Gene Expression Analysis of TaDREB1 in Egyptian Wheat (*Triticum aestivum*) Cultivars under Drought Stress

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ABSTRACT

Plants encounter various stressors, including salinity, drought, and pathogens, which can hinder growth and cause mortality, ultimately reducing crop yields globally. Understanding plant resistance mechanisms is crucial for enhancing resilience, promoting growth, and improving crop yield and quality, especially in the face of widespread phenomena like drought. DREB proteins (Dehydration-Responsive Element Binding), crucial transcription factors in plants, regulate genes essential for drought, high salinity, and low-temperature stress responses via binding to DRE/CRT elements. These factors have been employed to enhance wheat's drought tolerance, thus resulting in improved growth, yield, and physiological traits. In this context, the current study aimed to investigate how PEG-induced drought affects the transcript expression levels of TaDREB1-A, TaDREB1-B, and TaDREB1-D genes in seven Egyptian wheat cultivars, which exhibit varying degrees of drought resilience. Our findings reveal that the expression of the TDREB1-A gene was upregulated in three of the seven wheat cultivars (Sakha94, Sakha95, and Shandweel-1), demonstrating distinct levels of drought tolerance, while downregulated in the remaining four genotypes. Notably, TaDREB1-B and TaDREB1-D showed upregulation in one genotype (Shandweel-1) but downregulation in the other six genotypes. Thus, it deserves to be mentioned that Shandweel-1 is the promising cultivar to be introduced in breeding programs for drought tolerance improvement, and the highest correlation between the relative expression of DREB1 genes and relative water content (RWC) was recorded for TaDREB1-A. These results underscore the complex regulation of DREB1 gene expression within hexaploid wheat cultivars under drought stress, highlighting the need for further comprehensive investigations into the underlying regulatory mechanisms.

Keywords: Drought, DREB1 transcription factor, Gene expression, RWC, Wheat.

INTRODUCTION

Plants are subject to a multitude of stressors, encompassing abiotic factors such as salinity and drought, along with biotic elements like pathogens, leading to growth inhibition and mortality. Given the complexity and variability of field conditions, concurrent stresses often reduce crop yields worldwide (Vinocur & Altman, 2005 and Liu *et al.*, 2018). Therefore, understanding plant resistance mechanisms is critical for enhancing resilience, promoting growth and reproduction, and ultimately improving crop yield and quality. Drought is a widespread phenomenon globally, significantly contributing to plant loss, even in irrigated regions where water availability for irrigation is constrained (Sharma *et al.*, 2022). Investigating wheat's resilience to drought stress becomes crucial in this context. Plants respond to drought stress through intricate molecular mechanisms, activating metabolic pathways and synthesizing diverse stress-responsive proteins (Elseehy & El-Shehawi, 2018 and Xu *et al.*, 2019).

Several multigene families, notably bZIP (basic region lucin zipper), AP2 (APETALA2)/ERF (Ethylene Responsive Factor), MYB (Myeloblastosis)/MYC (Myelocytomatosis), NAC (**N**AM, **A**TAF1-2, and **C**UC2), and WRKY, have been implicated in conferring drought tolerance (Golldack *et al.*, 2011 and Xu *et al.*, 2011). Of particular significance is the plant-specific ethylene response element binding factor (AP2/ERF) family, which is crucial for both developmental processes and stress response pathways (Lata and Prasad, 2011). Structurally, members of this family typically feature an AP2 binding domain, characterized by a unique three-dimensional arrangement comprising approximately 60 amino acid residues arranged in three β-sheets and one α-helix (Allen *et al.*, 1998). The AP2/ERF is divided into five subfamilies based on

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domain number and characteristics: RAV, ERF, AP2, DREB, and Soloist (Dietz *et al.,* 2010).

