Journal of Plant Physiology and Breeding
2019, 9(1): 111-128 ISSN: 2008-5168

Induced genes expression pattern in response to drought stress at seedling stage of wheat

Borzo Kazerani and Saeid Navabpour*

Received: February 18, 2019 Accepted: June 3, 2019
Department of Plant Breeding and Biotechnology, Faculty of Plant Production, Gorgan University of Agricultural Science and Natural Resources, Gorgan, Iran.
*Corresponding author; Email: s.navabpour@gau.ac.ir

Abstract
Molecular studies have shown that several genes with various functions are induced by drought stress and various transcription factors are involved in the regulation of the stress-inducible genes. To evaluate the effects of drought stress on gene expression, CRTBF2, DREB6 and DBF transcription factors as well as the expression pattern of *Acyl*, *SuT5* and *SuT4* genes were studied using qRT-PCR. The experiment was carried out as factorial based on completely randomized design. One factor included drought stress levels of -1, -2, -4 and -6 bar imposed by polyethylene glycol plus a control. The second factor consisted of four wheat cultivars as follows: Qaboos and Koohdasht (as drought tolerant), and Ehsan and Morvarid (as drought sensitive). Assessing the seedling morphological traits, such as germination percentage, plumule length and radicle length showed that with an increase in drought stress, the tolerant cultivars of Qaboos and Koohdasht showed lower decrease for these traits compared to the sensitive cultivars of Ehsan and Morvarid. The results of evaluating the gene expressions demonstrated that the *DREB6* gene expression was higher in the tolerant cultivars of Qaboos and Koohdasht as compared to the sensitive cultivars of Ehsan and Morvarid, indicating the possible role of the product of this gene, as a transcription factor, in inducing promoters of drought tolerance genes and transcribing such genes. However, the *CRTBF2* and *DBF* genes acted as repressor transcription factors in expressing the genes involved in the drought stress tolerance in the cultivars under study. The expressions of the *Acyl* and *SuT5* genes were greater in the tolerant cultivars compared to the sensitive cultivars. The *SuT4* gene expression was lower in both tolerant and sensitive cultivars than the expression of this gene in the corresponding control treatment. Therefore, it seems that the *Acyl* and *SuT5* genes, through lipid decomposition and carbohydrate transport pathways, respectively, increased the tolerance of Qaboos and Koohdasht cultivars in this study.

Keywords: Drought stress; Gene expression; Seedling stage; Transcription factor; Wheat.

Citation: Kazerani B and Navabpour S, 2019. Induced genes expression pattern in response to drought stress at seedling stage of wheat. Journal of Plant Physiology and Breeding 9(1): 111-128.

Introduction

In plants, full recognition of the tolerance mechanisms and genes involved in stress conditions, obtained by using several techniques, such as genetic manipulation, can improve crops' tolerance to different stresses. One of the most important methods of controlling stress in plants is the regulation at the gene transcription stage. Transcription factors regulate the expression of many genes by binding to transcription elements in the DNA promoter. Therefore, they play a key role in plants' tolerance to stress (Shoushi Dezfuli and Kalantar Ahmadi 2018). A number of transcription factors have been identified over the past few years. This indicates their important role in regulating plants' responses to stress (Singh *et al.* 2002). One of the most crucial transcription factor families is AP2 (APETALA 2) /EREBP (Ethylene-Responsive Element Binding Proteins). AP2 has a highly conserved region called the AP2 domain, which contains 60 to 68 amino acids (Weigel 1995; Okamuro *et al.* 1997). The AP2

transcription factors, including DREB (Dehydration Responsive Element-Binding factor) and CBF (Core Binding Factor) proteins, control the expression of stress-responsive genes (Shinozaki and Yamaguchi-Shinozaki 2000). For this purpose, in the upstream 5' region (the promoter) of the drought-responsive genes, there are multiple cis-acting elements (located in the GCC box), such as DRE (Dehydration Responsive Element)/CRT (C-repeat), which are involved in the signal transduction cascades under osmotic stress conditions. The DRE/CRT cis-acting elements have been identified in the promoter region of drought-tolerance genes, such as *RD29A* (Responsive to Dehydration 29A) and *kin1* (Yamaguchi-Shinozaki and Shinozaki 1994; Yamaguchi-Shinozaki and Shinozaki 2005). In sum, it can be stated that DREB is a part of the AP2/EREBP subfamily and plays a vital role in response to abiotic stress by creating special bonds with the DRE/CRT regulator element (Mizoi *et al.* 2012). This action is performed by the trans-acting activity of DREB. DREBs are central regulators for responding to environmental stresses and tolerating adverse conditions that plants are exposed to. By regulating the expression of a large number of genes induced by osmotic stresses as well as adjusting the natural pathway response to stress, DREBs contribute to biochemical and physiological adaptations, and eventually, adaptation to osmotic stresses. In general, DREBs, in comparison with other stress-induced genes, increase the levels of plants' tolerance, which can be used for genetic engineering, and also marker assisted selection in breeding programs (Lata and Prasad 2011). A number of DREB homologs have been identified and they show different types of reactions to various kinds of abiotic stresses at different levels. For example, in Arabidopsis, the overexpression of *DREB1A* increases the tolerance to cold, drought and salinity stresses (Kasuga *et al.* 1999). Furthermore, according to Liu *et al.* (1998) and Gilmour *et al.* 2000), *DREB1B* is a cold-regulated gene. A variety of *DREB2* homologs induces tolerant genes at drought (Liu *et al.* 1998; Nakashima *et al.* 2000) and high salinity stresses (Nakashima *et al.* 2000). Among other transcription factors in the AP2/EREBP family, CRTBF2 can be mentioned. The *CRTBF2* gene is expressed by the occurrence of osmotic stresses and induces tolerance to drought stress (Ramezanpour *et al.* 2010). Proteins encoded by this gene are family members of the activating transcription. By binding to CRT regions of the drought tolerance gene promoter, the resulting proteins induce the expression of genes tolerant to drought stress (Glimour *et al.* 1998). Moreover, *DBF* (Dehydration responsive element binding factor) gene was introduced by Kizis and Pages (2002) as a transcription factor which activates drought stress tolerance genes. The DBF is also a part of the AP2/EREBP family and induces the *rab17* (Responsive to abscisic acid 17) gene expression under drought stress conditions (Kizis and Pages 2002). In other words, the protein resulted from the *DBF* gene is bound to the DRE cis-acting regions in the *rab17* gene promoter and, as a transcription factor, activates the expression of the gene and increases the drought tolerance. By applying drought stress and screening a wheat cDNA library, Xu *et al.* (2008) identified three new homologs of the *DBF* gene family and called them

TaAIDFs. Among other important genes involved in the genetic pathway of inducing tolerance to drought stress, genes involved in the decomposition of lipids, such as *Acyl*, and genes involved in the transport of carbohydrates, such as *SuT4* (Sucrose Transporter 4) and *SuT5* (Sucrose Transporter 5) can be mentioned (Navabpour *et al.* 2015). Increasing the expression of the *Acyl* gene causes an increase in the biosynthesis of free fatty acids and, thus, plays a role in the repair and biosynthesis of the cell membranes (Navabpour *et al.* 2015). The acyl-acyl carrier protein thioesterase product was used to enhance drought tolerance in transgenic tobacco (Zhang *et al.* 2012). *SuT4* gene encodes one of the enzymes involved in the transport of simple carbohydrates and polysaccharides during cold stress to induce plant tolerance (Ramezanpour 2007). *SuT5* gene is also one of the carbohydrate transporting genes involved in cold stress (Ramezanpour 2007). The enzyme encoded by *SuT5* gene interferes with the transport of carbohydrates accumulated to the highest level during cold stress (Ramezanpour 2007). By studying *OsSuT5* rice genes, Ibraheem (2011) showed that the expression of *OsSuT2* gene increased gradually by increasing the levels of drought and salinity stresses; however, the *OsSuT1*, *OsSuT4* and *OsSuT5* genes were expressed at low-stress levels. On the other hand, the *OsSuT3* gene expression under the stress conditions did not show any significant changes compared to the control conditions. *PtaSuT4* exists in the tonoplast of mesophyll cells in stems and leaves (Payyavula *et al.* 2011). Not only the transport of sucrose is necessary to supply cellular energy, but also it is essential for osmoprotectant activities during drought stress (Navabpour *et al.* 2015). Regarding the sensitivity of wheat to drought stress in the seedling stage, this study was conducted to evaluate the seedling traits (germination percentage, plumule length, radicle length) and to study the pattern of expressions of *CRTBF2*, *DREB6*, *DBF*, *Acyl*, *Sut4* and *SuT5* genes in response to drought stress at the seedling stage.

