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GENE EXPRESSION EVALUATION OF DHN GENE IN SOME WHEAT (*Triticum asetivum* L.) CULTIVARS UNDER ABIOTIC STRESS

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ABSTRACT

Drought is one of the major causes of dramatic yield loss in crop plants. Plants perceive and respond to stress .Upon perception of stress, a signal is communicated to downstream components resulting in change of gene expression and thereby of proteins required for the initial damage-repair and physiological re-programming for better adaptation. The relative water content (RWC) used as one of the most reliable indicators for defining both the sensitivity and the tolerance of wheat plants to water deficit.

The real-time quantitative PCR used to monitor the expression pattern of dehydrin gene in Triticum asetivum leaves under drought and ABA stress. The results showed a high quantitative up-regulation of dehydrin, Identifying the high tolerant varieties Vorobey and Giza 168 exhibited a higher activation of DHN and at the same time a high relative expression level of the gene. Similar gene expression profile was observed in Sakha 93. However, relative expression level was lower for Finisi, Gemmiza 7and Gemmiza 9 compared to other varieties.

Key words: Gene Expression Evaluation, DHN Gene, Wheat (*triticum asetivum l.*) Cultivars, Under Abiotic Stress

INTRODUCTION

Drought is a complex physical–chemical process, in which many biological macromolecules and small molecules, such as nucleic acids, proteins, carbohydrates, lipids, hormones, ions, free radicals and mineral elements are involved (Hong *et al*., 2006). The identification of drought tolerant or sensitive wheat genotypes was firstly based on the relative water content (RWC), water loss rate (WLR) and free proline content (Rampino *et al.*, 2006; Vasil, 2007; Jia *et al.*, 2008).

One way of increasing productivity in stressful environments is to breed crops more tolerant to stress. However, success in breeding for tolerance has been limited because tolerance to stress is controlled by many genes and their

simultaneous selection is difficult , complexity of the several tolerance mechanisms involved, tremendous effort is required to eliminate undesirable genes that are also incorporated during breeding and there is a lack of efficient selection procedures particularly under field conditions (Ribaut *et al*., 1997 ; Flowers *et al*., 2000).

The real-time quantitative PCR, the most recent technique to explore expression due to its ability of accurate and sensitive quantification of low copy number genes express at a minute scale, has emerged as having the most potential in this regard. Some low copy number genes express at such minute levels that no method except quantitative RT-PCR can detect expression accurately (Czechowski *et al.,* 2004). However real-time PCR (qPCR) technique is considered to be the most accurate and most reliable for what often serves to validate data obtained by other methods. Undoubtedly, its advantages are sensitivity, real time detection of reaction progress, speed of analysis and precise measurement of the examined material in the sample (Gachon et al. 2004). Moreover expression level for some genes is often so small that qPCR becomes the only technique that can detect such a small number of mRNA copies (Bustin *et al.,* 2009). Gene expression profiles of group 2 (dehydrins) and group 4 Late embryogenesis abundant (Lea)genes in developing seeds of *Triticum durum* and *T. aestivum* and in coleoptiles and coleorhizae of T. durum seedlings were monitored by real-time quantitative RT-PCR (Mohamed *et al.,* 2005).

Huggett *et al.,* (2005) and Radonic *et al.,* (2004) investigated nucleotide sequences, gene families, specificity, regulation and level of expression.

Northern blotting, microarray and real-time quantitative PCR (qRTPCR) are commonly employed for quantification of gene expression under different experimental conditions.

MATERIALS AND METHODS

Materials

A total of six wheat (*Triticum aestivum* L.) cultivars were used in the present study, four Egyptian cultivars, Sakha93, Gemmiza7, Gemmiza9 and Giza168; and two Mexican cultivars, Vorobey and Finisi Table 1. Grain samples were obtained from Field Crops Research Institute, Agriculture Research Center, Giza, Egypt. The Mexican cultivars were kindly obtained from CIMMYT.

