CATRINA (2010), 5 (1): 15-21
© 2010 BY THE EGYPTIAN SOCIETY FOR ENVIRONMENTAL SCIENCES

Differential Gene Expression and Physiological Adaptation of Two *Triticum aestivum* Cultivars for Drought Acclimation

Mohamed M. Ibrahim and Ibrahim Alaraidh
Science Department (biology section), Teachers college, King Saud University, Riyadh, KSA

ABSTRACT

Gene expression as a valuable stress marker in ecophysiological studies and rapid changes in gene expression in plants in response to stress are important for environmental adaptation. Two *Triticum aestivum* cultivars [drought sensitive (Sids-1) and drought tolerant (Veery)] were exposed to drought stress interrupted by rehydration period. The differentially expressed gene in drought stressed seedlings was strongly and rapidly induced in leaves of tolerant wheat (*cv*Veery) especially after rehydration, whereas induction was delayed and transcripts accumulated to a low level in sensitive one (*cv* Sids-1). Drought induced alteration in the expression of genes involved in signaling and in oxidative stress responses. A significant variability in drought tolerance was found between the two cultivars: while drought sensitive (Sids-1) oxidative stress indices were significantly increased after 4 days drought stress compared to the control, drought tolerant cultivar (Veery) indices increased after 8 days. After 2 days rehydration, plants exposed to 12 days drought showed better cultivar-dependent behavior, compared to those exposed to drought without rehydration and Veery cultivar showed better drought tolerance than Sids-1. This may be related to their antioxidant activity (smaller IC50 values for both 1,1-diphenyl-2-picrylhydrazyl and superoxide scavenging), associated with lower leaf MDA accumulation in comparison to sensitive cultivar. Taken together, results suggest that wheat may be interesting for production of antioxidant compounds, and that the cultivar-dependent capacity to induce antioxidative mechanisms in response to drought, may result in a corresponding variability for growth sustainability.
Key words: Gene expression, drought acclimation, *Triticum aestivum.*

INTRODUCTION

Drought is one of the major ecological factors limiting crop production and food quality globally, especially in the arid and semi-arid areas of the world. Drought is a major yield-limiting environmental factor identified across the globe and soils too dry for crop production cover 28 % of the Earth's land surface (Bray, 2004). There is continuous search for new genes and characterizations of their regulatory elements to design innovative strategies for better plant adaptation.

Differential display of mRNA was employed to identify genes for comparing the transcripts in several treatments simultaneously (Liang and Pardee, 1992). It is worth noting that differential display (DD) has been used successfully to identify gene imprinting, a phenomenon referring to-specific gene expression (Hagiwara *et al*., 1997 and Ivanova *et al*., 1998). Also differential display of mRNA was employed to identify such genes owing to the ease and speed of comparing the transcripts in several treatments simultaneously (Liang *et al* 1994). Among crop plants, wheat (*Triticum aestivum*), which often experiences water-limited conditions, is an attractive study system because of the natural genetic variation in traits related to drought tolerance (Labhilili *et al*., 1995; Loggini *et al*., 1999). Despite significant progress in molecular biology of the drought response, the genes and/or gene products required for dehydration tolerance remain unknown (Ingram and Bartels, 1996; Bray, 1997). One of the responses of plants to abiotic stresses such as drought and salinity is an alteration in gene expression as evidenced by changes in the abundances of specific mRNAs and newly synthesized proteins (Bray, 1993; Skriver and Mundy, 1990). In wheat, the differential gene expression was analyzed between two cultivars in both primary roots and seedling leaves. Differences were detected in gene expression patterns between cultivars and their parents, however it was found that the differential expression patterns are also dependent on the tissues tested and developmental stages (Sun *et al*., 1999 and Zhongfu *et al*., 2000).

