes responsive to drought stress in wheat leaves. Ninety-three genes that were differentially expressed between high and low progeny lines were identified. One fifth of these genes were markedly responsive to drought stress. Several potential growth-related regulatory genes, which were down-regulated by drought, were expressed at a higher level in the high TE (Tandem Expressed) lines than the low TE lines and are potentially associated with a biomass

production. Also, Walia *et al* (2006) studied the salt-tolerance in barley (*Hordeum vulgare*). Barley cultivar Morex was used for transcriptional profiling during salinity stress using microarray containing approximately 22,750 probe sets. The experiment was designed to target the early responses of genes to a salinity stress at seedling stage. They found a comparable number of probe sets up-regulated and down-regulated in response to salinity.

Therefore, the objectives of this study were to assess salt stress tolerance in ten wheat cultivars to determine the most salt tolerant and the most sensitive cultivars and to screen the modified gene expression under salt stress.

MATERIALS AND METHODS

Ten wheat cultivars were assessed for their salt tolerance according to the performance of some yield-related traits namely; plant height (PH), youngest elongate blade leaf length (YEBL), shoot fresh weight (SFW), shoot dry weight (SDW), main spike length (MSL) and main spike weight (MSW). These cultivars were Sakha 94, Sakha 61, Sakha 93, Gemmieza 10, Gemmieza 9, Gemmieza 7, Sids 1, Sohag 3, Giza 168 and Sahel 1. Seeds of these cultivars were kindly provided by Field Crops Research Institute, Agricultural Research Center (ARC), Giza, Egypt.

This study was carried out in a greenhouse experiment in the ARC from the middle of November 2006 to the first of March 2007.

The surface clay loam soil (0-30 cm) was collected from the Agricultural Research Center farm at Giza. The soil was air-dried, ground, passed through a 5-mm mesh screen, and thoroughly mixed. The soil consisted of 39 % clay, 27.5 % silt, 26.6 % fine sand and 2.32 % coarse and, and the organic matter content was 1.58 %.

Pots were salinized with two sodium chloride levels; control (no added NaCl) and 6000 ppm NaCl in the irrigation water. The irrigation rate was twice a week. Salt solutions were used for two successive times followed by irrigation with normal water (Tap water) and so on up to the end of the experiment (105 days of planting).

To avoid an osmotic shock for seedling emergence the topmost soil layer was not salinized until 10 days after sowing. Fifteen seeds were sown in each pot. One week after sowing, the seedlings were thinned to ten per pot. All treatments were replicated four times.

Data were statistically analyzed using SAS program to determine the most two contrasting cultivars in their salt tolerance.

RNA extraction

RNA was extracted according to Ashoub *et al* (2006); 0.2g of leaf tissue of each of the two selected cultivars (tolerant and sensitive) under both control and salt stress (Fig. 1.) were ground in liquid nitrogen, 4 volume of extraction solution (0.2M MOPS; pH 3-4) and equal volume of phenol/chloroform (1:1) saturated with 0.2 M MOPS were added. The mixture was incubated for 10 min at room temperature then centrifuged at 10000xg for 10 min at 4 °C. The aqueous phase was collected with an equal volume of chloroform and re-centrifuged. The resulting aqueous phase was collected and 1/10 volume of 3M sodium acetate (pH 5.2) and 1 vol of absolute isopropanol were added. Samples were stored at -20 °C for 2 h, RNA was collected by centrifugation at 10000xg for 15 min at 4 °C. The supernatant was discarded and the pellets were re-suspended in 100 µl of 3 M sodium acetate, at pH 5.2. RNA was precipitated; pellets were washed with 100 µl of 70% ethanol and then precipitated as above and re-suspended in 50 µl nuclease free H_2O. RNA quality was examined on denature agarose gel electrophoresis.