DREB (Dehydration-Responsive Element Binding) proteins constitute a significant subfamily of transcription factors that regulate genes by binding to the DRE/CRT cis-acting elements, enabling plants to respond effectively to drought, high salinity, and low-temperature stresses (Liu *et al.,* 1998). These proteins are characterized by three conserved domains: the EREBP/AP2 DNA binding domain, an N-terminal nuclear localization signal, and a Ser/Thr-rich region located adjacent to the EREBP (Ethylene Responsive Element Binding Protein)/AP2 domain (Sakuma *et al.,* 2002).

DREB1 transcription factors, successfully employed to enhance drought tolerance in transgenic wheat, represent a proven strategy for improving wheat's resilience to drought stress. The increased drought tolerance observed in AtDREB1A transgenic wheat plants, attributed to elevated relative water content (RWC), chlorophyll, sugar, and proline levels compared to non-transgenic plants, was complemented by their stable growth and yield performance across various environments (Noor *et al.,* 2018). Under water-limited field conditions, the GmDREB1 overexpressing wheat lines demonstrated superior growth and higher grain yields than non-transgenic plants, with noted enhanced photosynthesis in transgenic plants possibly due to prolonged leaf functionality, indicating a potential role for altered melatonin metabolism in the observed drought-related effects (Zhou *et al.,* 2020).

The TaDREB1 gene, which contains a single conserved EREBP/AP2 domain, exhibits similarity to DREB family members in Arabidopsis in terms of both amino acid sequences and the structural arrangement of DNA-binding motifs (Shen *et al.,* 2003). It was isolated from a drought-induced cDNA library of wheat and found to be present on chromosomes 3A, 3B, and 3D (Wei *et al.,* 2009). This gene has been observed to be induced by various stressors, including low temperature, abscisic acid (ABA), salinity, and drought (Kurahashi *et al.,* 2009 and Liu *et al.,* 2018).

In this regard, the present research aimed to investigate the impact of PEG-induced drought on the transcript expression levels of TaDREB1-A, TaDREB1-B, and TaDREB1-D genes in seven Egyptian wheat cultivars exhibiting varying levels of drought tolerance.

MATERIAL AND METHODS

Plant materials and Growth conditions

Seeds of seven Egyptian wheat (*Triticum aestivum*) cultivars (Shandaweel-1, Sakha-94, Sakha-95, Giza-171, Misr-3, Gemeiza-11, and Gemeiza-12) were treated with 10% sodium hypochlorite for 30 minutes and then rinsed with distilled water. Ten seeds of each cultivar were planted in plastic pots (25 cm in diameter and 20 cm in depth) containing sandy soil. Wheat seedlings were irrigated every 4 days to field capacity (0.5L/pot) until the application of drought stress treatment (Abd El-Moneim *et al.,* 2020).

Application of drought stress

Thirty-day-old seedlings were irrigated with 20% PEG-6000 (drought-stressed plants) and left without irrigation for 7 days, while control plants were irrigated regularly by water only, this treatment was conducted according to Abd El-Moneim *et al.* (2020) with some modifications. Morpho–physiological attributes of control and drought-stressed plants such as shoot length, root length, shoot fresh weight, root fresh weight, shoot dry weight, root dry weight, and Leaf relative water content (RWC) were assessed. Leaf relative water content (RWC) of control and drought-stressed plants was calculated using the equation developed by González and González-Vilar (2001):

$$RWC\ (\%) = (FW - DW)/ (TW - DW) \times 100$$

The leaves of control and drought-stressed plants were excised and immediately stored at −80 ◦C for further analysis.

RNA extraction and gene expression analyses

Total RNA was extracted from leaves by using a total RNA mini kit (Geneaid, UK) according to the manufacturer's instructions. First-strand cDNA was synthesized with TOPscript™ cDNA Synthesis Kit (Enzynomics, Republic of Korea) using an oligo (dT18).