Furthermore, the imposition of biotic and abiotic stress conditions can give rise to excess concentrations of active oxygen species (AOS), resulting in oxidative damage at the cellular level. Drought shows a particular affinity for sulfhydryl groups and also reacts with hydroxyl groups resulting in alterations in function of membrane by inducing changes in lipid peroxidation suggesting that the tissue suffered from oxidative stress (Mukherjee and Choudhuri, 1983). Therefore, a consequence of water stress is usually accompanied by the formation of active oxygen species (AOS) (Smirnoff, 1993) such as the superoxide radical, H_2O_2, and the hydroxyl radical (Foyer *et al*., 1994; Asada, 1997). However, plants can respond and adapt to water stress by altering their cellular metabolism and invoking various defense mechanisms (Bohnert and Jensen, 1996). Thus, plants are equipped with complex and a highly efficient antioxidative defense system composed of protective non-enzymatic and enzymatic protection

* *Corresponding author: mibrahim@ksu.edu.sa*

mechanisms function to interrupt the cascades of uncontrolled oxidation in some organelles (Noctor and Foyer, 1998). These mechanisms serve to maintain the antioxidants in their reduced functional state (Schwanz *et al*., 1996) that efficiently scavenge AOS and prevent damaging effects of free radicals (Shalata and Tal, 1998).

Increased concentration of free proline probably have several functions, such as osmotic adjustment, stabilization of subcellular structures, scavenging of free radicals, retaining of energy and also as a stress signal molecule during environmental conditions causing water deficiency in plant tissues (Siripornadulsil *et al*., 2002).

Triticum aestivum is one of the most important grain for direct human consumption all over the world. Only small percentage of the growing area is well watered, and water deficit is the abiotic stress that is most limiting for grain production. Therefore, physiological and molecular studies of drought acclimation and tolerance in *Triticum aestivum* are needed to find the traits and genes involved in drought acclimation. A differential display was used in this study to analyze alterations in gene expression, as well as some physiological parameters, in two wheat varieties after drought and subsequent re-watering in *Triticum aestivum* plants are reported.

MATERIALS AND METHODS

Plant materials and treatment

Seeds were grown in plastic pots (15 cm diameter x 20cm height). Seeds were soaked in continuously aerated distilled water for 24 h in darkness. By the end of soaking period, twelve seeds were sown in each pot containing 1700 g sterilized sandy soil (70%) and 30% vermiculite under 16/8 h day/night cycle. Light intensity was 420µmol m-2s-1 at the canopy of plant supplied by a mixture of fluorescent and incandescent lamps and at controlled temperature of 28/26˚C and 55/60% relative humidity.

The pots were irrigated by distilled water. After fifteen days from sowing, the pots were irrigated with half strength of Hoagland solution only up to twenty eight days then the pots for each cultivar were grouped into two sets. In the first set, control (non-stressed) plants were grown under these conditions throughout the whole experimental period. In the second set, plants were drought pretreated (drought acclimated) by cessation of watering for 8 days. After this drought period, the plants were rewatered for 48 h then subjected to second drought period extended for 12 days.

Oxidative stress indices

Determination of scavenging activity (Antiradical activity)

Scavenging activity was determined for 1,1-Diphenyl-2-picrylhydrazyl (DPPH·) and superoxide anion radical and the effect of methanolic extracts on DPPH· degradation was estimated according to Hanato *et al*. (1988). Superoxide anion derived from dissolved oxygen by a pure methanol stock (PMS)/NADH coupling reaction reduces nitro blue tetrazolium (NBT).

The antiradical activity was expressed as IC_{50} (mg ml^{-1}), the antiradical dose required to cause a 50% inhibition (three replicates per treatment). A lower IC50 value corresponds to a higher antioxidant activity of plant extract (Patro *et al*., 2005). The ability to scavenge the DPPH·radical was calculated using the following equation:

$$\text{DPPH}\cdot \text{ scavenging effect} = \frac{A_0 - A_1}{A_0} \times 100$$

where A_0 is the absorbance of the control at 30 min, and A_1 is the absorbance of the sample at 30 min.

Superoxide anion scavenging activity was assessed using the method described by Duh *et al*. (1999). Evaluating the antioxidant activity in root extract was based on IC_{50}. The IC_{50} index value was defined as the amount of antioxidant necessary to reduce the generation of superoxide radical anions by 50%. The IC_{50} values (three replicates per treatment) were expressed as mg ml^{-1}. As for DPPH·, a lower IC_{50} value corresponds to a higher antioxidant activity of plant extract.

Detection and isolation of differentially expressed wheat cDNAs

A differential display technique to isolate differentially expressed genes was done according to what reported by Liang and Pardee (1992).

Extraction of total RNA

Genomic DNA contamination of the total RNA from roots of drought-stressed and non-stressed plant samples was removed by the treatment of 100 µg RNA with 20 U DNAse I, purchased from Amersham- Pharmacia Biotech (France). After phenol extraction, the DNA-free total RNA was used for differential display experiments.