Materials and Methods

The varieties used in this study were commercial cultivars of Golestan Province, proposed by Golestan Agricultural and Natural Resources Research and Education Center (GANRREC 2018) to farmers for planting. Qaboos and Koohdasht cultivars and Ehsan and Morvarid cultivars were suggested to be planted under rainfed and irrigated conditions, respectively (GANRREC 2018). The experiment was carried out as factorial at a laboratory of the Plant Breeding and Biotechnology Department, Gorgan University of Agricultural Sciences and Natural Resource, Iran, using a completely randomized design with three replications. Experimental factors were five levels of osmotic potential (0, -1, -2, -4 and -6 bar), and four wheat cultivars. Distilled water was used as the control. Different levels of drought stress were applied by polyethylene glycol (PEG 6000). The PEG concentration required for preparing the water potential was obtained by the following formula (Michel and Kaufman 1974):

$$QS=-(1.18 \times 10^{-2})\, C - (1.18 \times 10^{-4})\, C^2 + (2.67 \times 10^{-4})\, CT + (8.39 \times 10^{-7})\, C^2T$$

where, QS= osmotic potential (bar); C= PEG concentration (g/l); T= temperature (^oC). Twenty-five seeds were selected from each cultivar. After

being disinfected with 2.5% sodium hypochlorite and washed by distilled water, these seeds were placed in Petri dishes. Each Petri dish was considered as a replication. At the bottom of each Petri dish, there were 2 Whatman filter papers. 10 ml of the PEG solution with the desired potential was added to each Petri dish. After eight days, 15 seedlings were randomly chosen from each Petri dish and plumule and radicle lengths were measured using a ruler and recorded in centimeters. The radicle with 2 mm length or higher was regarded as seed germination (Soltani *et al.* 2001). Accordingly, the germination percentage was calculated by counting the germinated seedlings. To measure the expression of the genes under study (Table 1), leaf samples were taken from various treatments and were frozen in liquid nitrogen. The leaf samples were stored at -80 °C until the RNA extraction stage.

To determine the pattern of the expression of drought stress pathway genes, the entire RNA was initially extracted from the leaf sample using the p-BIOZOL buffer (Bioflux, Japan) and according to the manufacturer's instructions. To eliminate the remaining DNA in the RNA samples, the DNase I enzyme (fermentas) was used. The reverse transcriptase reaction was performed by the M-MuLV enzyme (fermentas) according to the manufacturer's instructions. RNA was extracted with high quality by electrophoresis on 1.5% agarose gel. The formation of the 28S and 18S ribosomal RNA on the gel showed the high purity of the RNA. A spectrophotometer was used to quantitatively analyze the extraction. The levels of photo-absorption were read at wavelengths of 260, 280 and 320 nm and the purity of the RNA was determined. The ratio of the photo-absorption at the wavelength of 260/280 was in the range of 1.8

Table 1. Genes, primer sequence and accession number in NCBI.

Gene	Primer sequence	PCR production length	Melting temperature (ºC)	NCBI accession number
GAPDH For	5′TCACCACCGACTACATGACC3'	3119 bp	59.11	EF592180
GAPDH Rev	5′ACAGCAACCTCCTTCTCACC3'		59.60	
CRTBF2 For	5'GACAACCGATGACGAGAAGG3'	Unknown	58.37	AY572831
CRTBF2 Rev	5'ACAGGCCCTCCGAGTAGAAC3'		60.97	
DREB6 For	5'AGGCACCAGACACAAGCAC3'	Unknown	60.53	AY781361
DREB6 Rev	5'ACATGGGCCTTTGGACCT3'		58.39	
DBF For	5'CGGAGATGCAGCTTCTTGATT3'	723 bp	58.71	DQ021908
DBF Rev	5'TCACTTTGGACGAGCTGTGG3'		60.25	
Acyl For	5'AAAACCGTCGGTGGAGAAC3'	2943 bp	58.00	Unknown
Acyl Rev	5'GAGGTCGGCATCAACAAGA3'		57.47	
SuT5 For	5'GAAGGCTGCAACAAAACCTC3'	47 bp	57.86	Unknown
SuT5 Rev	5'TTTGCCAAGGCTCTACTGTG3'		58.10	
SuT4 For	5'AGTGCCTTGACAACCTTGCT3'	3939 bp	60.11	Unknown
SuT4 Rev	5'GGTTCCGACATCCAGAACAT3'		57.60	

to 2 µg/µl, indicating the acceptable purity of the RNA. qRT-PCR was applied to multiply the transcripts of defense genes in the drought stress pathway by using specific primers (Table 1) and the quantitative method conducted by the iQ5 device (Bio-Rad). A 20 µl of the reaction solution was prepared in special tubes. This solution contained 3 µl of cDNA (dilution 1:20), 0.5 µl of each primer (10 µM), 9.9 µl of SYBR Green qRT-PCR Master Mix (2x), 0.5 µl of DMSO and 5 u/µl of *Taq* DNA enzyme polymerase. In this regard, the amount of the reaction solution was adjusted to 20 µl using twice-boiled distilled water, free of RNase, and used for the reaction. The first stage, called pre-denaturation, consisted of one cycle at 95 °C for 300 seconds. The second, third and fourth stages were 40 cycles including the denaturation stage at 95 °C for 10 seconds, annealing stage at primer annealing temperature for 20 seconds and the extension stage at 72 °C for 10 seconds. The final extension stage consisted of one cycle at 72 °C for 300 seconds. Then, melting step included 81 cycles at primer melting temperature (Table 1) for 10 seconds.

GAPDH (glyceraldehyde 3-phosphate dehydrogenase), as a reference gene, and non-stressed samples, as control samples, were used to calculate the relative expression level of drought stress pathway genes. The concentration of the primers and their binding temperature are regarded as fundamental factors in optimizing the qRT-PCR reaction. Optimum conditions were provided in a way that they allow the reaction efficiency to be at the best possible and no non-specific product was produced during the reaction. This was confirmed by using the melting curve, because a single melting peak was observed for each treatment (Figure 1).

The relative level of expression of the studied genes compared to the reference control genes (*GAPDH*) was analyzed using REST (Pfaffl *et al.* 2002) based on the following equation:

$$R=\frac{(E\ target)^{\Delta c\ P\ target\ (Mean\ control-Mean\ sample)}}{(E\ ref)^{\Delta c\ P\ ref\ (Mean\ control-Mean\ sample)}}$$

Before analysis of variance, the normality of errors and equality of error variances were verified. Means were compared by the least significant difference (LSD) method, using SAS 9.3 software. Charts were drawn by Excel 2016.