Quantitative Polymerase Chain Reaction (qPCR) was conducted for every target gene, with three replicates for each treatment (control and drought-stressed plants) across all seven cultivars. Expressions of the three DREB1 genes were quantified using SYBR Green PCR Master Mix (TOP realTM qPCR 2X PreMIX, Enzynomics, Republic of Korea) according to the manufacturer's instructions and the 18S rRNA gene served as the internal reference gene. QRT-PCR reaction mixture consists of 10 µL SYBR Green mix, 1 µl of cDNA, 1µl (10 pmol/ul) of each forward and reverse primer, and the total volume adjusted up to 20 ul by nuclease-free water. Three primer pairs, previously described by Menglin *et al.* (2019), were used for the amplification of TaDREB1-A, TaDREB1-B, and TaDREB1-D genes (Table1). Thermo-cycling conditions were initial denaturation at 95°C for 12 min, followed by 45 cycles at 95ºC for 15 s, annealing temperature (Table 1) for 15 s, and elongation at 72ºC for 20 s. The relative gene expression was analyzed using the comparative CT method (Livak and Schmittgen, 2001).

Table 1. Primer sequences, and annealing temperatures for each primer pair which are used in real-time PCR

Gene name	Forward Primer sequence (5′–3′)	Reverse Primer sequence (5′–3′)	Annealing temperature
TaDREB1-A	GCACCTCCATTGCTGAC	AATCATTGCTCACTTCTTTC	64°C
TaDREB1-B	GATGTCTAATGGGGCAACT	TAATCATAACTTACATTCGCTT	57°C
TaDREB1-D	TCCTTCTCTTATCTCAAATGC	ATCATAACTTACGTTCGCTG	53 °C
18S rRNA	CTTCCGTCAATTCCTTTAAG	GCAAGTCTGGTGCCAGCAGCC	58 °C

Statistical analysis:

The drought tolerance evaluation experiment was conducted in a random complete block design, and a two-way analysis of variance (ANOVA) was used to test the differences between the means of different variables. The differences among treatment means were compared by using Fisher's least significant difference (LSD) test ($p \leq 0.05$) (Sokal and Rohlf, 1981), with distinct letters indicating significant variations between treatments. The correlation between RWC and the relative expression of the studied genes was calculated using the Pearson correlation coefficient.

RESULTS AND DISCUSSION

The comparison of morpho-physiological traits among wheat cultivars under drought stress was assessed. Drought, recognized as a substantial abiotic stressor, profoundly obstructs plant growth and developmental processes, resulting in a subsequent decline in crop yield. Different plant species exhibit a spectrum of physiological and morphological adaptations aimed at mitigating the deleterious impacts of drought stress (Chowdhury *et al.,* 2021 and Sharma *et al.,* 2022).

The analysis of variance presented in table (2) showed that differences among the PEG treatments were highly significant for all morpho-physiological traits except for root fresh weight, which was not significant (Table 2). The seven Egyptian wheat cultivars indicated highly significant differences for all morpho-physiological traits except for shoot length and shoot dry weight. The interaction between the PEG treatments and Egyptian wheat cultivars was not significant for most morpho-physiological traits, but it was significant for germination percentage (%), root length, and root fresh weight traits (Table 2).

Table (3) indicates that the PEG treatment (20%) gave the lowest significant values for all morpho-physiological traits except for root fresh weight across the seven Egyptian wheat cultivars.

Table 2. Analysis of variance for morpho-physiological traits as influenced by the PEG, wheat cultivars, and their interaction

S.O.V.	D.F.	Mean Squares								
		Germination Percentage (%)	Shoot length. (cm)	Root length (cm)	Shoot fresh weight (g)	Root fresh weight (g)	Turgid weight (g)	Shoot dry weight (g)	Root dry weight (g)	RWC
PEG levels (A)	1	100.60^{**}	121.72^{**}	517.30^{**}	36.66^{**}	$5.52^{n.s}$	34.11^{**}	1.40^{**}	0.67^{*}	1473.49^{**}
Cultivars (B)	6	4.39^{**}	$7.43^{n.s}$	16.46^{**}	2.37^{*}	91.07^{**}	7.02^{*}	$0.65^{n.s}$	1.8^{**}	87.21^{*}
AXB	6	4.82^{**}	$7.87^{n.s}$	30.86^{**}	$1.41^{n.s}$	10.32^{**}	$4.22^{n.s}$	$0.10^{n.s}$	$0.18^{n.s}$	$27.72^{n.s}$
Error	28	0.5	4.85	3.70	1.49	1.81	3.60	0.67	0.10	26.16

n.s,*, **: not significant, significant at $p \leq 0.05$ and 0.01, respectively.

