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ISOLATION AND EXPRESSION OF DEHYDRIN AND DREB2 TRANSCRIPTION FACTOR GENES IN SOME WHEAT (*Triticum asetivum* L.) CULTIVARS.

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ABSTRACT

Drought is a worldwide problem, constraining global crop production and quality seriously. Water drives agricultural production in many parts of the world. Two genes from Triticum asetivum, were isolated under drought conditions. These drought responsive genes selected for expressional analyses can be classified into two groups. DHN gene with length 360 bp belong to the first group includes functional proteins already known to be involved in the response to water. DREB2 transcription factor gene with length 1256 bp belong to the second group that comprises protein factors involved in the regulation of signal transduction and gene expression.

Key words: Isolation & Expression, Dehydrin & Dreb2 Transcription Factor Genes, *Triticum asetivum* L. Cultivars**.**

INTRODUCTION

One of the main cellular events occurring during water deficit is extensive modification of gene expression resulting in a strict control of all the physiological and biochemical responses to the stress. Several genes specifically involved in stress response have been identified. Among these are the genes encoding the so-called LEA proteins. LEA proteins accumulate under stress conditions such as drought, salinity and low temperatures, but they are present also in ABA-treated vegetative plants. To this family belong the DHN genes, which are up-regulated during the stress (Zhu *et al.,* 2000). Further investigations, including other DHN gene expression analysis as well as quantification of their expression, are needed to add new insight to the molecular aspects of drought tolerance in plants and to clarify the role of single members of DHN gene family in the general pattern of drought stress response. Modification in the expression level of five dehydrin (DHN) genes was also analysed by reverse transcription–polymerase chain reaction (RT-PCR) (Rampino *et al.,* 2006).

Molecular study of regulatory networks suggests the existence of ABA independent and ABA-dependent signal transduction pathways that convert the initial stress signal into cellular responses (Seki *et al.,* 2007). Some transcription factors are expressed through abscisic acid pathways, while others have independent expression to abscisic acid hormone (Nakashima and Shinozaki, 2006).Most ABA-inducible genes such as rd22 contain a conserved cis acting element named ABRE in their promoter regions and are regulated by transcription factors such as bZIP, MYB and MYC (Shinozaki and Yamaguchi-Shinozaki, 1997). However, ABA independent stress inducible genes such as RD29A are regulated by other transcription factors like DREB (Narusaka *et al.,* 2003). Regulatory networks study shows that many of these signal pathways overlapped each other (Shinozaki and Yamaguchi-Shinozaki, 2000 & Knight and Knight, 2001). Dehydration responsive element binding factors, DREB transcription factors, are members of the AP2/ERF family which consist of many important regulatory and stress responding genes. DREB transcription factors have a sharp and transient response to abiotic stress condition and induce the expression of downstream functional genes, which are involved in abiotic stresses. In fact, they specifically bind to a distinct region in the promoter of target genes known as DRE/CRT sequence, which activate the transcription of genes (Shinozaki and Yamaguchi-Shinozaki , 2000).

MATERIALS AND METHODS

Materials:

A total of four wheat (*Triticum aestivum* L.) cultivars were used in the present study, two Egyptian cultivars, Giza168 and Gemmiza7 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 sterilized 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 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

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

No.	Cultivar	Pedigree	Origin
2	Giza168	MRL/BUC//SERI CM93046-8M-0Y-0M-2Y-0B	Egypt
3	Gemmiza7	7CMH74A630/SX//SERI82/AGENT	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

after 0, 4, 8, 12 h of drought treatment; control plants were well-watered and harvested at the same time.

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).

Second Strand cDNA Synthesis:

Specific primers for drought inducible genes DHN and DREB2 was used to synthesize the second strand cDNA (Table 2). Each PCR reaction mixture of 25 µ1 contained 2 µl of the first-strand cDNA, 5 µl of PCR reaction buffer (5x), 1.5 mM of MgCl2, 0.5 mM of dNTPs, 0.5 µl of each

Table 2: List of RT- PCR primers

Primer name	Primer sequence
DHN	F: 5´-GCGTCATGGAAAGCATCAC-3′ R: 5´-GTCCAGGCAGCTTGTCCTT-3′
DREB2	F: 5´-AAGAAAACAGGCGACAAGAT-3´ R: 5´-ACGAAGCACAAAAAACTAGC-3´

primer (100 µM) and 1 U GoTaq® DNA Polymerase (Promega, Madison, USA). The amplification was started in PCR (Biometra, Germany) with a 5 min denaturation step at 94 °C, followed by 30 cycles of 94 °C for 60 s, 59 °C for 45 s, and 72 °C for 60 s, and a final extension step at 72 °C for 5 min.

The products were separated on 1.5 % agarose gel electrophoresis stained with ethidium bromide.