Results

Morphological traits

The results of the analysis of variance for morphological traits demonstrated that the drought stress, cultivar and their interactions were significant for germination percentage, plumule length and radicle length at 1% significance level. The significant differences among the cultivars in the measured traits indicated a significant genetic differences among the studied cultivars at the germination stage with regard to drought stress. The significant interaction of cultivars with drought stress indicated that the differences among the cultivars were not similar at different levels of drought stress (Table 2).

Germination percentage:

The Qaboos and Ehsan cultivars, with the means of 74.2% and 63.4%, had the highest and lowest germination percentage, respectively, on the average of stress levels. There were no significant differences among the Qaboos, Koohdasht and

Morvarid cultivars in terms of germination percentage. Moreover, the germination percentages of Koohdasht and Morvarid were not significantly different from Ehsan; however, Qaboos had significantly higher germination percentage than Ehsan (Table 3).

Under non-stress conditions (control), Ehsan, Morvarid, Koohdasht and Qaboos, with means of 100%, 99%, 98% and 98%, had the highest to lowest germination percentages, respectively. With the onset of stress (-1 bar), the germination percentages of Ehsan and Morvarid

Table 2. Analysis of variance for morphological traits in wheat cultivars under different drought stress levels.

Source of variation	df	Mean squares		
		Germination percentage	Plumule length	Radicle length
Stress	4	273.1**	0.912**	2.47**
Variety	3	344.7**	1.249**	3.22**
Stress × Variety	12	169.2**	0.749**	1.91**
Error	40	34.35	0.149	0.431
CV (%)		8.5	5.4	7.1

**significant at 1% level of probability.

Table 3. Values of morphological traits in wheat cultivars, averaged over drought stress levels.

Trait	Ehsan	Morvarid	Koohdasht	Qaboos	LSD (5%)
Germination percentage (%)	63.4	66.00	72.2	74.2	9.69
Plumule Length (cm)	6.5	6.64	7.6	7.9	0.639
Radicle Length (cm)	7.98	8.08	10.42	10.5	1.086

.

cultivars significantly decreased compared to the corresponding controls; however, the germination percentages of Koohdasht and Qaboos cultivars were not significantly reduced as compared to the controls. Under -2 bar, the germination percentages of Ehsan and Morvarid cultivars were significantly lower than the control at the 1% significance level, while the Koohdasht and Qaboos cultivars were significantly lower than the control at 5% significance level. At the high levels of stress (-4 and -6 bar), germination percentages of all cultivars reduced significantly compared to the respective controls. Under -6 bar, Qaboos, Koohdasht, Morvarid and Ehsan had the germination percentages of 24%, 21%, 14% and 10%, respectively (Figure 1). The drought-sensitive cultivars (Ehsan, Morvarid) showed a greater decrease in the germination percentage compared to the drought tolerant cultivars (Qaboos, Koohdash).

Plumule length: Qaboos and Ehsan cultivars, with the means of 7.9 and 6.5 cm, showed the highest and lowest plumule lengths, respectively. There was no significant difference between the plumule lengths of the Qaboos and Koohdasht cultivars. Additionally, there was no significant difference between the plumule lengths of Morvarid and Ehsan cultivars; however, Qaboos and Koohdasht were significantly different from Morvarid and Ehsan (Table 3). Under non-stress conditions (control), the Ehsan cultivar had the highest plumule length (15 cm), followed by Morvarid, Koohdasht and Qaboos cultivars (14, 13.5 and 13 cm, respectively. Under the -1 bar stress, the plumule length of Qaboos cultivar was not

significantly different from the control; however, under this condition, the plumule length of Morvarid, Koohdasht and Qaboos was significantly different from that of the corresponding control. Under -2, -4 and -6 bar, the plumule length of all cultivars were significantly different from that of the corresponding control. At -6 bar, the Qaboos, Koohdasht, Morvarid and Ehsan cultivars had the following values of plumule length (2, 1.7, 0.8 and 0.5 cm, respectively (Figure 2).

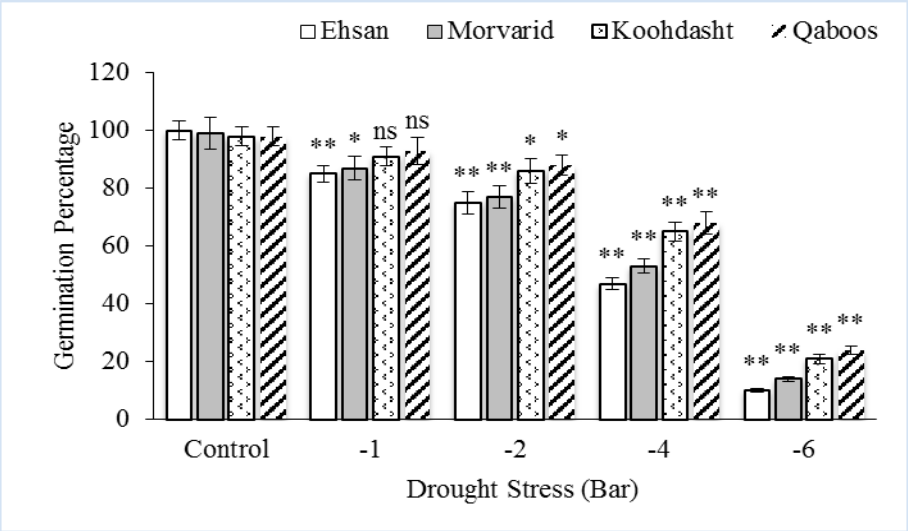

Figure 1. Germination percentage of Ehsan, Morvarid, Koohdasht and Qaboos cultivars at different drought stress levels; bars represent standard error of the means (n= 3); ns, * and ** indicate lack of significance and significant at 0.05 and 0.01 probability levels, as compared to the corresponding control.

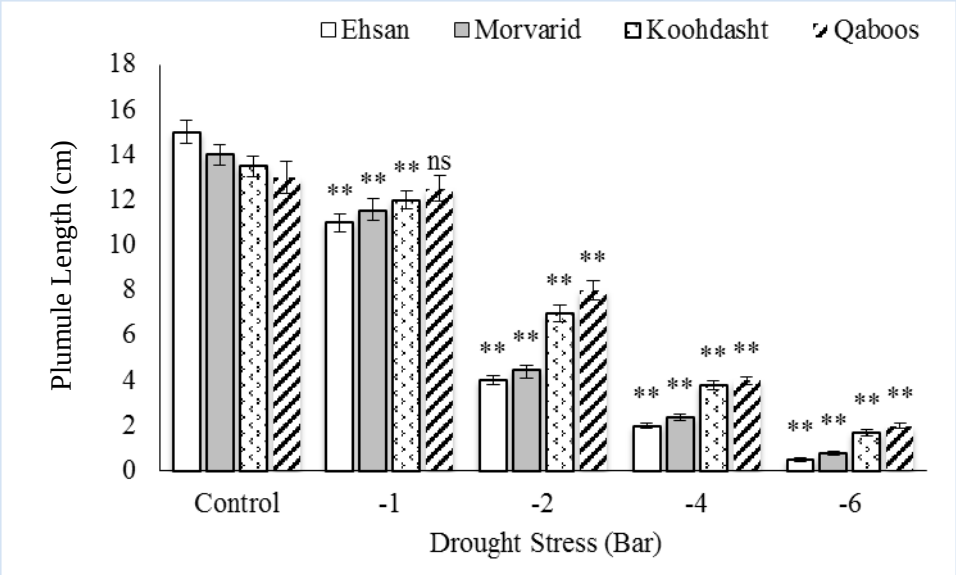

Figure 2. Plumule length of Ehsan, Morvarid, Koohdasht and Qaboos cultivars at different drought stress levels; bars represent standard error of the means (n= 3); ns, * and ** indicate lack of significance and significant at 0.05 and 0.01 probability levels, as compared to the corresponding control.