Table 3. Means of morpho-physiological traits as influenced by the PEG level overall wheat cultivars

PEG. levels	Germination Percentage (%)	Shoot length (cm)	Rootlength (cm)	Shoot fresh weight (g)	Root fresh weight (g)	Turgid weight (g)	Shoot dry weight (g)	Root dry weight (g)	RWC
Control	9.71^{a}	19.90^{a}	13.5^{a}	5.11^{a}	3.08^{a}	8.12^{a}	0.94^{a}	0.60^{a}	57.57^{a}
20%	6.62^{b}	16.50^{b}	6.5^{b}	3.24^{b}	2.35^{a}	6.32^{b}	0.58^{b}	0.35^{b}	45.72^{b}
L.S.D. 0.05	0.45	1.39	1.3	0.77	n.s	1.19	0.16	0.20	3.23

Means with the same letter are not significantly different.
n.s: not significant

There are significant differences between Shandawel-1 and the remaining six cultivars (Gemmiza-11, Gemmiza-12, Giza-171, Misr-3, Sakha-94, and Sakha-95) in root length and root fresh weight under drought stress. However, there are insignificant differences among all seven cultivars in shoot dry weight under drought stress (Table 4). Additionally, there are insignificant differences among Gemmiza-11, Giza-171, Misr-3, Sakha-94, and Sakha-95 in shoot length, root length, shoot fresh weight, root fresh weight, shoot dry weight, and relative water content (RWC) under drought stress. Similarly, there are insignificant differences between Shandawel-1 and Giza-171, Misr-3, and Sakha-95 in shoot length, shoot fresh weight, shoot dry weight, and RWC under drought stress (Table 4). However, significant differences exist between Shandawel-1 and Gemmiza-12 in shoot length, root length, shoot fresh weight, root fresh weight, root dry weight, and RWC under drought stress (Table 4).

Under drought stress conditions, Shandawel-1, Giza-171, Misr-3, and Sakha-95 exhibited comparable responses in four out of nine morpho-physiological traits, whereas Gemmiza-11, Giza-171, Misr-3, Sakha-94, and Sakha-95 demonstrated similar responses in six out of nine morpho-physiological traits (Table 4). These findings underscore the extent of similarity among these cultivars regarding their adaptation and resistance to drought.

The interaction between the PEG treatments and Egyptian wheat cultivars was significant for three morpho-physiological traits: germination percentage, root length, and root fresh weight traits (Table 2). The Shandawel-l wheat cultivar gave the highest values for three morpho-physiological traits: germination percentage, root length, and root fresh weight traits, on both PEG treatments (Table 5).

Table 4. Means of morpho-physiological traits as influenced by wheat cultivars' overall PEG levels