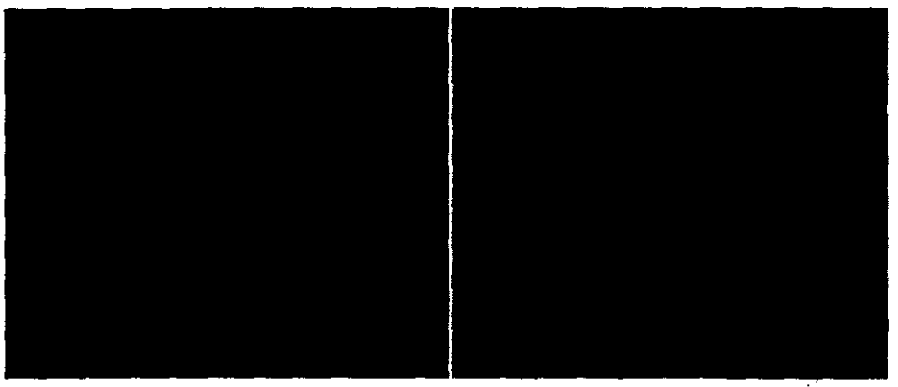

Fig. 1. Sakha 93 (the most salt tolerant cultivar) and Sakha 61 (the most salt sensitive cultivar), under control and salt stress conditions, under the greenhouse conditions.

RT-PCR

RNA of each of the four aforementioned samples was reverse transcribed (RT) in the presence of 5 mM MgCl, 1X PCR Buffer, 1 mM dNTPs, 25 u MuLV Reverse Transcriptase, 4 u RNA-guard Ribonuclease inhibitor and 2.5 µl of 20 PM anchor poly T primer (RNA image, Genhunter product) as reverse primer in a final reaction volume of 30 µl.

Reactions were carried out at 42°C for 30 min, followed by a 10 min step at 94°C to denature the enzyme, then was cooled to 4°C.

PCR conditions for differential display

Equal amounts of the produced cDNA (2µg) of each sample were amplified using reaction mixture, which contains 1 unit Taq, 1x buffer, 1.5 mM MgCl, dNTPs in the presence of the anchor poly T primer (RNA image, Genhunter product) ended with G or C or A (R) and random primer AB1 as (F). Reactions were carried out in the following conditions; the first cycle for 4 min at 94°C, followed by 35 cycles as follows, 94°C for 1 min, 40°C for 1 min and 2 min at 72 °C then holded at 4°C. Each set of reactions always included a no-sample negative control. A negative control containing RNA instead of cDNA to rule out genomic DNA contamination was always performed. PCR products were resolved in polyacrylamide denaturing gel (Sequencing gel) and the banding patterns was visualized using Silver stain kit (Promiga Product) and photographed using gel documentation system. Banding patterns were scored as 1 for the present band and 0 for the absent one.

RESULTS AND DISCUSSION

Under salinity stress, the examined varieties differed in the six yield-related measured traits compared to the control conditions (Table 1), the increase of these traits under salt stress conditions lead to consistent tolerant ranking for these varieties. The highest average of plant height (PH) under salinity conditions was recorded by cv. Sakha 93 (treated was 140.6 % of the control), while the lowest was obtained by Giza 168 (treated was 86.3 % of the control). Also, under salt- stressed condition, the highest average of the youngest elongate blade leaf (YEBL) was recorded by cv. Sakha 93 (treated was 165.8 % of the control), while the lowest one was obtained by Gemmieza 9, while Giza 168 recorded the lowest increase compared to the control due to its decrease in this trait (treated was 69.1 % of the control), so its rank was the lowest. The highest average of shoot fresh weight (SFW) under 6000 ppm, cv. Sakha 93 recorded the first rank depends on the rate of its increase compared to the control (treated was 133.3 % of the control), while Gemmieza 9 was the lowest rank (treated was 44 % of the control). For the shoot dry weight (SDW), the results indicated that the highest rank cultivar was Sakha 93 (treated was 137.0 % of the control), while the lowest was obtained by Sids1 (treated was 61.1% of the control). On the other hand, main spike length (MSL) indicated that the highest rank was scored by cv. Sohag 3 (treated was 175.0 % of the control), while the lowest was obtained by cv. Giza 168 (treated was 59.1 % of the control). In addition,

Table 1. The average of the *six measured traits under salt* stress (6000 ppm) and control conditions, % of increase or decrease as compared to control conditions in the ten wheat cultivars and ranking of each trait and cultivar.