Reverse transcription

Reverse transcriptase (RT) enzyme has 5'-3' polymerase activity and uses single stranded mRNA as a template in the presence of a primer to synthesize a complementary DNA strand. Three reverse transcription reactions per sample are set for the first strand cDNA synthesis using the genomic DNA-free mRNAs from leaves and roots of control and stressed seedlings, as a template, and a pool of two-base anchored oligo-dT primers. The reverse transcription (RT) reactions were set per RNA sample using 10 µg freshly diluted RNA in water and incubated at 80 °C for 5 min, and transferred to ice. To the denaturated RNA was added 1× RT buffer (50 mM Tris-HCl pH 8.3, 75 mM KCl, 3 mM $MgCl_2$, and 10 mM DTT), a dNTPs mix (10 mM each), and

2μM each of T10GN,T10CN,T10ANoligonucleotides (where N is the 3-fold degenerate for A, G, and C). A ribonuclease inhibitor (50 U) was added together with 200 U reverse transcriptase to each RT reaction and incubated at 37 °C for 1 h. The resulting first strand cDNAs were used as templates in PCR amplification of radioactive fragments.

Polymerase chain reaction (PCR)

PCR was performed by setting up a 25-μL reaction containing 2 μL corresponding first-strand synthesis cDNA, 1× PCR buffer (10 mMTris-HCl pH 8.3, 50 mM KCl, 1.5 mM$MgCl_2$), 20 μM of each dNTP, 1 μM of the respective anchored oligo-dT in combination with 0.5 μM of the random 8-mer primer, 3.7·105 Bq [35S]-dATP (Amersham) and 1 U Taq DNA polymerase. The denaturation, annealing and primer extension steps of the PCR program were 94°C for 30 s, 37°C for 2 min and 72°C for 30 s, respectively for 45 cycles, followed by a final extension step at 72°C for 10 min. The radioactive samples (3.5 μL) were mixed with 2 μL DNA sequencing stop solution and heated to 70°C for 10 min and stored on ice prior to loading on 6 % denaturing polyacrylamide DNA sequencing gel. Electrophoresis was performed at constant 1 400 V for 3 h, then the gel was dried without fixation and exposed to a Kodak X-Ray film for 2 d. The radioactive cDNA bands corresponding to over-expressed sequence in roots of the stressed sample from the R1 seedlings were isolated from the dried sequencing gel. PCR product was visualized and photographed using short UV radiation with gel documentation system (Alpha Chem., USA).

Determination of proline content

Proline content was determined by the method described by Bates *et al*. (1973) with minor modification. Segments from barley root tips (60 segments/500 mL) were excised into 3% aqueous sulphosalicylic acid and homogenized using a cold pestle and mortar. The homogenate was centrifuged at 12,000 g for 10 min. The reaction mixture containing 100 mL of supernatant, 100 mL glacial acetic acid and 100 mL ninhydrin reagent (2.5% ninhydrin in 60% phosphoric acid) was incubated at 98 °C for 30 min and terminated by cooling the tubes on ice. The absorbance was determined at 518 nm.

Statistical analysis

The percentages of a given differential expression pattern were computed for each of the two cultivars. Simple correlations were estimated to determine the relations between a specific expression pattern using data obtained from the two cultivars.

RESULTS

Drought stress and antioxidant capacity assay

Antioxidant activities were evaluated by the effect of extract samples on Diphenyl-2-picrylhydrazyl (DPPH·) radical and superoxide radical anion scavenging activities after all drought stress exposures. In control samples, DPPH· radical-scavenging activity was similar in both cultivars (IC50 values 0.72 and 0.77 mg ml^{-1}) in Sids-1 and Veery, respectively (Table 1). In extracts from Veery, the scavenging activity was poorly modified by drought stress, with IC_{50} values increased to 1.1- and 1.3-fold more than control. On the contrary, it was strongly diminished in Sids-1, with IC_{50} values augmented to 2- and 3-fold increase compared to control value after 4 and 8 days drought respectively. On the other hand, tolerant cultivar (Veery), after rehydration and at the end of the second drought phase, showed insignificant change and IC_{50} values are nearly similar to the corresponding control. Whereas, scavenging activity in the sensitive cultivar (Sids-1) changed significantly, but to a lesser extent than the values obtained after 8 days drought without rehydration (Table 1).