Cultivars	Germination Percentage (%)	Shoot length(cm)	Root length(cm)	Shoot fresh weight (g)	Root fresh weight(g)	Turgid weight (g)	Shoot dry weight (g)	Root dry weight (g)	RWC
Sakha 94	8.5 ^{a}	18.41 ^{ab}	9.33 ^{b}	4.39 ^{abc}	1.14 ^{b}	7.93 ^{abc}	0.77 ^{a}	0.61 ^{b}	50.05 ^{bc}
Sakha 95	8.83 ^{a}	19.17 ^{a}	10.00^{b}	4.09^{abc}	1.17 ^{b}	6.78^{abc}	0.76 ^{a}	0.29 ^{bc}	55.28^{ab}
Giza 171	8.33 ^{a}	18.50 ^{ab}	8.92 ^{b}	4.70 ^{ab}	1.31 ^{b}	8.10 ^{ab}	0.87 ^{a}	0.24 ^{bc}	53.27 ^{ab}
Misr 3	8.33 ^{a}	17.50 ^{ab}	10.17 ^{b}	4.29 ^{abc}	1.24 ^{b}	7.36 ^{abc}	0.83 ^{a}	0.19 ^{c}	51.60 ^{ab}
Shandawell-1	9.17 ^{a}	19.75 ^{a}	13.50^{a}	5.34 ^{a}	11.54 ^{a}	8.60 ^{a}	0.85 ^{a}	1.66 ^{a}	56.40 ^{a}
Gemmiza 11	7.17 ^{b}	17.66^{ab}	9.77 ^{b}	3.38^{bc}	1.02^{b}	6.07 ^{bc}	0.65^{a}	0.14^{c}	49.83 ^{bc}
Gemmiza 12	6.83 ^{b}	16.41^{b}	8.42 ^{b}	3.08 ^{c}	1.59 ^{b}	5.70 ^{c}	0.59 ^{a}	0.21 ^{c}	45.10 ^{c}
L.S.D. 0.05	0.83	2.60	2.27	1.44	1.59	2.24	0.30	0.37	6.04

Table 5. Means for morpho-physiological traits interaction as affected by the PEG levels and wheat cultivars

PEG Levels	Cultivars	Germination percentage (%)	Root length (cm)	Root fresh weight (g)
Control	Sakha 94	9.67	12.17	0.60
	Sakha 95	9.67	14.0	1.06
	Giza 171	9.67	10.33	0.87
	Misr 3	9.33	14.00	1.46
	Shandawell-1	10.00	21.67	14.72
	Gemmiza 11	10.00	12.33	0.62
	Gemmiza 12	9.67	10.17	2.22
20%	Sakha 94	7.33	6.50	1.69
	Sakha 95	8.0	6.0	1.28
	Giza 171	7.0	7.5	1.76
	Misr 3	7.3	6.33	1.02
	Shandawell-1	8.3	5.33	8.36
	Gemmiza 11	4.3	7.2	0.42
	Gemmiza 12	4.0	6.67	0.96
L.S.D. 0.05		1.67	4.30	2.45

To characterize the expression patterns of the three TaDREB1 genes and their relationship with drought stress tolerance in wheat seedling leaves, qPCR analysis was conducted. This method was utilized to assess the transcript levels of TaDREB1-A, TaDREB1-B, and TaDREB1-D across seven wheat genotypes that exhibit varying levels of drought stress tolerance. In this investigation, 20% PEG 6000 induced gene expression in Shandweel-1, Sakha95, and Sakha 94. This concentration was screened based on the previous results, which mentioned that this treatment induced the expression of DREB1 genes in most varieties of wheat (Vuković *et al.,* 2022). In addition, the expression of many genes, such as WRKY genes, was induced by using a 20% PEG treatment (Okay *et al.,* 2014). The expression of the TaWRKY10 gene in wheat cv. Chinese Spring was induced and reached a maximum level at 1 h after 20 % PEG treatment, and TaWRKY10 overexpression enhanced the tolerance to drought stress in transgenic tobacco (Wang *et al.,* 2013). Wheat-inbred line seedlings revealed high drought tolerance with 20% PEG stimulation (Ismail *et al.,* 2023).