Methods

Seeds were surface esterilized in 10% sodium hypochloride for 30 min and then rinsed with ddH2O for 1 min 15 times. Plants were grown in soil composed of sand and clay (1:1) and watered daily under controlled conditions (28ºC day/25ºC night, 12-h photoperiod, ~ 300 $\mu molm^{-2}s^{-1}$ light intensity, with a 14 h

Table 1: The six bread wheat cultivars utilized in this study, their pedigree and their origin.

NO	Cultivar	Pedigree	Origin
1	**Sakha93**	SAKHA92/TR810328 S88-71-1S-2S-0S	Egypt
2	**Giza168**	MRL/BUC//SERI CM93046-8M-0Y-0M-2Y-0B	Egypt
3	**Gemmiza7**	7CMH74A630/SX//SERI82/AGENT	Egypt
4	**Gemmiza9**	ALD"s"/HUAC//CMH74A-630/SX CGM4583-56M-1GM-0GM	Egypt
5	**Vorobey**	CROC_1/AE.SQUARROSA(224)// OPATA/3/PFAU/SERI//BOW	Mexico
6	**Finisi2000**	THB//MAYA/NAC/3/RABE/4/VS73.600/ MIRLO/3/BOW//YE/TRF	Mexico

photoperiod and 83% relative humidity) according to Ayman A. Diab *et al.,* 2007.

Drought treatment was applied as described by Ozturk *et al.,* 2002. Whole ten day old seedlings. Plants were removed from soil, washed carefully and placed on paper towels under the same conditions. Leaves were harvested after 24 h of drought treatment, control plants were well-watered and harvested at the same time. For ABA treatment. phytohormone treatment was by spaying the plants with 100 µmol/L ABA solution, leaf tissues were collected after 24 h of treatment according to (Borovskii *et al.,* 2002) All the samples were frozen in liquid nitrogen immediately after collection, and then stored at -80°C.

Leave Relative Water Content (RWC):

Leaves samples were taken as bulks from both control and drought stressed plants. Then the Relative Water Content (RWC) was assessed as described by Siddique *et al*. (2000); using flag leaves after imposing to drought conditions. Fresh weights were determined within 2 h after excision. Turgid weights were obtained after soaking leaves in distilled water in test tubes for 16 to 18 h at room temperature (about 20°C) and under low light conditions. After soaking leaves were quickly and carefully blotted dry with tissue paper in preparation for determining turgid weights. Dry weights were obtained after oven drying of leaf samples for 72 h at 70°C. RWC was calculated from the equation proposed by Siddique *et al*. (2000).

RWC = (Fresh weight – Dry weight) / (Turgid weight – Dry weight) x 100.

Total RNA Isolation*:*

Total RNA was isolated from the control and treated plant.. The total RNA was isolated using the Trizol reagent (Ambion, **USA** Cat. no.15596-026) and according to their instructions. 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, Madison, USA).

First cDNA Strand Synthesis:

The isolated RNA was used to synthesize the first strand cDNA through Reverse Transcription (RT) reactions. The reactions of each RNA sample contained 2 µg of freshly diluted RNA, 1 µM of polynucleotide $(dT)_{18}$ primer. The tubes containing the above mentioned components were incubated at 65°C for 5 minutes and then were used for the cDNA synthesis according to the H¯ Revert aid first strand cDNA synthesis kit (Fermentas INC., Maryland, USA**).** RT reaction samples were incubated in a PCR thermocycler programmed for one cycle at 45°C for 60 minutes, 70°C for 5 minutes and then the first strand cDNA solution was stored at 4°C. The PCR device model belonged to Biometra Ltd. Company (TPersonal Thermocycler, Biometra, Goettingen, Germany).