Table (1): Antioxidant activities (IC_{50} values) in roots of two wheat cultivars subjected to drought stress periods, first for 8 days without irrigation and second for 12 days drought after 2 days rehydration. Values (means of three replicates) of each parameter followed by at least one same letter are not significantly different at $p < 0.05$.

Drought (Days)	DPPH-Scavenging Activity IC50 (mg ml^{-1})		Superoxide Scavenging Activity IC50 (mg ml^{-1})	
	Sids-1	Veery	Sids-1	Veery
Control	0.72±0.19	0.77±0.11^{c}	4.10±0.11^{bc}	4.57±0.86^{bc}
D_4	1.44±0.23^{b}	0.86±0.02^{c}	6.10±1.08^{b}	2.70±0.29^{c}
D_8	2.13±0.06^{a}	0.99±0.48^{c}	15.90±3.04^{a}	4.90±0.11^{bc}
D_8 after 2d Rewatering	1.54±0.23^{b}	0.91±0.02^{c}	5.90±1.08^{b}	2.89±0.29^{c}
D_{12} after 2d Rewatering	1.22±0.19^{b}	0.83±0.1^{b}	7.39±1.03^{b}	3.11±0.95^{c}

Drought stress and proline content

The proline content was significantly increased in the first drought period extended for 8 d in sensitive stressed roots more than tolerant cultivar. After 8 d drought stress, proline content in sensitive cultivar, representing 260% compared with control, while the corresponding value for tolerant cultivar was only 190% of the control. With subsequent drought, after 2d rehydration, its amount was apparently near to the control value, representing 110 % and 125% for tolerant and sensitive cultivars, respectively (Fig. 1).

The concentration of extract from control plants required for 50% scavenging superoxide radical generation was similar in the two cultivars (4.1 and 4.57 mg ml^{-1} in Sids-1 and Veery, respectively). After 4 d drought, Veery cultivar exhibited a nearly two-fold increase of its scavenging activity but was slightly reduced compared to control after 8 days drought (Table 1). The response of Sids-1 differed on two points from that of Veery. First, the scavenging activity was diminished at both 4 and 8 d drought stress. Second, the magnitude of this effect was very important, since the IC50 values were 3-4-fold higher than in Veery for 4 and 8 d drought, respectively. Prolonged drought extended for 12 days after rehydration resulted in a significant amelioration in the scavenging activity of tolerant cultivar (Veery) more better than sensitive cultivar (Sids-1) (Table 1). After 2 d rehydration, the superoxide scavenging activity for tolerant cultivar (Veery) exposed to 12 d drought was nearly similar to control (3.11 mg ml^{-1}). The corresponding values for sensitive cultivar was 7.39. These results were better than their values obtained after 8 days drought without rehydration.

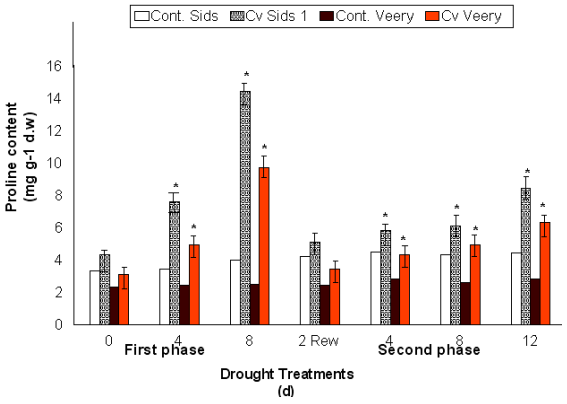

Figure (1): Proline content in roots of well watered and drought stressed wheat cultivars *CV* Sids-1 (drought sensitive) and *CV* Veery (drought Tolerant) subjected to two drought phases interrupted by 2d dewatering. Each value represents the mean ±SE of five replicates. Significant differences ($P<0.05$) between treatments according to LSD test are shown by an asterisk.

Differential display

A great variation in the number and intensity of the expressed bands in response to drought duration and cultivar- dependent was noticed as shown in (Fig. 2).

Two common bands with a molecular mass 87 and 147 bp were clearly expressed especially in the tolerant cultivar in both control and drought stressed plants either before or after rehydration period. Following two days rehydration and after 12 days drought, two new bands with a molecular mass of 284 and 351 bp were expressed only in the roots of tolerant cultivar (Veery) in comparison with control (Table 2).