Variety	Parameters	PH	YEBL	SFW	SDW	MSL	MSW	cultivar ranking
Sakha 94	S	52.00±3.74	17.20±1.84	0.51±0.05	0.41±0.05	4.43±0.53	0.13±0.02	
	C	48.33±2.46	16.42±2.72	1.02±0.14	0.64±0.08	6.75±0.35	0.18±0.02	
	%	107.59	104.75	50.00	64.06	65.63	73.33	
	Ranking	3	3	7	7	9	6	6
Gemmieza 10	S	61.50±0.84	20.60±1.28	0.97±0.02	0.60±0.06	11.00±0.33	0.10±0.02	
	C	58.44±1.33	18.60±1.65	1.04±0.12	0.74±0.10	10.56±0.59	0.18±0.02	
	%	105.24	110.75	93.27	81.08	104.17	55.56	
	Ranking	5	2	4	6	2	10	4
[illegible]	S	58.13±2.70	21.71±2.29	0.92±0.15	0.76±0.13	9.25±1.27	0.20±0.03	
	C	64.00±2.16	29.83±3.16	2.02±0.15	1.21±0.09	10.83±0.50	0.26±0.03	
	%	90.83	72.78	45.54	62.81	85.41	75.38	
	Ranking	[illegible]						
Gemmieza 9	S	56.20±1.18	12.00±0.99	0.55±0.05	0.44±0.05	5.60±0.60	0.14±0.02	
	C	54.00±1.49	14.80±2.27	1.25±0.09	0.71±0.05	5.41±0.51	0.15±0.02	
	%	104.07	81.08	44.00	61.97	103.51	93.33	
	Ranking	6	7	10	8	3	3	7
Sids 1	S	55.00±1.20	16.17±1.18	0.76±0.05	0.58±0.04	4.75±0.38	0.11±0.01	
	C	51.33±1.82	17.67±2.17	1.55±0.16	0.95±0.09	6.75±0.50	0.17±0.02	
	%	107.15	91.51	49.03	61.05	70.37	67.06	
	Ranking	4	6	8	10	8	8	9
Sohag 3	S	50.83±2.76	14.00±0.38	1.05±0.14	0.86±0.08	7.70±0.30	0.11±0.02	
	C	49.35±2.14	18.50±1.90	1.29±0.11	0.70±0.06	4.40±1.03	0.11±0.02	
	%	103.09	75.68	81.40	122.86	175.00	100.00	
	Ranking	7	8	6	4	1	2	2
Gemmieza 7	S	60.43±0.99	20.57±0.94	1.14±0.22	0.87±0.16	4.50±0.30	0.09±0.01	
	C	52.00±3.17	21.00±0.72	1.11±0.16	0.80±0.13	6.33±0.77	0.14±0.02	
	%	116.21	97.95	102.70	108.75	71.09	64.29	
	Ranking	2	5	3	5	7	9	5
[illegible]	S	61.60±0.46	36.14±0.46	0.96±0.03	0.63±0.05	5.40±0.36	0.12±0.01	
	C	43.80±1.50	21.8±2.14	0.72±0.06	0.46±0.04	5.45±0.34	0.08±0.01	
	%	140.64	165.78	133.33	136.96	99.08	150.91	
	Ranking	[illegible]						
Giza 168	S	44.25±0.89	14.50±0.75	0.91±0.01	0.78±0.08	3.63±0.32	0.11±0.00	
	C	51.29±4.87	21.00±4.29	0.97±0.15	0.57±0.09	6.14±0.77	0.14±0.02	
	%	86.27	69.05	93.81	136.84	59.12	81.43	
	Ranking	10	10	4	2	10	4	8
Sahel 1	S	45.00±7.21	13.75±1.65	0.79±0.20	0.54±0.15	4.80±0.82	0.08±0.01	
	C	45.50±3.44	14.00±1.31	0.74±0.22	0.44±0.08	5.42±0.67	0.11±0.01	
	%	98.90	98.21	106.76	123.64	88.56	71.82	
	Ranking	8	4	2	3	5	7	3

PH= plant height (cm), YEBL =youngest elongate blade leaf length (cm), SFW =shoot fresh weight (g), SDW =shoot dry weight (g), MSL =main spike length (cm), MSW = main spike weight (g)
S= under salt-stress conditions C= under Controlled conditions % =100 – [(C-S)/C X 100]

cv. Sakha 93 (treated was 150.0 % of the control), while the lowest rank was scored by cv. Gemmieza 10 (treated was 55.6 % of the control). As shown in Table (1) also, the ten varieties were arranged in descending order

according to the average of their arrangement overall the six investigated traits and it could be concluded that Sakha 93 was the highest cultivar in growth characters under salt stress (the most salt tolerant cultivar) while, Sakha 61 was the lowest one in growth characters under salt stress (the most salt sensitive cultivar) as detected from the results.