Radicle length: The Qaboos and Ehsan cultivars, with the means of 10.5 and 7.98 cm, had the highest and lowest radicle lengths, respectively. There was no significant difference between Qaboos and Koohdasht, and also between Morvarid and Ehsan, in terms of radicle length; however, Qaboos and Koohdasht had significantly greater radicle length than Morvarid and Ehsan (Table 3). Under normal conditions (control), the radicle length of Ehsan, Morvarid, Koohdasht and Qaboos cultivars were in the descending order of 17, 16, 15 and 14.5 cm, respectively. At -1 bar, radicle length of Koohdasht, Morvarid and Ehsan was lower than the corresponding controls; the radicle length of the Qaboos cultivar was not significantly different from the corresponding control. Under -2, -4 and -6 bar, the radicle length of all cultivars was significantly lower than that of the corresponding controls (Figure 3).

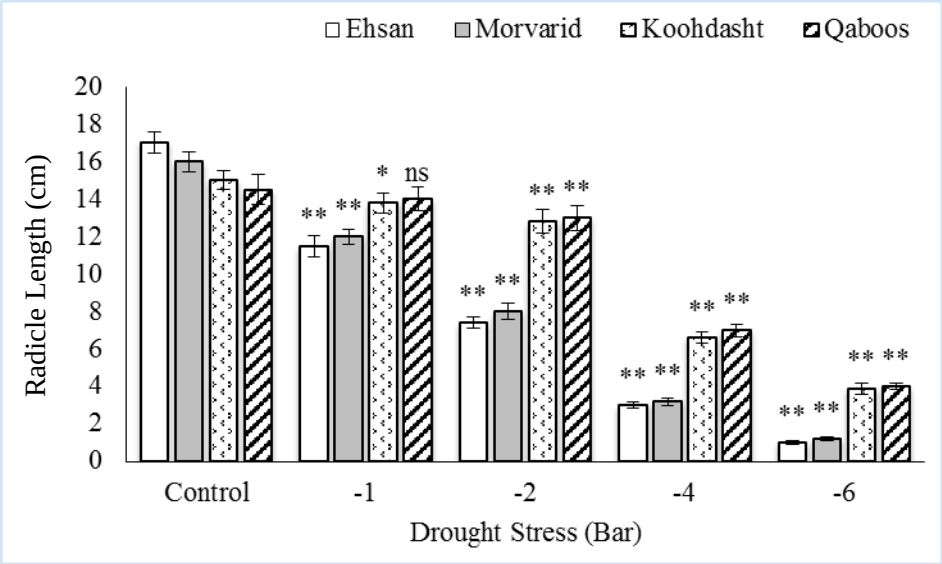

Figure 3. Radicle length of Ehsan, Morvarid, Koohdasht and Qaboos cultivars at different drought stress levels; bars represent standard error of the means (n= 3); ns, * and ** indicate lack of significance and significant at 0.05 and 0.01 probability levels, as compared to the corresponding control.

Induced genes expression pattern

The results of the analysis of variance for the gene expression data demonstrated that the drought stress, cultivar and their interaction were significant for *CRTBF2*, *DREB6*, *DBF*, *Acyl*, *SuT4* and *SuT5* genes at 1% significance level. The significant mean squares among the cultivars for the measured traits indicated a significant genetic difference among the studied cultivars at the germination stage, averaged over the drought stress levels. The significance of cultivars × drought stress interaction indicated that the differences among cultivars varies from one drought stress level to another (Table 4).

***CTBF2* gene:** The Ehsan and Qaboos cultivars, with the means of 4.38 and 1.00, had the highest and lowest relative CRTBF2 gene expression, respectively (Table 5). By applying drought stress, Ehsan and Morvarid showed ascending trends in

Table 4. Analysis of variance for the gene expression of wheat cultivars under different drought stress conditions.

Source of Variation	df	Mean squares					
		CRTBF2	DREB6	DBF	Acyl	SuT4	SuT5
Stress	4	0.0761**	0.061**	0.0163**	0.017**	0.006**	0.057**
Variety	3	0.905**	0.0812**	0.019**	0.0193**	0.009**	0.081**
Stress × Variety	12	0.065**	0.048**	0.0138**	0.014**	0.004**	0.045**
Error	40	0.014	0.0073	0.00325	0.00318	0.00077	0.00763
CV (%)		4.5	5.1	3.8	5.3	4.8	4.2

**significant at 1% probability level.

Table 5. Different gene expression values of wheat cultivars under study, averaged over drought stress levels.

Gene	Ehsan	Morvarid	Koohdasht	Qaboos	LSD (5%)
CRTBF2	4.38	4.08	1.20	1.00	0.196
DREB6	0.74	0.80	2.48	2.70	0.142
DBF	2.64	2.48	0.46	0.42	0.094
Acyl	0.808	0.972	1.160	1.318	0.093
SuT4	0.534	0.550	0.592	0.636	0.046
SuT5	1.28	1.48	2.64	2.92	0.145

the *CRTBF2* gene expression up to -2 bar and then, by increasing the severity of the drought stress, they showed descending trends for the expression of this gene. The sensitive cultivars of Ehsan and Morvarid showed significant differences with the corresponding control in the *CRTBF2* gene expression under -1, -2 and -4 bar; however, under -6 bar, they showed no significant difference with the corresponding control. From the onset of the drought stress until -2 bar, the tolerant cultivars of Qaboos and Koohdasht showed significantly lower expression than the corresponding control. Under -4 bar conditions, while the Koohdasht cultivar was significantly different from the corresponding control, Qaboos did not show any significant difference with the related control. On the other hand, Koohdasht and Qaboos showed significant increase in the gene expression compared to the related controls at the -6 bar stress (Figure 4).

***DREB6* gene:**

The Qaboos and Ehsan cultivars, had the highest and lowest relative *DREB6* gene expressions (2.7 and 0.744, respectively). Qaboos and Koohdasht showed significantly greater *DREB6* gene expression than Ehsan and Morvarid (Table 5). By applying drought stress, the *DREB6* gene expression in the tolerant cultivars of Qaboos and Koohdasht had ascending trends until the -2 bar stress level, and then decreased by increasing the severity of drought stress. The Qaboos and Koohdasht cultivars showed significantly sharper increase in the *DREB6* gene expression at all levels of drought stress compared to the corresponding controls. The expression of this gene in Ehsan and Morvarid at drought stress conditions were lower than the control and increased significantly only under the -6 bar level in relation to the Morvarid cultivar (Figure 5).