Table (2): Effect of drought on the presence or absence of different amplified cDNA bands in roots of two wheat cultivars.

Cultivar	Number of band	Drought (Days)	Primer dimmer (87 bp)	Band-2 (147bp)	Band-3 (139bp)	Band-4 (194bp)
Sensitive (Sids-1)	1	0	(+)	(+)	(-)	(-)
	2	4	(+)	(+)	(-)	(-)
	3	8	(+)	(+)	(-)	(-)
	4	12d After Rew.	(+)	(+)	(+)	(+)
Tolerant (Veery)	5	0	(+)	(+)	(+)	(-)
	6	4	(+)	(+)	(-)	(-)
	7	8	(+)	(+)	(-)	(-)
	8	12d After Rew.	(+)	(+)	(+)	(+)

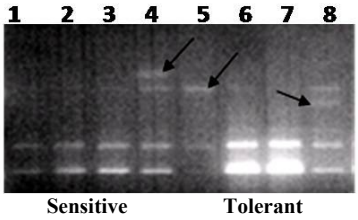

Figure (2): Differential display (DDRT-PCR) and qualitative expression patterns from roots of two wheat varieties. Lane 1, unstressed leaves (control); lane 2, drought stressed roots for 4 d; lane 3 represent, 8 d drought and lane 4 represents 12 days drought after 2 days rehydration, respectively for sensitive cultivar(sids-1). Lane 5, represent unstressed leaves (control); lane 6; after 4 days drought; lane 7, drought stressed roots for 8 days and lane 8 represents 12 days drought after 2 days rehydration, respectively for tolerant cultivar (Veery).

DISCUSSION

Drought-induced growth variations are correlated with cultivar types and parallel variations in anti-oxidative ability as well as with opposite changes in lipid peroxidation. Thus, as expected, the drought stress is effective in augmenting extractable, active anti-oxidants from wheat roots, and there is variability for the accumulation of these compounds in response to drought. Furthermore, some causal relationship is linked between the content of oxidative stress indices and antioxidant scavenging activities. In this study, the DPPH$^{\cdot}$ and $O_2^{\cdot-}$ (PMS/NADH-NBT system) methods were selected to evaluate the antioxidant activities of plant extracts as they are among the most effective methods for evaluating the concentration of radical-scavenging materials active by a chain-breaking mechanism (Anagnostopoulou *et al*., 2006). Plants of the tolerant cultivar (Veery) exposed to drought stress showed a higher antiradical ability against both DPPH$^{\cdot}$ radical and superoxide anion, in contrast to Sids-1 cultivar (Table 1). These results suggest that in Sids-1 plants under prolonged drought stress, an imbalance between generation of (AOS) and their scavenging systems might have occurred. Such a hypothesis was corroborated by other previous results obtained by Al-Ghamdi, 2009, that evidenced increased malondi-aldehyde (MDA) and H_2O_2 contents in Sids-1 leaves, compared to Veery plants. The better response of tolerant cultivar (Veery) under drought stress, especially after rehydration period, was correlated to their better antioxidant activities and these activities may be directly linked to their free radical scavenging activities (Huang *et al*., 2006). Our results also suggest that there is variability in the capacity to induce antioxidative mechanisms in response to drought before and after rehydration, leading to a corresponding variability for growth protection (Table1). Another explanation would be that the two cultivars escape from drought stress unlikely. The degree of oxidative cellular damage in plants exposed to abiotic stress is controlled by the capacity for protection against oxidative agents. Drought tolerance seems to be preferred by an increased antioxidative capacity to detoxify reactive oxygen species (Smirnoff, 1993; Noctor and Foyer, 1998).