Jamshid *et al* (1995) reported that high grain yield under salinity stress appeared to be a better selection criterion for salinity tolerance than biomass yield or harvest index. Several accessions, differing in geographical origin within Iran, had consistently high ranking for grain yield in the control and two salinity treatments. Hu *et al* (2005) reported that salinity greatly reduced the leaf cross-sectional area of wheat (*Triticum aestivum*) during its development, which may lead to variation in the architectural properties of growing leaves that would result in a change in leaf physiological functions.

Differential display

Differential display was performed using cDNA produced using RNA templates extracted from salt-treated and control plants of the two selected wheat cultivars, i.e. the most salt tolerant cultivar (Sakha 93) and the most salt sensitive one (Sakha 61). In addition to the cDNA template for the PCR products, three poly T anchor primers ended with A or C or G were used as 5' primer and the random primer AB1 as a 3' primer.

Differential display was conducted to screen the modification of gene expression under salt stress as compared to control conditions either up or down regulated gene expression and the newly synthesized bands under salt stress. It was also performed to analyze the sequence of the modified fragments and to know the nature of these genes and how those genes act under salt conditions to increase salt tolerance of wheat plants.

Results presented in Table (2) and Fig. (2) showed that PCR produced 41 amplified fragments with different lengths with the use of anchor primer A. From these fragments, bands No. 12, 15, 20, 25 were upstream regulated under salt stress. On the other hand, two bands; No. 13 and No. 34 were newly synthesized under salt stress in both the tolerant and the sensitive cultivars.

The results of PCR products using the same poly T anchor ended with C showed a total of 40 amplified fragments with different lengths. Some of these fragments indicated modifications in gene expression under salt stress as compared to control conditions; one of these is band No. 2 which is upstream regulated under salt stress. On the other hand, band No. 23 was newly synthesized under salt stress in both of the two cultivars, but bands No. 33 and No. 35 were found in the plants grown under control

Table 2. Banding patterns for the tolerant and the sensitive wheat cultivars under control and salinity conditions as detected from the differential display gel using three different anchor primers.

Band No.	Anchor A				Anchor C				Anchor G			
	Tt	St	Tc	Sc	Tt	St	Tc	Sc	Tt	St	Tc	Sc
1	1	1	1	1	1	1	1	1	1	1	1	1
2	1	1	0	0	1^+	1^+	1	1	1	1	1	1
3	1	1	1	1	1	1	1	1	1	1	1	1
4	1	1	1	1	1	1	1	1	0	0	1	1
5	1	1	1	1	1	1	1	1	1	1	1	1
6	1	1	1	1	1	1	1	1	1	1	1	1
7	1	1	1	1	1	1	1	1	1	1	1	1
8	1	1	1	1	1	1	1	1	1	1	1	1
9	1	1	1	1	1	1	1	1	1	1	1	1
10	1	1	1	1	1	1	1	1	1	1	1	1
11	1	1	1	1	1	1	1	1	1	1	1	1
12	1^+	1^+	1	1	1	1	1	1	1	1	1	1
13	1	1	1	1	1	1	1	1	1	1	1	1
14	1	1	1	1	1	1	1	1	1	1	1	1
15	1^+	1^+	1	1	1	1	1	1	1	1	1	1
16	1	1	1	1	1	1	1	1	1	1	1	1
17	1	1	1	1	1	1	1	1	1	1	1	1
18	1	1	1	1	1	1	1	1	1	1	1	1
19	1	1	1	1	1	1	1	1	1	1	1	1
20	1^+	1^+	1	1	1	1	1	1	1	1	1	1
21	1	1	1	1	1	1	1	1	1	1	1	1
22	1	1	1	1	1	1	1	1	1	1	1	1
23	1	1	1	1	1	1	0	0	1	1	1	1
24	1	1	1	1	1	1	1	1	1	1	1	1
25	1^+	1^+	1	1	1	1	1	1	1	1	1	1
26	1	1	1	1	1	1	1	1	1	1	1	1
27	1	1	1	1	1	1	1	1	1	1	1	1
28	1	1	1	1	0	0	1	0	1	1	1	1
29	1	1	1	1	1	1	1	1	1	1	1	1
30	1	1	1	1	1	1	1	1	1	1	1	1
31	1	1	1	1	1	1	1	1	1	1	1	1
32	1	1	1	1	1	1	1	1	1	1	1	1
33	1	1	1	1	0	0	1	1	1	1	1	1
34	1	1	1^+	1^+	1	1	1	1	0	0	1	1
35	1	1	1	1	0	0	1	1	1	1	1	1
36	1	1	1	1	1	1	1	1	1	1	1	1
37	1	1	1	1	1	1	1	1	1	1	1	1
38	1	1	1	1	1	1	1	1	1	1	1	1
39	1	1	1	1	1	1	1	1	1	1	1	1
40	1	1	1	1	1	1	1	1	-	-	-	-
41	1	1	1	1	-	-	-	-	-	-	-	-