***DBF* gene:** Ehsan and Qaboos had the highest and lowest relative *DBF* gene expression (2.64 and 0.42, respectively). There was no significant difference between the Qaboos and Koohdasht cultivars in terms of the relative *DBF* gene expression (Table 5). Considering the sensitive

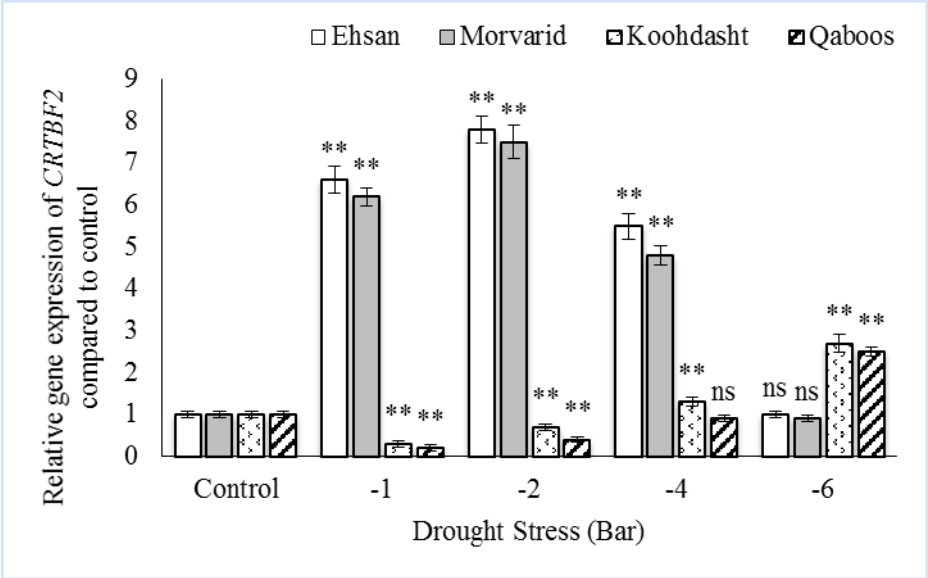

Figure 4. Quantitative expression pattern of *CRTBF2* gene for wheat cultivars under study at different drought stress conditions; bars represent standard error of the means (n= 3); ns, * and ** indicate lack of significance and significant at 0.05 and 0.01 probability levels, as compared to the corresponding control.

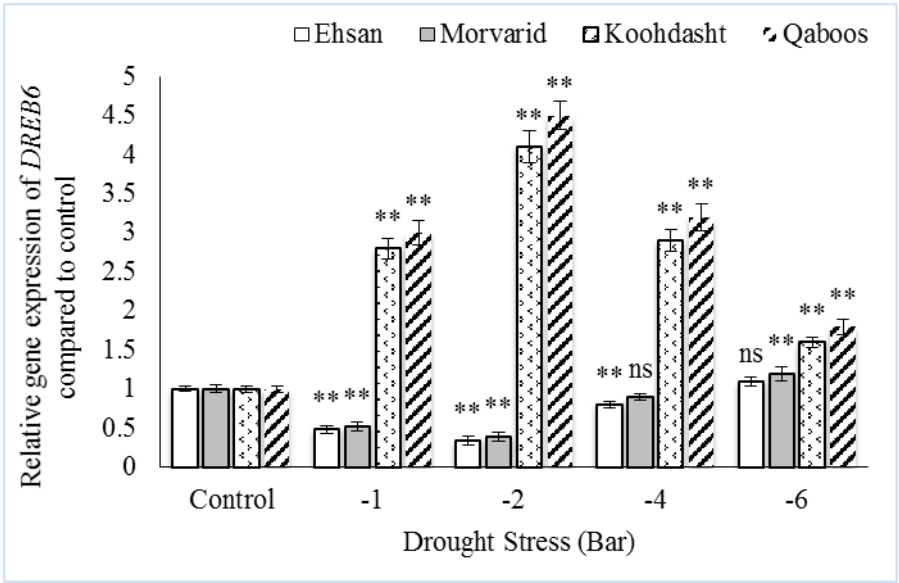

Figure 5. Quantitative expression pattern of *DREB6* gene for wheat cultivars under study at different drought stress conditions; bars represent standard error of the means (n= 3); ns, * and ** indicate lack of significance and significant at 0.05 and 0.01 probability levels, as compared to the corresponding control.

cultivars of Ehsan and Morvarid, the *DBF* gene expression showed a significant increase over the corresponding control at all stress levels; however, this increase was much higher at the -1 bar stress level, and was lower at higher levels of drought stress. The *DBF* gene expressions in the tolerant cultivars of Qaboos and Koohdasht decreased significantly compared to the corresponding controls (Figure 6).

***Acyl* gene:** The Qaboos and Ehsan cultivars, with means of 1.318 and 0.808, had the highest and lowest relative *Acyl* gene expressions, respectively (Table 5). The *Acyl* gene expression in the sensitive cultivars of Ehsan and Morvarid under -1 bar was significantly lower than the corresponding control conditions, but it increased under the -2 bar stress. However, at -4 bar the gene expression decreased again significantly compared to the corresponding control. Although both cultivars showed reduction under -6 bar, but this decrease was only significant for the Ehsan cultivar relative to the respective control. The *Acyl* gene expression showed significant increase in Qaboos and significant decrease in Koohdasht compared to the corresponding control at -1 bar stress. The values of both Qaboos and Koohdasht cultivars significantly decreased at -2 and -4 bar, but sharply increased at -6 bar in relation to the respective controls (Figure 7).

***SuT4* gene:** The Qaboos and Ehsan cultivars, with the highest and lowest means of 0.636 and 0.534, respectively, were significantly different in terms of the *SuT4* gene expression; however, there was no significant difference between Qaboos and Koohdasht cultivars. Moreover, there was no significant difference between Koohdasht and Morvarid in terms of relative expression of *SuT4* gene (Table 5). At all drought stress levels, the expression of *SuT4* gene in all cultivars was significantly lower than the corresponding control treatment (Figure 8).

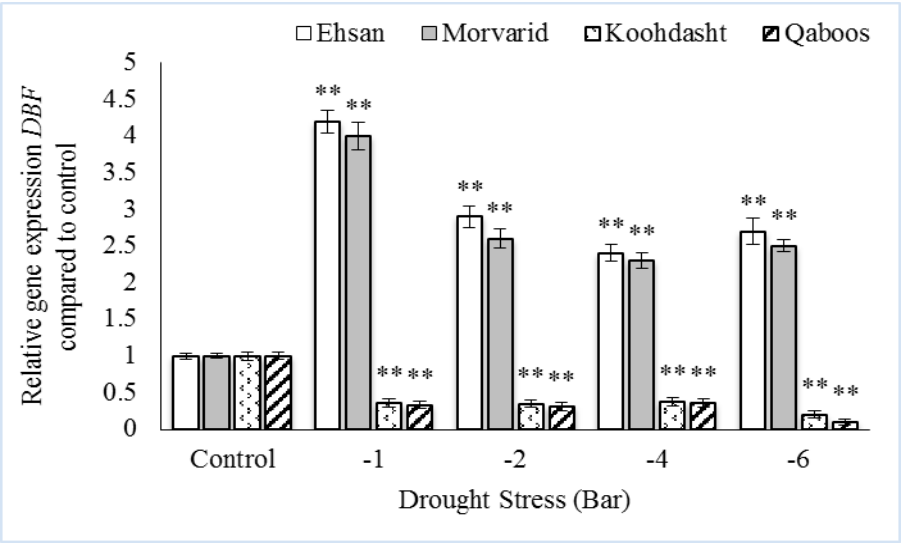

Figure 6. Quantitative expression pattern of *DBF* gene for wheat cultivars under study at different drought stress conditions; bars represent standard error of the means (n= 3); ns, * and ** indicate lack of significance and significant at 0.05 and 0.01 probability levels, as compared to the corresponding control.