Real Time RT-PCR:

DHN gene in leaf tissues were investigated with qRT-PCR. The leaf samples of ten day old plants were harvested after 24 h of drought treatment and after 24h treatment with 100 µM ABA. Total RNA was isolated from whole leaf tissues by using the Trizol reagent (Ambion**,** USA Cat. no.15596-026) and according to their instructions. To eliminate residual genomic DNA, each RNA sample was treated with 10 U of RNAse-free DNaseI (Roche Applied Science GmbH, Germany) for 20 min at 37 0C and was quantified with spectrophotometer (Nanodrop,USA). After checking the integrity on formaldehyde agarose gel electrophoresis, the RNA samples with sufficient integrity and quality was used as template for cDNA synthesis according to the H¯Revert aid first strand cDNA synthesis kit (Fermentas INC**.,** Maryland, USA). The efficiency of cDNA synthesis reactions were assessed through the use of wheat Tubulin primers for end-point RT-PCR (More details about the primer sequences could be seen at Table 2).The primers for qRT-PCR were designed from the sequenced cDNA fragments by using Fast PCR program (Kalendar *et al.,* 2009; www.biocenter.helsinki.fi/bi/Programs/). Each RTPCR reaction was set up in total volume of 25 µl, containing 12.5 µl SYBR Green PCR SuperMix (BioRad Laboratories, Hercules, USA Laboratories, Hercules, USA), 10 pmole of each primer and 75–200 ng of the cDNA. qRT-PCR reactions were carried out with an IQ5 System (BioRad Laboratories, Hercules, USA Laboratories, Hercules, USA) with cycling parameters: 95 °C for 3 min,

Table 2: List of Real Time PCR primers

Primer name	Primer sequence	
Wdhn13	F: 5´-GCGTCATGGAAAGCATCAC-3′ R: 5´-GTCCAGGCAGCTTGTCCTT-3′	
Tubulin	F: 5´- AGAACACTGTTGTAAGGCTCAAC -3´ R: 5´- GAGCTTTACTGC CTCGAACATGG -3´	

followed by 40 cycles of 95 °C for 30 s, 55 °C for 30 s, and 72 °C for 40 s. Each cDNA sample was analyzed in three different reactions with three technical replicates and negative controls. In order to determine, if there are significant differences in expression at the different time points, three biological replications have been performed for each transcript. The relative abundance level of DHN transcript for different reactions were normalized with respect to the loading standard, Tubulin. The relative fold expression differences were calculated by using the comparative CT method (Schmittgen *et al.,* 2000).

The ΔCT values for all of the transcripts were averaged across all treatments and experimental replicates. Finally, Student's t-test was applied to check for the statistical significance between treated and –untreated groups.

RESULTS AND DISCUSSION

Relative Water Content (RWC) Measurement:

The Relative water content (RWC) estimates for the present six wheat cultivars under drought stress condition (after 24 h of drought treatment) were calculated and summarized in Table 3. The highest RWC estimates under stress conditions were detected for cultivar Vorobey (52.55) and Giza 168 (48.97). The lowest RWC estimates (under stress conditions) were obtained for Gemmiza 7 (29.52), Finisi F (32.66) and Gemmiza9(33.89). While no significant differences between Sakha 93 (45.01) and Giza168 (48.97) can be considered as resistant. All cultivars gave higher RWC values at the controls. The ANOVA analysis for RWC of these six wheat cultivars under stress showed that there were highly significant differences between these six wheat cultivars ($P < 0.0007$). The interaction "treatment x cultivar" was also highly significant ($P = 0.0027$).

According to these results, it can be suggested that Vorobey, Giza 168; and Sakha93 are relatively resistant to drought, but Gemmiza7, Finisi F and Gemmiza9 are sensetive. Siddique *et al.* (2000) found that the exposure of plants to drought led to noticeable decreases in leaf water potential and relative water content (RWC) with concurrent increase in leaf temperature. Liu *et al.* (2005)

Table 3: Comparison of relative water content (RWC) in the six wheat cultivars, under control (C) and water stress conditions (T).