RESULTS AND DISCUSSION

DHN gene expression:

The expression of all the DHN gene isolated was studied by RT-PCR analysis on RNAs isolated from control and stressed seedlings in the *Triticum asetivum* genotypes resistant (*i.e*. Giza 168 and Vorobey) or sensitive (*i.e.* Gemmeza 7and Finisi) to dehydration stress. The results reported in (Figure 1) show that the presence of one amplified band with length of 360 bp and show that the different expression patterns were observed among the analysed gene. The expression levels were found to be related to both the severity of the stress and the genotype. DHN gene expression was induced in all the resistant genotypes under control and all stress treatments .In all the sensitive genotypes the expression of DHN gene induced after 8 h and 12h treatment.

The data showed that, in the sensitive genotypes DHN gene was not expressed in the well watered plants but only in the stressed plants, on the contrary the resistant genotypes DHN gene was expressed in the well watered plants and the stressed plants confirming the role of early production of these proteins in the protection mechanisms activated by plants in response to drought stress. Rampino *et al*., (2006) showed that, the transcription of TdDHN13 was visible in the resistant genotypes Claudio

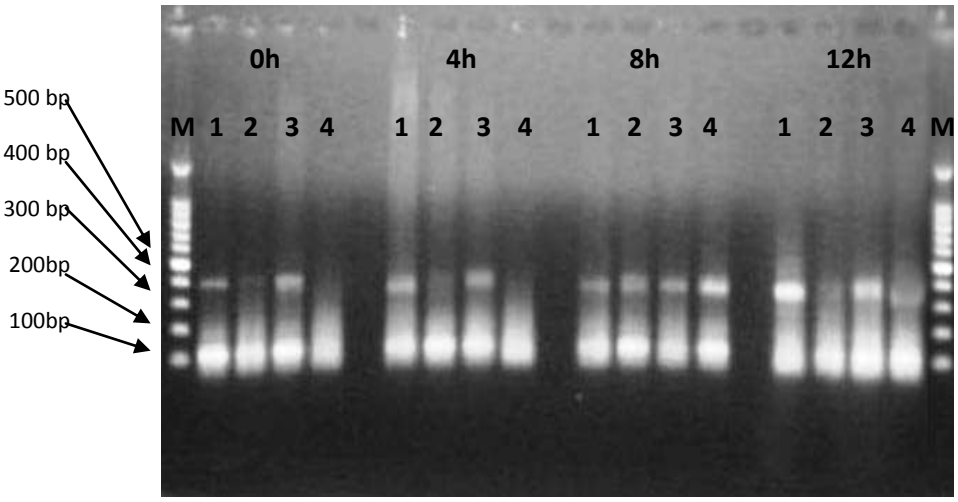

Figure 1. Gel electrophoresis of PCR product of DHN, M = GeneRuler 100bp DNA ladder and 360 bp amplified by RT-PCR is shown, wheat cultivars (1) Giza168, (2) Gemmiza7, (3)Vorobey and (4)Finisi, drought stress (0 (control), 4, 8 and 12 hours)

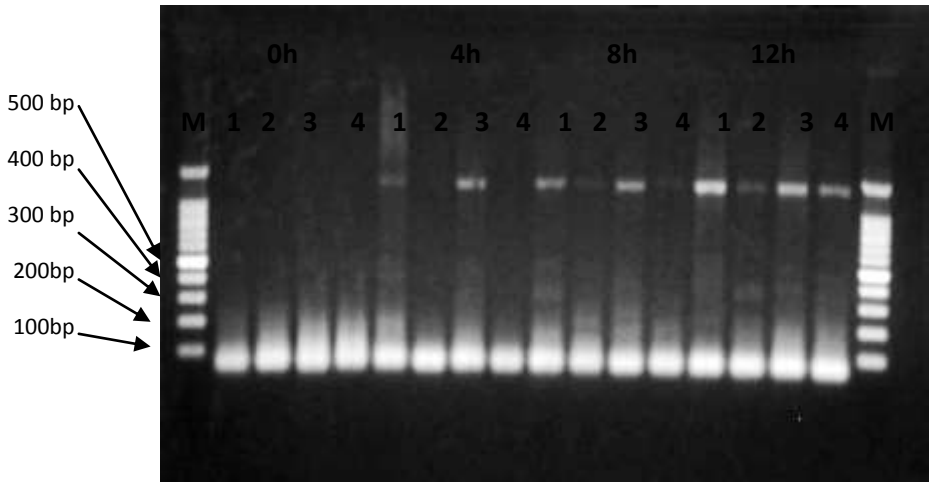

Figure 2. Gel electrophoresis of PCR product of DREB2, M = GeneRuler 100bp DNA ladder and 1256 bp amplified by RT-PCR is shown, wheat cultivars (1) Giza168, (2) Gemmiza7, (3) Vorobey and (4) Finisi**,** drought stress (0 (control), 4, 8 and 12 hours)