1 (present), 1^+ (present and accelerated) and 0 (absent)
Tolerant treated (Tt), Tolerant control (Tc)
Sensitive treated (St), Sensitive control (Sc)

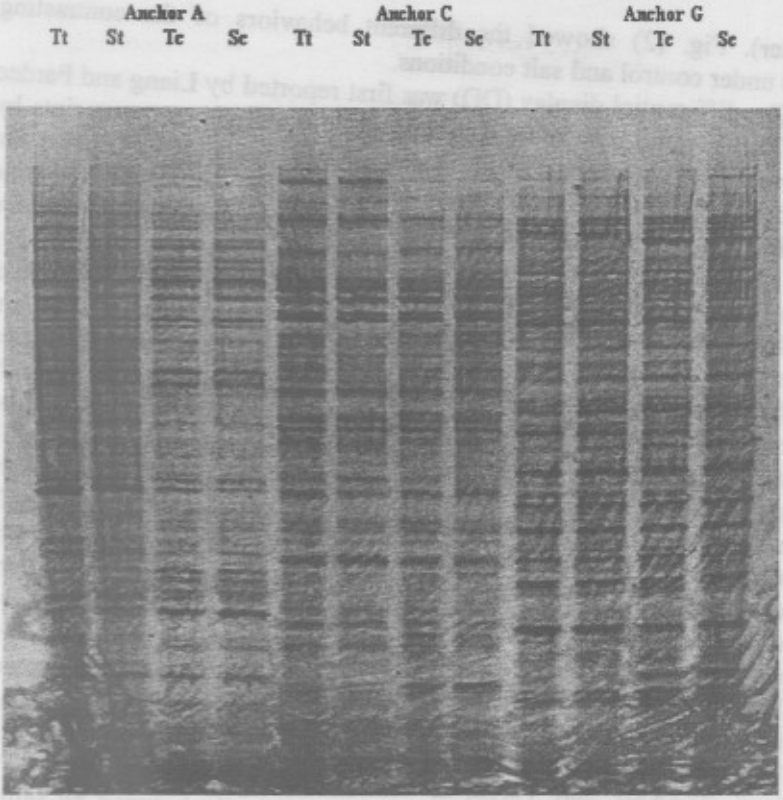

Fig. 2. Differential display gel using three different anchor primers and cDNA templates from the tolerant and the sensitive wheat cultivars under control and salt treatment conditions.

conditions, but not in salt-treated plants of the two cultivars under investigation.

Results of differential display using poly T anchor ended with G showed 39 amplified fragments and bands No. 4 and No. 34 were present only under the control conditions, but they were absent under salt stress in both of the two cultivars (down-regulated). On the other hand, band No. 5 was newly synthesized under salt stress in the two cultivars; these modified bands were eluted from the gel and were cloned for further investigations (sequencing of newly synthesized and upstream regulated bands will be

done later). Fig. (2) showed the different behaviors of the contrasting cultivars under control and salt conditions.