Cultivar	Control plants (C)	Water-stressed plants (T)
Vorobey	**94.67**	**52.55**
Finisi F	**93.37**	**32.66**
Sakha 93	**95.62**	**45.01**
Giza 168	**92.77**	**48.97**
Gemmiza 7	**90.47**	**29.52**
Gemmiza 9	**90.35**	**33.89**

studied the relative water content in two wheat cultivars (Miannong 4 and Miannong 5) under progressive water stress. They showed that Miannong 4 and Miannong 5 had relatively high tolerance to water stress. Rampino *et al*. (2006) studied *Triticum* and *Aegilops* seedlings differing in their response to drought stress at the physiological and molecular levels. They suggested that sorting of resistant and sensitive genotypes can be firstly based on the relative water content (RWC) measurement.

Expression Profile of Dehydrin (DHN) Gene Response to Water Stress:

Expression of gene encoding dehydrin was up-regulated in response to water deficit. After stress treatment, mRNA levels of the DHN13 gene were up-regulated and unchanged at 24 h drought and ABA stress in comparison with controls (Figure 1). Tolerant genotypes reveal a more rapid onset or a higher level of expression of corresponding gene in dependence of stress conditions. After 24 h drought stress, the DHN13 gene was up-regulated approximately 5-fold for Giza 168, 4.35-fold for Sakha 93, 1.5-fold for Gemmiza 9, 0 .81-fold for Gemmiza 7, 4.89-fold for Vorobey and 1.09-fold for Finisi F, response to water stress. Likely, stress ABA treatment, the DHN13 gene was up-regulated approximately 1.54-fold for Giza 168,1.2-fold for Sakha 93, 0.53-fold for Gemmiza 9, 0.42-fold for Gemmiza 7, 1.51-fold for Vorobey and 1.09-fold for Finisi F, response to water stress in comparison to the indicated control. Collectively, the high tolerant varieties were Vorobey and Giza 168 exhibited a higher activation of DHN and at the same time a high relative expression level of the gene. Similar dynamics of gene

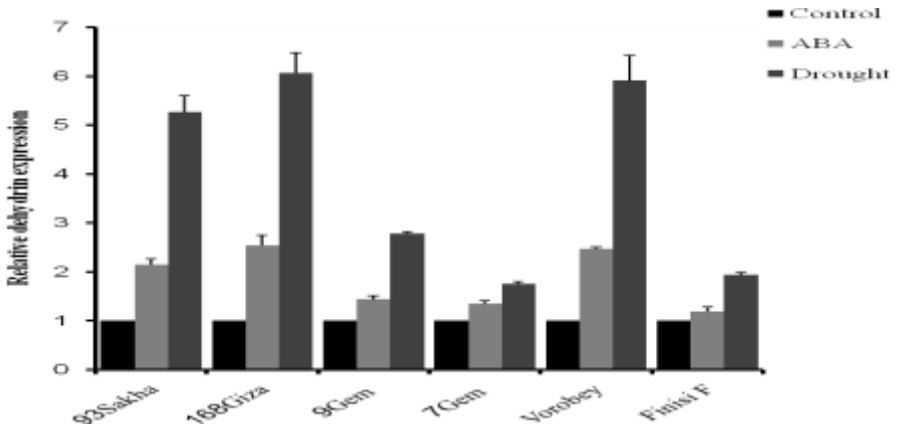

Figure 1: Evaluation of the relative expression of DHN under drought and ABA stress in wheat plants.

expression was observed in Sakha 93. However, relative expression level was lower for Finisi, Gemmiza 7 and Gemmiza 9 as compared to the other varieties. The increased activity of the gene was detected at a time when the plants were still maintaining a high water content in the leaves.

Consequently, dehydrins proteins have different expression patterns in responses to drought stress. Previously, Ali-Benali *et al.,* (2005), found that Td11 and Td16 were up regulated under water deficit in coleorhizae of Triticum durumseedlings, accompanied with the highest induction observed in Td16 after 2 days of water stress. High activity of indicated gene was detected at a time when the plants still maintaining a high water content in the leaves. Rampino *et al.,* (2006) reported a similar activation of genes expression and phenomenon in durum wheat from the COR/LEA group with more drought tolerant genotypes.