Proline accumulation is another characteristic feature of water-stressed tissues. In our study, its accumulation was increased significantly in sensitive cultivar (Sids-1) more than tolerant one (Veery), throughout the first drought stress phase (Fig. 1). On the contrary, after rehydration, the proline content decreased sharply in both cultivars. Caballero *et al*., 2005 evidenced that in plant roots, proline accumulation was observed only during severe water stress, while at moderate stress conditions proline accumulated only in leaves. Similarly, in many plant species, increases in the concentration of free proline probably have several functions, such as osmotic adjustment, stabilization of subcellular structures, scavenging of free radicals, retaining of energy and also as a stress signal molecule during environmental conditions causing water deficiency in plant tissues (Siripornadulsil *et al*., 2002). Although the expression of a number of genes was shown to be enhanced by exposure to drought stress (Doebley and Lukens, 1998), differential responses to dehydration have been documented in only a few studies comparing expression profiles between drought-stressed and non stressed organs by means of differential mRNA display. In contrast to the original DDRT-PCR results, two amplicons corresponding to a presumptive drought-responsive gene were shown to be constitutively expressed in the tissues examined by qualitative RT-PCR. This discrepancy between experimental methods is probably due to the limitations of DDRT-PCR, such as the somewhat indiscriminant amplification of a population of similarly sized cDNAs and the difficulty in excising a single amplification product from the differential display gel (Ninghui *et al*., 1996 and 1997; Schmidt *et al*., 2002). Even those genes whose expression was up-regulated or down regulated by drought before and after rehydration were expressed at different levels under individual stress conditions (Erdei *et al*., 2002). It has been proposed that high-throughput stress-specific gene expression analysis is important for understanding gene function (Rossby *et al*., 2001; Chaves *et al*., 2003). The results reported here suggest that organ, as well as, stress-specific gene expression analyses are necessary in gene identification and characterization studies.

The results demonstrated that some patterns of differential expression detected in roots of both cultivars are correlated with drought stress in some traits and some other traits are correlated with differential expression patterns only detected in roots of either of the cultivars (Fig. 2 and Table 2).

As Somerville, 1999 stated: “we speculate that the hybrids will exhibit significant differences in the

expression of clusters of functionally related genes and that will have different patterns of expression". Results reported here also support this speculation. Further investigation is needed to isolate these differentially expressed genes, and to characterize their physiological functions leading to the drought acclimation.

In conclusion, results reported here demonstrated that changes in gene expression do occur in the two cultivars in response to drought, and these differentially expressed genes, though functionally not known yet, may play important roles for cultivars to exhibit its response to drought stress before and after rehydration.

REFERENCES

AL-GHAMDI, A.A. 2009. Evaluation of Oxidative Stress Tolerance in two Wheat Cultivars (*Triticum aestivum*) in Response to Drought, Int. J. Agri. Biol., 10(6).

ANAGNOSTOPOULOU, M.A., P. KEFALAS, V.P. PAPAGEORGIOU, A.N. ASSIMOPOULOU, D. BOSKOU 2006. Radical scavenging activity of various extracts and fractions of sweet orange peel (*Citrus sinensis*), Food Chem. **94:** 19-25.

ASADA, K. 1997. The role of ascorbate peroxidase and monodehydroascorbate reductase in H_2O_2 scavenging in plants. *In* JG Scandalios, Ed., Oxidative Stress and the Molecular Biology of Antioxidant Defenses. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 715-735.

BATES, L.S., R.P. WALDREN, AND I. D. TEARE. 1973. Rapid determination of free proline for water stress studies. Plant and Soil **39:** 205-207.

BOHNERT H.J., R.G. JENSEN. 1996. Strategies for engineering water-stress tolerance in plants. Trends in Biotechnology **14:** 89–97.

CABALLERO, J.I., C.V. VERDUZCO, J. GALAN, AND E.S.D. JIMENEZ. 2005. Proline accumulation as a symptom of drought stress in maize: A tissue differentiation requirement. J. Exp. Bot., **39:** 889–97.

CHAVES M.M., J.P. MAROCO AND J.S. PEREIRA. 2003. Understanding plant responses to drought-from genes to the whole plant, Funct. Plant Biol., **30:** 239-264.

DOEBLEY, J., L. LUKENS. 1998. Transcriptional regulators and the evolution of plant form, Plant Cell **10:** 1075-1082.

DUH, P., Y.Y. TU, AND G.C. YEN. 1999. Antioxidant activity of water extract of harng jyur (*Chrysanthemum morifolium*, Ramat), Lebensm.-Wiss, Technol **32:** 269-277.

ERDEI, L.; I. TARI, AND J. CSISZAR. 2002. Osmotic stress responses of wheat species and cultivars differing in drought tolerance: some interesting genes (advices for gene hunting), Acta Biol. Szeged., **46 (3/4):** 63-65.

FOYER, C. H., P. DESCOURVIERES, AND K.J. KUNERT. 1994. Protection against oxygen radicals: an important defense mechanism studied in transgenic plants. Plant, Cell Environ. **17:** 507-523.