The differential display (DD) was first reported by Liang and Pardee (1992). This method can be used to amplify low abundance transcripts by polymerase chain reaction (PCR). Yamazaki and Saito (2002) studied the application of the differential display to investigate gene expression in plants. They reported that genes involved in physiological events, stress responses, signal transduction and secondary metabolism have been isolated and characterized using differential display method. Some of the isolated genes encoded transcription factors, membrane proteins and rare enzymes that were previously difficult to purify. These results suggest that differential display is a powerful tool that can be used to investigate the rare genes involved in the plant life cycle without using information from proteins. Kamiya *et al* (2004) investigated transcriptional regulation in response to high-salinity stress in *Arabidopsis* with a cDNA microarray containing 7000 independent full-length *Arabidopsis* cDNAs. The transcripts of 11 genes were increased more than 5-folds within 5 h after treatment with 0.1M NaCl. On the other hand, the transcripts of 57 genes were down-regulated to less than one-third within 5 h after treatment with 0.1 M NaCl. Venn diagram analysis revealed that 11 genes were significantly induced by NaCl. Differences in gene expression between salinity stressed and normally grown wheat seedlings were also compared by Wang *et al* (2005) using differential display (DD) technique. Two genes of wheat, TaASN1 and TaASN2, were further isolated by the RT-PCR approach. They found that TaASN1 was dramatically induced by salinity, osmotic stress in wheat seedlings. TaASN2 transcripts were very low in all detected tissues and conditions. In order to assess global changes in gene expression patterns in stress-induced tissues, Mochida *et al* (2006) conducted large-scale analysis of expressed sequence tags (ESTs) in common wheat. Twenty-one cDNA libraries derived from stress-induced tissues, such as callus, as well as liquid cultures and abiotic stress conditions were constructed. Four hundred and ninety genes showing five fold induction or 218 genes for suppression in comparison to the control expression level were selected. They also reported that this method can be applied to different stress-treated tissues. Then, the method was applied to screen genes in response to abiotic stresses such as drought and salinity. Silico selection of screened genes from virtual display should provide a powerful tool for functional plant genomics.

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اصطياد بعض الجينات متفاوتة التعبير الجيني تحت تأثير الإجهاد الملحي في القمح

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تم تقييم عشرة أصناف قمح لدرجة تحملها للملوحة على أساس بعض الصفات المرتبطة بالمحصول وهى : طول النبات و طول أصغر ورقة تم اكتمال استطالة تحملها و الوزن الغض و الجاف للساق و طول و وزن السنبلة الرئيسية ، وذلك بتعريض النباتات لإجهاد ملحي باستخدام تركيزين من كلوريد الصوديوم: صفر و 6000 جزء في المليون، في تجربتين في البيوت الزجاجية في مركز البحوث الزراعية (في الموسم الشتوي 2006-2007). أظهرت النتائج أن الصنف سخا 93 هو الأكثر تحملا للملوحة أما الصنف سخا 61 كان الأكثر حساسية تبعا لأدائهم تحت إجهاد الملوحة. تم استخلاص الحمض النووي RNA من الصنفين الأكثر تحملا و الأكثر حساسية للملوحة. ثم تم إجراء نسخ عكسي لهما باستخدام بادئ تفاعل متعدد النيوكليتيدات كبادئ من الطرف 5 وكذلك باستخدام إنزيم النسخ العكسي لإنتاج الخيط الأول من الـ cDNA المكمل. تم استخدام الخيط الأول من الـ cDNA المكمل الناتج من الخطوة السابقة كقالب لتفاعل الـ PCR للكشف عن الجينات المتفايرة التعبير. أظهرت نتائج الجينات المتغايرة التعبير عن العديد من التغايرات بين الصنفين في التعبير الجيني تحت ظروف الإجهاد الملحي، كما أظهرت وجود جينات تم التعبير عنها تحت الإجهاد الملحي ولم تكن تعبر عن نفسها في الظروف الطبيعية، كما تم الكشف عن جينات أخرى يزداد حجم التعبير الجيني بها، وكذلك بعض الجينات الأخرى التي ينخفض حجم التعبير الجيني لها كاستجابة للإجهاد الملحي في نباتات القمح. وسوف يتم إن شاء الله تعريف هذه الجينات فى دراسة لاحقة.

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