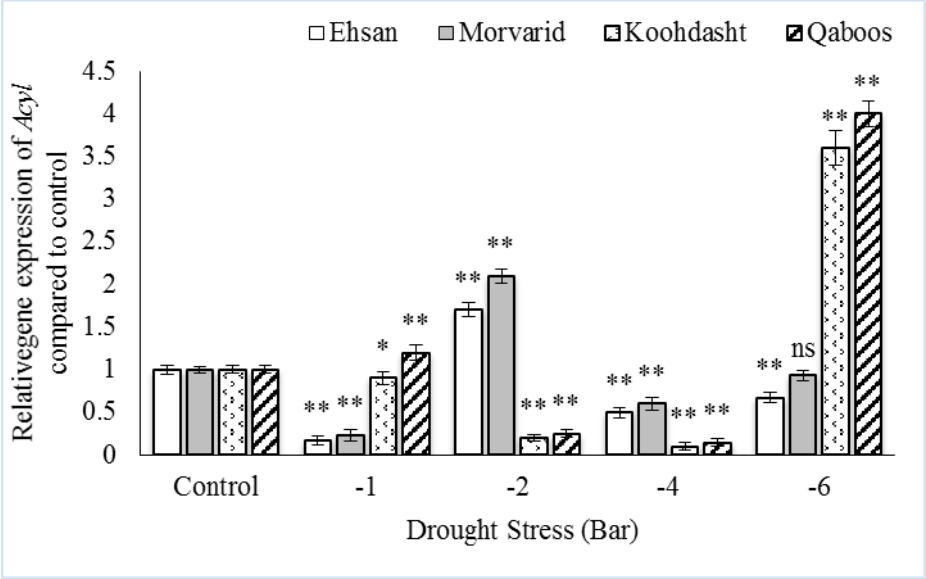

Figure 7. Quantitative expression pattern of *Acyl* gene for wheat cultivars under study at different drought stress conditions; bars represent standard error of the means (n= 3); ns, * and ** indicate lack of significance and significant at 0.05 and 0.01 probability levels, as compared to the corresponding control.

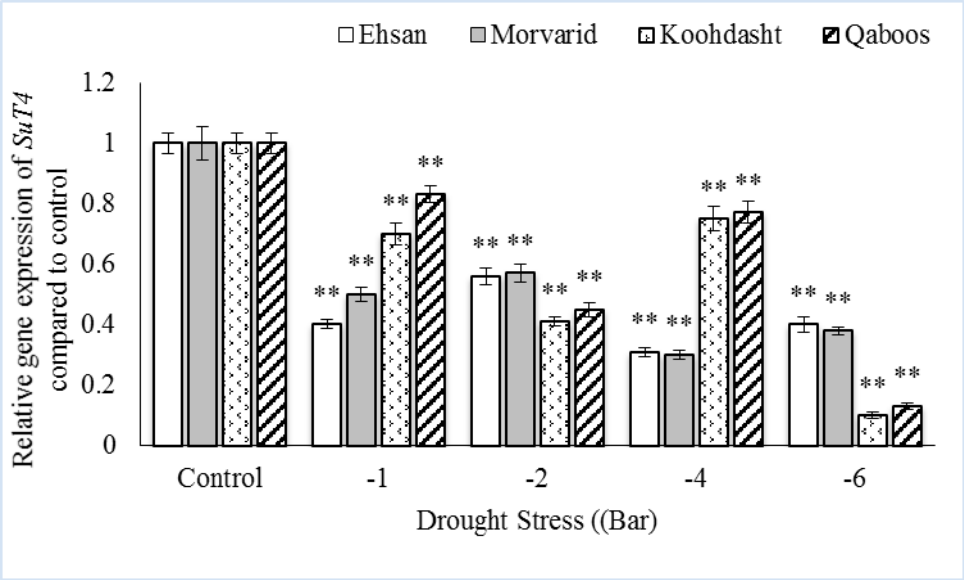

Figure 8. Quantitative expression pattern of *SuT4* gene for wheat cultivars under study at different drought stress conditions; bars represent standard error of the means (n= 3); ns, * and ** indicate lack of significance and significant at 0.05 and 0.01 probability levels, as compared to the corresponding control.

***SuT5* gene:** The relative *SuT5* gene expressions of Qaboos and Koohdasht cultivars (2.92 and 2.64, respectively) were much higher than the sensitive cultivars of Morvarid and Ehsan (1.48 and 1.28, respectively), averaged over the drought stress levels (Table 5). This difference was also reflected at separate stress levels. The *SuT5* gene expressions of Koohdasht and Qaboos were highly increased at all drought stress treatments compared to the corresponding controls. Although the

sensitive cultivars of Ehsan and Morvarid showed increase in the expression of this gene at -1, -2 and -4 bar stresses, but this increase was not comparable to the increase in gene expression of the tolerant cultivars; even, their expression decreased significantly at -6 bar as compared to the to the respective controls (Figure 9).

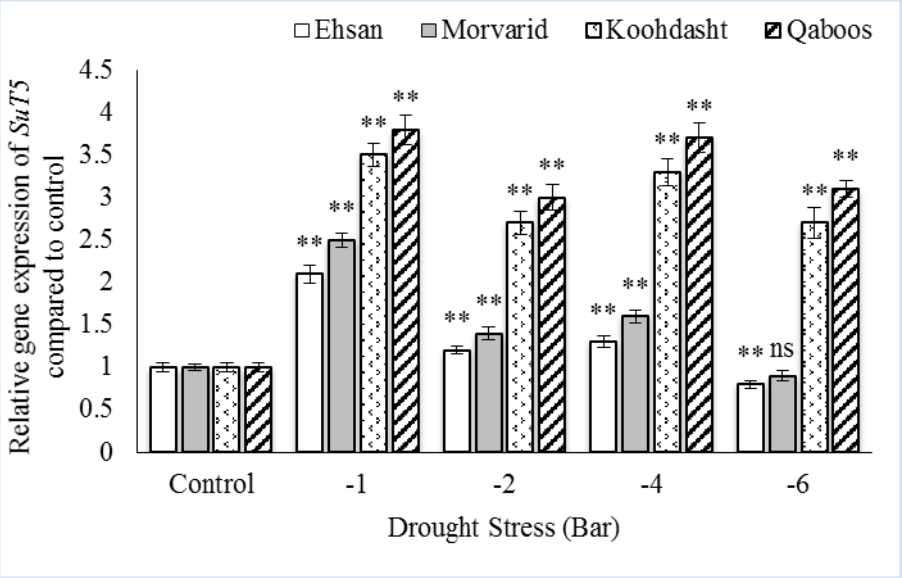

Figure 9. Quantitative expression pattern of *SuT5* gene for wheat cultivars under study at different drought stress conditions; bars represent standard error of the means (n= 3); ns, * and ** indicate lack of significance and significant at 0.05 and 0.01 probability levels, as compared to the corresponding control.

Discussion

By increasing the drought stress levels, descending trends for germination percentage, plumule length and radicle length of the cultivars under investigation were observed. Other workers have also reported the reduction in germination percentage (Prisco *et al.* 1992; Rauf *et al.* 2007), plumule length (Rauf *et al.* 2007; Vakili Bastam *et al.* 2017) and radicle length (Vakili Bastam *et al.* 2017) of wheat by increasing the drought stress level.

It was noted that the decrease in the germination percentage, plumule length and radicle length of the tolerant cultivars (Koohdasht and Qaboos) were smaller than the sensitive cultivars (Ehsan and Morvarid). Therefore, the cultivars with smaller reduction in plumule length can be considered as more tolerant to drought stress at the seedling stage. The smaller reduction of radicle length in tolerant cultivars enables them to absorb more water from the soil, which increases the metabolic activities for germination (El-Sharkawi and Springuel 1977).