HAGIWARA, Y., M. HIRAI, K. NISHIYAMA, I. KANAZAWA, T. UEDA, Y. SAKAKI, AND T. ITO. 1997. Screening for imprinted genes by allelic message display: identification of a paternally expressed gene impact on mouse chromosome 18. Proc. Natl. Acad. Sci. USA **94:** 9249-9254.

HANATO, T., H. KAGAWA, T. YASUHARA, AND T. OKUDA. 1988. Two new flavonoids and other constituents in licorice root: their relative astringency and radical scavenging effect, Chem. Pharm. Bull. **36:**1090-1097.

HUANG, Y.C., Y.H. CHANG, AND Y.Y. SHAO. 2006. Effects of genotype and treatment on the antioxidant activity of sweet potato in Taiwan, Food Chem. **98:** 529-538.

INGRAM, J. AND D. BARTELS. 1996. The molecular basis of dehydration tolerance in plants. Annual Review of Plant Physiology and Plant Molecular Biology **47:** 377–403.

IVANOVA, A.V., M.J. BONADUCE, S.V. IVANOV, AND A.J.S. KLAR. 1998. The chromo and SET domains of the Clr4 protein are essential for silencing in fission yeast. Nat. Genet. **19:** 192-195.

LABHILILI, M., P. JOUCHIER, AND M.F. GAUTIER 1995. Characterization of cDNA encoding *Triticum drum* dehydrins and their expression patterns in cultivars that differ in drought tolerance. Plant Sci **112:** 219-230.

LIANG P., A.B. PARDEE. 1992. Differential display of eukaryotic messenger RNA by means of polymerase chain reaction. Science **257:** 967–971.

LOGGINI, B., A. SCARTAZZA, E. BRUGNOLI, AND F. NAVARI-IZZO. 1999. Antioxidant defense system, pigment composition, and photosynthetic efficiency in two wheat cultivars subjected to drought, Plant Physiol. **119:** 1091–1099.

MUKHERJEE, S.P, AND M.A. CHOUDHURI. 1983. Implication of water stress- induced changes in the levels of endogenous ascorbic acid and hydrogen peroxide in Vigna seedlings, Physiologia Plantarum **58:** 166-170.

NINGHUI, C., JINSHUI, Y., YANGPING, G., MINGLIANG, X. AND Q. MIN. 1996. Differential display of mRNA between hybrid F1 and its parental inbred lines, Chinese Science Bulletin **41:** 939–943.

NINGHUI, C., G. YANGPING, Y. JINSHUI, Q. MIN, AND G. KOULIN 1997. Alteration of gene expression in rice hybrid F1 and its parental seedlings, Acta Botanica. Sinica **39:** 379–382.

NOCTOR G, AND C.H. FOYER. 1998. Ascorbate and glutathione: keeping active oxygen under control. Ann. Rev. Plant Physiol. Plant Mol. Biol., **49:** 249-279.

PATRO, B.S., A.K. BAURI, S. MISHRA, AND S. CHATTOPADHYAY. 2005. Antioxidant activity of

Myristica malabarica extracts and their constituents, J. Agricultural Food Chemistry **53:** 6912-6918.

ROSSBY, P.; R.C. LIANG, D.H. SHELTON, A. MANIER, CHAKRABARTI, AND F. SULSER. 2001. Detection of an mRNA polymorphism by differential display. Molecular Biotechnology. **19:** 121-124.

SCHMIDT, J.V., P.G. MATTESON, B.K. JONES, X.J. GUAN, AND S.M. TILGHMAN. 2002. The Dlk1 and Gtl2 genes are linked and reciprocally imprinted. Genes Dev. **14:** 1997-2002.

SCHWANZ, P., C. PICON, P. VIVIN, E. DREYER, J. GUEHL, AND A. POLLE. 1996. Responses of antioxidative systems to drought stress in Pendunculate Oak and Maritim Pine as modulated by elevated CO_2. Plant Physiology. **110:** 393-402.

SHALATA, A. AND M. TAL. 1998. The effects of salt stress on lipid peroxidation and antioxidants in the leaf of the cultivated tomato and its wild salt-tolerant relative *Lycopersicon pennellii*. Physiol. Plant., **104:** 169–174.