Dehydrins are a family of plant proteins that are induced by stimuli that have a dehydrative component such as drought, low temperature, salinity, and ABA (Close, 1996). A wheat dehydrin has been shown to increase dehydration tolerance in transgenic rice (Cheng *et al.,* 2002). In addition ،drought-induced accumulation of dehydrin proteins has been associated with drought tolerance in many plant species, especially annual crops (Close *et al.,* 1993; Lopez *et al.,* 2001; Lopez *et al.,* 2003). DHNs have been extensively studied in barley, where a dispersed family of 12 Dhn genes was characterized (Choi *et al.,* 1999 ؛Choi & Close, 2000; Zhu *et al.,* 2000). Most of the barley Dhn genes are up-regulated by dehydration and ABA ،while others are cold-induced or embryo-specific. Also, the ectopic expression of a wheat dehydrin (DHN-5) in Arabidopsis plants improved their tolerance to high salinity and water deficit (Brini *et al.,* 2007). Our

results underline the fact that during drought stress, variations in pattern expression of dehydrin gene (LEA group2) areobserved. We revealed that each of them exhibited a different transcription pattern in response to stress .Dehydrins belonging to the same family may respond differently to the same stress conditions. The dramatic increase of the Td16 gene in the current study indicate the importance of this gene in response to drought stress ،thus the synthesis of dehydrins is a major part of global stress response to protect cell under water deficit.

CONCLUSION

Six bread wheat (*Triticum aestivum* L) cultivars were subjected to drought and ABA stress after ten day old, while the controls were continuously maintained under optimal condition. Relative Water Content (RWC) and the real-time quantitative PCR used to monitor the expression pattern of dehydrin gene in *Triticum asetivum* leaves under drought and ABA stress. Highly significant differences among the wheat cultivars ($P < 0.0007$) were detected for RWC. The high tolerant varieties were Vorobey and Giza 168 exhibited a higher activation of DHN and at the same time a high relative expression level of the gene. Similar dynamics of gene expression was observed in Sakha 93. However, relative expression level was lower for Finisi, Gemmiza 7 and Gemmiza 9 as compared to the other varieties. The increased activity of the gene was detected at a time when the plants were still maintaining a high water content in the leaves.

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تقييم التعبير الجيني لجين DHN في بعض القمح (***Triticum asetivum* L.**) المحصول وتحت الضغط غير الحيوية

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يعتبر الجفاف أحد الأسباب الرئيسية لفقدان عائد كبير في نباتات المحاصيل. إن النباتات تواجه الإجهاد عن طريق الإدراك والاستجابة للإجهاد، بعد إدراك الإجهاد يتم تبليغ إشارة إلى مكونات تلعب دور في تغيير التعبير الجيني، وبالتالي هناك بروتينات لازمة لإصلاح الضرر وإعادة البرمجة الفسيولوجية لتحسين التكيف. تم تقدير المحتوى المائي النسبي(RWC) باعتبارها واحدة من المؤشرات الأكثر موثوقية لتحديد كل من حساسية تحمل نباتات القمح إلى نقص المياه. كذلك تم استخدام ال PCR الكمي لتعين نمط التعبير الجيني لجين ال DHN في القمح *Triticum asetivum* تحت ظروف الجفاف والمعاملة بحامض الابسيسك . أظهرت النتائج ارتفاع التعبير الجيني لل DHN تحت ظروف المعاملة وكان الأصناف الأكثر تحملا "جيزة ١٦٨ " و "Vorobey" أعطت مستوى تعبير جيني أعلى ، وقد لوحظ تشابه في التعبير الجيني في الصنف "سخا ٩٣". وكان أدنى مستوى تعبير جيني في الأصناف "جميزة ٧" و " Finisi" مقارنة بالأصناف الأخرى.