The results of examining the seedling traits revealed significant adaptations with the gene expressions of the drought stress tolerance pathways. Also, the dual and different role of transcription factors in increasing or decreasing the expression of the downstream genes was demonstrated in the current study. In fact, some transcription factors had an inductive role in activating promoters with cis-acting factors, while

others repressed the activation of the promoters. In this study, the differential pattern of expressing *DREB6* gene demonstrated that the expression of this gene in the tolerant cultivars was more than the sensitive cultivars; therefore, the DREB6 transcription factor can induce the expression of at least one promoter of the drought tolerance genes and increase its activity after the stress treatment. Since the *RD29A* gene expression under drought stress encodes proteins that act as tolerance-inducing agents, the DRE agents present in the *RD29A* gene promoter contribute to the rapid expression of this gene during the drought stress. By examining the expression of the *DREB* gene family in Arabidopsis during drought stress, it was found that the proteins derived from the genes of this family activated the transcription of the reporter genes, which transport the DRE region (Liu *et al.* 1998). Given that *DREB6* gene belongs to this gene family and the proteins derived from it act as transcription factors activating the expression of the genes, which have the DRE region, it can be concluded that by increasing drought stress, the expression level of this gene and the induction of the expression of the drought-tolerant genes in the Qaboos and Koohdasht cultivars has increased and contributed to the tolerance of the Qaboos and Koohdasht cultivars. On the other hand, the gene expression pattern in the sensitive cultivars of Ehsan and Morvarid showed that the expression of *DREB6* gene in these cultivars was lower than the corresponding control at -4 bar drought stress, which can be one of the reasons for the sensitivity of these cultivars. However, the differential pattern of the expression of *CRTBF2* and *DBF* genes were different from *DREB6* gene; the expression of *CRTBF2* and *DBF* genes decreased in the tolerant cultivars and increased in the sensitive cultivars. This indicates the repressive role of *CRTBF2* and *DBF* genes in inducing their downstream genes, which is due to the fact that some transcription factors can have a repressive role in the gene expression (Delessert *et al.* 2005). On the other hand, the overexpression of these genes in the sensitive cultivars compared to the tolerant cultivars could be due to the interference of the products of this gene as transcription factors in expressing other genes regulating cell activities during the stress. This requires carrying out further studies to investigate the nature of this gene and its upstream 5' region. In general, it can be argued that the transcription factors can act as stimulants or repressors of their downstream genes. Since the usual mechanism for controlling the gene expression and regulating transcription is carried out by phosphorylation and dephosphorylation of specific factors, it is likely that transcription factors may be different with respect to their amino acid composition. The transcription factors that have a higher percentage serum composition, have higher performance because these sequences act as expected sites for phosphorylation. Therefore, the induction of a promoter of a gene is dependent on multiple cis-acting factors and collaborations among them (Kizis and Pages 2002). Our findings about the expression of *CRTBF2*, *DREB6* and *DBF* genes are consistent with the results of a study conducted by Ramezanpour *et al.* (2010).

In this study, some drought-tolerance pathway genes such as *Acyl* had a different pattern than the transcription factors studied. Among the most

important events that happen during drought stress, is the increase in the destruction rate of the lipids and the reduction in their production. One of the causes of this reduction is the decrease in water absorption by roots, which limits the amount of phosphate (De Paula *et al.* 1993). Therefore, enhancing the *Acyl* gene expression under the most severe drought treatment (-6 bar) in the tolerant cultivars of Qaboos and Koohdasht, may have led to an increase in the biosynthesis of free fatty acids, which is used in the repair and biosynthesis of the cell membranes.

Another plant action in the face of drought stress is the accumulation of carbohydrates. In the present study, the expression of *SuT4* gene didn't increase in both tolerant and sensitive cultivars, but decreased under drought stress. Nonetheless, Navabpour *et al.* (2015) reported an increased expression of this gene in the stem elongation and anthesis phases of tolerant wheat cultivars. Therefore, this gene is probably expressed at the maturity stage. However, the *SuT5* gene expression was higher in both of the sensitive and tolerant cultivars compared to the corresponding controls, but the increase was much higher in the Qaboos and Koohdasht cultivars. According to Danyluk and Sarhan (1990), mRNAs that are expressed under freezing stress conditions, can be generally divided into two groups. The first group includes mRNAs that have a differential expression between the tolerant and sensitive cultivars. The second group includes mRNAs that are expressed in both cultivars and their resulting polypeptides play a role in cellular regulation during the stress process, and are not related to the increased stress tolerance. This type of division in gene function is likely to be similar in other abiotic stresses, such as drought stress. Accordingly, the genes *DREB6* and *SuT5* can be considered in the first group, which play an important role in governing tolerance to drought stress.

In conclusion, the Koohdasht and Qaboos tolerant cultivars, compared to the sensitive cultivars of Ehsan and Morvarid, had better characteristics in terms of seedling traits and genetic potential in expressing drought tolerant induction genes under drought stress conditions. The transcription factors and genes related to drought tolerance, studied in this experiment, may be useful for selecting drought tolerant varieties at the seedling stage of wheat.

References

Danyluk G and Sarhan F, 1990. Differential mRNA transcription during the induction of freezing tolerance in spring and winter wheat. Plant and Cell Physiology 31(5): 609-619.

Delessert C, Kazan K, Wilson LW, Van Der Straeten D, Manners J, Dennis ES and Dolferus R, 2005. The transcription factor ATAF2 represses the expression of pathogenesis-related genes in Arabidopsis. The Plant Journal 43: 745-757.

De Paula FM, Pham Thi AT, Zuily-Fodil Y, Ferrari-Iliou R, da Silva V Jr and Mazliak P, 1993. Effects of water stress on the biosynthesis and degradation of polyunsaturated lipid molecular species in leaves of *Vigna unguiculata*. Plant Physiology and Biochemistry 31: 707-715.

El-Sharkawi HM and Springuel I, 1977. Germination of some crop plant seeds under reduced water potential. Seed Science and Technology 5: 677-688.

Gilmour SJ, Sebolt AM, Salazar MP, Everard JD and Thomashow MF, 2000. Overexpression of the *Arabidopsis* CBF3 transcriptional activator mimics multiple biochemical changes associated with cold acclimation. Plant Physiology 124(4):1854-1865.

GANRREC, 2018. A report of Golestan Agricultural and Natural Resources Research and Education Center. Retrieved December 17, 2018, from http://www.ganrrc.org.ir (In Persian).

Ibraheem O, 2011. Differential expression and regulation of sucrose transporters in rice (*Oryza sativa* L.) during environmental stress conditions. Ph.D. Thesis. Department of Biochemistry and Microbiology. University of Fort Hare. South Africa. 125p.

Kasuga M, Liu Q, Miura S, Yamaguchi-Shinozaki K and Shinozaki K, 1999. Improving plant drought, salt, and freezing tolerance by gene transfer of a single stress-inducible transcription factor. Nature Biotechnology 17(3): 287-291.

Kizis D and Pages M, 2002. Maize DRE-binding proteins DBF1 and DBF2 are involved in rab17 regulation through drought-responsive element in an ABA-dependent pathway. The Plant Journal 30(6): 679-689.

Lata C and Prasad M, 2011. Role of DREBs in regulation of abiotic stress responses in plants. Journal of Experimental Botany 62(14): 4731-4748.

Liu Q, Kasuga M, Sakuma Y, Abe H, Miura S, Yamaguchi-Shinozaki K and Shinozaki K, 1998. Two transcription factors, DREB1 and DREB2, with an EREBP/AP2 DNA binding domain separate two cellular signal transduction pathways in drought- and low-temperature-responsive gene expression, respectively, in Arabidopsis. The Plant Cell 10: 1391-1406.

Michel BE and Kaufmann MR, 1974. The osmotic potential of polyethylen glycol 6000. Plant Physiology 51: 914-916.

Mizoi J, Shinozaki K and Yamaguchi-Shinozaki K, 2012. AP2/ERF family transcription factors in plant abiotic stress responses. Biochimica et Biophysica Acta 1819(2): 86-96.

Nakashima K, Shinwari ZK, Sakuma Y, Seki M, Miura S, Shinozaki K and Yamaguchi-Shinozaki K, 2000. Organization and expression of two Arabidopsis DREB2 genes encoding DRE-binding proteins involved in dehydration- and high-salinity-responsive gene expression. Plant Molecular Biology 42(4): 657-665.