SIRIPORNADULSIL, S., S. TRAINA, D.P.S. VERMA, AND R.T. SAYRE. 2002. Molecular mechanisms of proline-mediated tolerance to toxic heavy metals in transgenic microalgae. Plant Cell **14:** 2837–2847.

SKRIVER K. AND MUNDY J. 1990. Gene expression in response to abscisic acid and osmotic stress, Plant Cell **2:** 503–512.

SMIRNOFF, N. 1993. The role of active oxygen in the response of plants to water deficit and desiccation. New Phytologist. 125: 27-58.

SOMERVILLE, C. AND S. SOMERVILLE 1999. Plant functional genomics, Science **285:** 380-383.

SUN, Q., Z. NI, AND Z. LIU. 1999. Differential gene expression between wheat hybrids and their parental inbreds in seedling leaves, Euphytica, **106:** 117-123.

ZHONGFU, N., S. QIXIN, AND W. LIMIN. 2000. Differential gene expression between wheat hybrids and their parental inbreds in seedling leaves of early and vigorous tillering stages, J. China Agricultural University, **5:** 1–8.

Received October 20, 2009
Accepted January 30, 2010

تباين التعبير الجيني والتأقلم الفسيولوجي في بادرات سلالتين من القمح كنتيجة للتأقلم علي الجفاف

محمد إبراهيم و إبراهيم العريض
كلية العلوم – جامعة الملك سعود – الرياض – المملكة العربية السعودية
الرمز البريدي ١١٤٩١- ص.ب. ٤٣٤١

إن التعبير الجيني يعتبر دلالة هامة في الدراسات الفسيولوجية البيئية للنباتات وتعتبر التغيرات في التعبيرات الجينية رداً سريعاً من النبات علي الإجهاد الذي يتعرض له وكوسيلة للتكيف البيئي. عُرضت سلالتين من نبات القمح أحدهما حساس للجفاف (Sids-1) والآخر مقاوم للجفاف (Veery) إلى فترتين من إجهادِ الجفافِ الأولي لمدة ٨ أيام بعدها أعيد ري النباتات لفترة يومين ثم عرضت النباتات إلي فترة جفاف ثانية امتدت إلي ١٢ يوم. كان التعبير الجيني لدي السلالة المقاومة للجفاف (Veery) له دلالات معنوية أفضل منه في حالة السلالة الحساسة (cv Sids-1) لنبات القمح خاصة بعد تعرض النباتات لفترة الجفاف الثانية بعد إعادة الري. تسبب الجفاف في حدوث تعديلاً في تعبير الجينات كإشارة ودلالة علي حدوث الإجهاد التأكسدي نتيجة للتعرض لإجهاد الجفاف. وجدت تباينات ذات دلالة معنوية بين السلالتين نتيجة للتعرض لإجهاد الجفاف في الفترتين الأولي والثانية، حيث أظهرت السلالة الحساسة للجفاف دالات الإجهاد التأكسدي بعد ٤ أيام من فترة الجفاف الأولي بينما ظهرت بعد ٨ أيام جفاف في السلالة المقاومة للجفاف.

بعد إعادة الري لمدة يومين أظهرت النتائج سلوكاً معتمداً علي نوع السلالة وكانت الاستجابة لتحمل الجفاف أفضل في كلا السلالتين منه في النباتات التي استمر تعرضها للجفاف بدون إعادة الري، كذلك كانت السلالة المقاومة للجفاف (Veery) أفضل في الاستجابة من تلك الحساسة للجفاف(Sids-1). يمكن أن نرجع تلك القدرة لدي النباتات علي المعيشة والتأقلم إلي النشاط المضاد للأكسدة لهذه النباتات التي تعرضت للإجهاد وقد استدل علي ذلك بقياس معدل الكبح للجذور الأكسوجينية النشطة وتحلل بعض مركبات الإجهاد التأكسدي بقياس النسبة IC_{50} ، وكذلك نقص كمية المالون داي ألدهيد في أوراق السلالة المقاومة للجفاف مقارنة بالسلالة الحساسة. من مجمل المعلومات التي حصلنا عليها تقترح نتائج هذه الدراسة أن نبات القمح يستطيع أن يصنع المركبات المضادة للأكسدة كاستجابة لتأثيرات الجفاف وهذه القدرة علي مواجهة إجهاد الجفاف تعتمد علي نوع السلالة وقد تؤدي إلي تغير مطابق لاستمرارية النمو للنبات.