and Svevo even at high RWC, and in Claudio the accumulation of transcripts paralleled the decrease in RWC. In the sensitive genotypes, the transcription of TdDHN13 was also evident but the expression of this gene occurred at lower RWC, and in the case of 5BIL90 the expression was very low until the severity of the stress increased. Patrizia *et al.,* (2006) results identified resistant and sensitive genotypes based on RWC %. Further characterization of genotypes differing in their response to water stress, and studied water loss rate (WLR) and free proline content after different periods of dehydration. Under drought stress, the water content of wheat leaves decreased, but membrane permeability increased. Western blot analysis showed that there was a specific protein of 28 ku under drought stress, the expression of dehydrin in weak drought tolerant wheat leaf was earlier than that in strong drought tolerant wheat leaf, and the content of dehydrin in strong drought tolerant wheat was higher than that in weak drought tolerant wheat. After redehydration, the water content of wheat leaves increased, the membrane permeability decreased, and this dehydrin could exist in plant for some time. It showed that harm to wheat under drought stress and expression of this dehydrin is closely related to drought resistance in wheat. For yield traits, Christopher *et al.,* (2008) conducted six detailed experiments to compare the growth, development and yield traits of cultivars SeriM82 and Hartog. The yield of SeriM82 was 6-28% greater than Hartog.

DREB2 gene expression:

After drought stress treatment RNA extraction and cDNA synthesis, DREB2 fragment with 1256 bp length. The results reported in (Fig. 2) show that in control (non-stressed) seedlings, no expression of any of the DREB2 gene was observed. On the contrary, drought (12 h) induced the expression of DREB2 gene in the sensitive genotypes analysed, while the expression of DREB2 gene induced in the resistant genotypes after 4 hours, 8 hours and 12 hours drought stress treatment. the accumulation of transcripts of DREB2 gene paralleled the severity of the stress.

WDREB2 transcription factor codes a DRE/CRT binding protein and have an important role in abiotic stress responses (Nakashima *et al.,* 2009). Zhao *et al.,* (2009) identified DREB1/CBF and DREB2 homologue genes in various plants such as rice, wheat, diploid wheat (*Triticum monococcum*), barley, wild barley (*Hordeum spontaneum*), maize, sorghum (*Sorghum bicolor*), rye (*Secale cereale*) and oat (*Avena sativa*). The WDREB2 expression is activated by cold, drought, salt and exogenous ABA treatment. It activates Cold responsive/ late embryogenesis abundant (Cor/Lea) genes (Egawa *et al.,* 2006). Two isoforms of WDREB2 were

isolated from Triticum aestivum cDNA source and molecularly characterized (Sima Sazegari and Ali Niazi, 2012).

CONCLUSION

Four bread wheat (*Triticum aestivum* L.) cultivars were subjected to drought stress(4h, 8h and 12h) after ten days old seedlings, while the controls were continuously maintained under optimal condition. The RT-PCR used to monitor the expression pattern of dehydrin and DREB2 gene in *Triticum asetivum* leaves under drought stress. For the DHN gene the results showed that the presence of one amplified fragment with length of 360 bp and show that the different expression patterns were observed among the analysed gene. The expression levels were found to be related to both the severity of the stress and the genotype. DHN gene expression was induced in all the resistant genotypes under control and all stress treatments .In all the sensitive genotypes the expression of DHN gene induced after 8 h and 12h treatment. For the DREB2 gene the results showed that the presence of one amplified fragment with 1256 bp in length, drought (12 h) induced the expression of DREB2 gene in the sensitive genotypes analysed, while the expression of DREB2 gene induced in the resistant genotypes after 4 hours, 8 hours and 12 hours drought stress treatment. The accumulation of transcripts of DREB2 gene paralleled the severity of the stress**.** On the contrary,control (non-stressed) seedlings no expression of was observed**.**

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تعبير و عزل جين DHN و جين عامل النسخ DREB2 لبعض أصناف القمح (*Triticum asetivum* L.)

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يعتبر الجفاف مشكلة عالمية تقييد إنتاج وجودة المحاصيل جديا، لذا فإن المياه توجه الإنتاج الزراعي في أجزاء كثيرة من العالم. تم عزل اثنين من الجينات من نبات القمح *Triticum asetivum* تحت ظروف الجفاف. هذه الجينات التي تستجيب للجفاف تم عزلها لتحليل التعبير الجينى يمكن تصنيفها إلى مجموعتين. جين ال DHN بطول ٣٦٠ نيوكليوتيدة ينتمي إلى المجموعة الأولى وتشمل بروتينات وظيفية معروفة بالفعل للمشاركة في الرد على نقص الماء. جين عامل النسخ DREB2 بطول ١٢٥٦ نيوكليوتيدة ينتمي إلى المجموعة الثانية التي تضم عوامل البروتين التى تشارك في تنظيم نقل الإشارة وتنظيم التعبير الجيني.

التوصية: تم عزل الجينات التى تنتج بروتين ال dehydrin وعامل النسخ DREB2 بنجاح من القمح وتشير النتائج إلى أن dehydrins وDREB2 يشاركون في استجابة النبات للإجهاد، مما يشير إلى إمكانية استخدام جين ال dehydrin وجين عامل النسخ DREB2 في التحسينات المعدلة وراثيا فى النباتات المعرضه للاجهاد. كذلك تستخدم فى حصر الأصناف بالكشف عن جين ال dehydrin وجين ال DREB2 لتسهيل تحديد الطرز الوراثية في برامج التربية.