Navabpour S, Ramazanpour SS, Soltanloo H and Vakili Bastam S, 2015. Drought stress changed expression profile of some genes in tillering and pollination stages of adult wheat. Applied Science Reports 9(2): 100-109.

Okamuro JK, Caster B, Villarroel R, Van Montagu M and Jofuku DK, 1997. The AP2 domain of APETALA2 defines a large new family of DNA binding proteins in Arabidopsis. PNAS USA 94(13): 7076-7081.

Payyavula RS, Tay KHC, Tsai CJ and Harding SA, 2011. The sucrose transporter family in *Populus*: the importance of a tonoplast PtaSUT4 to biomass and carbon partitioning. The Plant Journal 65(5): 757-770.

Pfaffl MW, Horgan GW and Dempfle L, 2002. Relative expression software tool (REST©) for group-wise comparison and statistical analysis of relative expression results in real-time PCR. Nucleic Acids Research 30(9): e36. doi: 10.1093/nar/30.9.e36.

Prisco JT, Haddad CRB and Bastos JLP, 1992. Hydration-dehydration seed pre-treatment and its effects on seed germination under water stress conditions. Revista Brasileira de Botanica 15(1): 31-35.

Ramezanpour SS, 2007. Quantitative expression profile of wheat genes under cold stress. PhD Thesis. Faculty of Agricultural Sciences and Engineering. Department of Agronomy and Plant Breeding, Tehran University, Iran (In Persian with English abstract).

Ramezanpour SS, Charkazi F and Soltanloo H, 2010. Expression profile of some dehydration responsive element-binding factor under drought stress induced by PEG in wheat seedlings. American-Eurasian Journal of Agricultural and Environmental Sciences 7(5): 600-606.

Rauf M, Munir M, Ul-Hassan M, Ahmad M and Afzal M, 2007. Performance of wheat genotypes under osmotic stress at germination and early seedling growth stage. African Journal of Biotechnology 6(8): 971-975.

Shinozaki K and Yamaguchi-Shinozaki K, 2000. Molecular responses to dehydration and low temperature: differences and cross-talk between two stress signalling pathways. Current Opinion in Plant Biology 3(3): 217-223.

Shoushi Dezfuli AA and Kalantar Ahmadi SA, 2018. Gene expression patterns of some transcription factors (MYB and WRKY) under salt stress in the seedling stage of alfalfa using qPCR. Journal of Crop Biotechnology 8(23): 27-41 (In Persian with English abstract).

Singh KB, Foley RC and Onate-Sanchez L, 2002. Transcription factors in plant defense and stress responses. Current Opinion in Plant Biology 5(5): 430-436.

Soltani A, Zeinali E, Galeshi S and Latifi N, 2001. Genetic variation for and interrelationship among seed vigor traits in wheat from the Caspian Sea coast of Iran. Seed Science and Technology 29(3): 653-662.

Vakili Bastam Sh, Ramezanpour SS, Soltanloo H, Zeinalinezhad Kh and Borzouei A, 2017. Study of drought tolerance in a wheat mutant line at the germination and adult stages. Environmental Stresses in Crop Sciences 10(1): 45-53 (In Persian with English abstract).

Weigel D, 1995. The APETALA2 domain is related to a novel type of DNA binding domain. The Plant Cell 7: 388-389.

Xu ZS, Ni ZY, Liu L, Nie LN, Li LC, Chen M and Ma YZ, 2008. Characterization of the *TaAIDFa* gene encoding a CRT/DRE-binding factor responsive to drought, high-salt, and cold stress in wheat. Molecular Genetics and Genomics 280(6): 497-508.

Yamaguchi-Shinozaki K and Shinozaki K, 1994. A novel cis-acting element in an Arabidopsis gene is involved in responsiveness to drought, low-temperature, or high-salt stress. The Plant Cell 6: 251-264.

Yamaguchi-Shinozaki K and Shinozaki K, 2005. Organization of cis-acting regulatory elements in osmotic- and cold-stress-responsive promoters. Trends in Plant Science 10(2): 88-94.

Zhang LH, Jia B, Zhuo RY, Liu JL, Pan HY, Baldwin TC and Zhang SH, 2012. An acyl-acyl carrier protein thioesterase gene isolated from wintersweet (*Chimonanthus praecox*), *CpFATB*, enhances drought tolerance in transgenic Tobacco (*Nicotiana tobaccum*). Plant Molecular Biology Reporter 30(2): 433-442.

الگوی بیان ژن‌های القایی در پاسخ به تنش خشکی در مرحله گیاهچه گندم

برزو کازرانی[1] و سعید نواب‌پور[2*]

۱- دانش آموخته دکتری، گروه اصلاح‌نباتات و بیوتکنولوژی، دانشکده تولید گیاهی، دانشگاه علوم کشاورزی و منابع طبیعی گرگان، گرگان.
۲. دانشیار، گروه اصلاح‌نباتات و بیوتکنولوژی، دانشکده تولید گیاهی، دانشگاه علوم کشاورزی و منابع طبیعی گرگان، گرگان.
*مسئول مکاتبه؛ Email: s.navabpour@gau.ac.ir

چکیده

مطالعات مولکولی نشان داده‌اند که تعدادی ژن با عملکرد گوناگون به وسیله تنش خشکی القا شده و عوامل رونویسی مختلف در تنظیم ژن‌های القا شده از تنش نقش دارند. به منظور ارزیابی اثر تنش خشکی بر بیان ژن، عوامل رونویسی CRTBF2، DREB6 و DBF و الگوی بیان ژن‌های Acyl، SuT5 و SuT4 با استفاده از qRT-PCR مطالعه شد. آزمایش به صورت فاکتوریل در قالب طرح کاملا تصادفی اجرا شد. فاکتور تنش شامل شاهد و ۱-، ۲-، ۴- و ۶- بار و فاکتور رقم شامل ارقام گندم قابوس و کوهدشت (متحمل به خشکی)، و احسان و مروارید (حساس به خشکی) بود. ارزیابی صفات مورفولوژیکی گیاهچه، از جمله درصد جوانه‌زنی، طول ساقه‌چه و طول ریشه‌چه در گیاهان مورد مطالعه نشان داد که با افزایش تنش خشکی، ارقام متحمل قابوس و کوهدشت از نظر این صفات نسبت به ارقام حساس احسان و مروارید کاهش کمتری داشتند. نتایج ارزیابی بیان ژن نشان داد که بیان ژن DREB6 در ارقام متحمل قابوس و کوهدشت در مقایسه با ارقام حساس احسان و مروارید بیشتر بوده و نشان‌دهنده نقش محصول این ژن به عنوان عامل رونویسی در القای راه‌انداز ژن‌های تحمل به خشکی و رونویسی از ژن‌ها بود. از طرف دیگر بیان ژن‌های CRTBF2 و DBF حاکی از آن بود که این ژن‌ها در بیان ژن‌های دخیل در تحمل به تنش خشکی در ارقام مورد بررسی، ظاهرا به عنوان عامل رونویسی بازدارنده عمل می‌کنند. بیان ژن‌های Acyl و SuT5 در ارقام متحمل نسبت به ارقام حساس بیشتر بود. با این حال، بیان ژن SuT4 در ارقام متحمل و حساس کمتر از بیان این ژن در تیمار شاهد مربوطه بود. بنابراین، ژن‌های Acyl و SuT5 به‌ترتیب از طریق مسیرهای تجزیه چربی و انتقال کربوهیدرات، تحمل ارقام قابوس و کوهدشت را افزایش دادند.

واژه‌های کلیدی: بیان ژن؛ تنش خشکی؛ عامل رونویسی؛ گندم؛ مرحله گیاهچه.