In this study, it was found that DREB1-A exhibited upregulation in three cultivars, namely Sakha-94, Sakha-95, and Shandweel-1, while it was downregulated in the remaining four cultivars: Gemmiza-11, Gemmiza-12, Giza-171, and Misr-3 (Fig. 1A). Both DREB1-B and DREB1-D demonstrated downregulation across all cultivars, except Shandweel-1, under drought stress conditions when compared to non-stress conditions (Fig. 1 B and C). The fold change in DREB1-A gene expression was notably higher in the Shandweel-1 cultivar (7.06) compared to both Sakha-95 (2.30) and Sakha-94 (1.44). Among the three genes, the highest level of expression was observed in Shandweel-1, with DREB1-B exhibiting the highest expression (9.54), followed by DREB1-A (7.06), and DREB1-D (4.75). Strikingly, DREB1-A, DREB1-B, and DREB1-D were downregulated in the four cultivars: Gemmiza-11, Gemmiza-12, Giza-171, and Misr-3.

The TaDREB1 genes were found to be located on chromosomes 3A, 3B, and 3D (Wei *et al.,* 2009). Additionally, the expression of TaDREB1-A, TaDREB1-B, and TaDREB1-D genes exhibits variation between the leaves and roots of wheat seedlings when subjected to identical stress conditions (Menglin *et al.,* 2019). TaDREB1A has been observed to be induced by drought using ABA and PEG, salinity, and low temperature in wheat leaves (Shen *et al.,* 2003; Kurahashi *et al.,* 2009 and Menglin *et al.,* 2019). Conversely, TaDREB1-D is predominantly expressed in roots (Menglin *et al.,* 2019), and high transcript expression of this gene was detected in wheat seeds under osmotic stress, but TaDREB1-A and TaDREB1-B transcript expression were not detected, suggesting an important role of this gene in roots and seed germination (Liu *et al.,* 2018). In the current study, DREB1-A exhibited upregulation under PEG-induced drought in Sakha-94, Sakha-95, and Shandweel-1 genotypes, while being downregulated in Gemmiza-11, Gemmiza-12, Giza-171, and Misr-3 genotypes (Fig. 1). Yousfi *et al.* (2016) revealed a significant contrast in the transcriptional regulation of TaDREB1A between genotypic variants exhibiting susceptibility and those demonstrating tolerance. Their study underscores that the elevated expression of TaDREB1A in susceptible genotypes is indicative of an impaired metabolic state relative to the tolerant genotypes.

Both DREB1-B and DREB1-D were suppressed across all cultivars under drought stress conditions, except for Shandweel-1 (9.54-fold for DREB1-B and 4.75-fold for DREB1-D), when compared to the non-stress condition (Fig. 1). TaDREB1A is upregulated in three cultivars (Sakha-94, Sakha-95, and Shandweel-1), whereas TaDREB1-B and TaDREB1-D are upregulated in one cultivar (Shandweel-1) in leaves. This result was supported by Menglin *et al.* (2019), who found that the level of gene expression of TaDREB1A was the highest in leaves compared to TaDREB1-B and TaDREB1-D, indicating a high response to high levels of abiotic stress such as drought, low temperature, and salinity. TaDREB1-B showed up-regulation under PEG and NaCl treatments but down-regulation under ABA and cold stresses, whereas TaDREB1-D exhibited down-regulation under PEG, NaCl, and ABA stress conditions, while up-regulation was observed under cold stress.

It has been reported that significant diversity exists among different drought-tolerant Egyptian genotypes regarding TaDREB1 gene haplotypes (Ahmed *et al.,* 2022). Additionally, Yousfi *et al.* (2016) reported the presence of inconsistent heterogeneity in TaDREB1 gene haplotypes and nucleic acid polymorphisms within the TaDREB1 gene in wheat among drought-resistant materials. These findings highlight the intricacy of gene expression, demonstrating that a single stressor can elicit varying levels of gene expression responses. This suggests that the drought tolerance observed in Giza-171, a superior cultivar for drought tolerance, and Misr-3 genotypes, which are relatively tolerant compared to other genotypes (Seleiman & Abdel-Aal, 2018 and Ghanem & Al-Farouk, 2024) may not solely be attributed to the expression of the TaDREB1 genes but could involve other drought-related genes as well. Further molecular analyses are necessary to identify additional genes associated with drought stress in these cultivars.

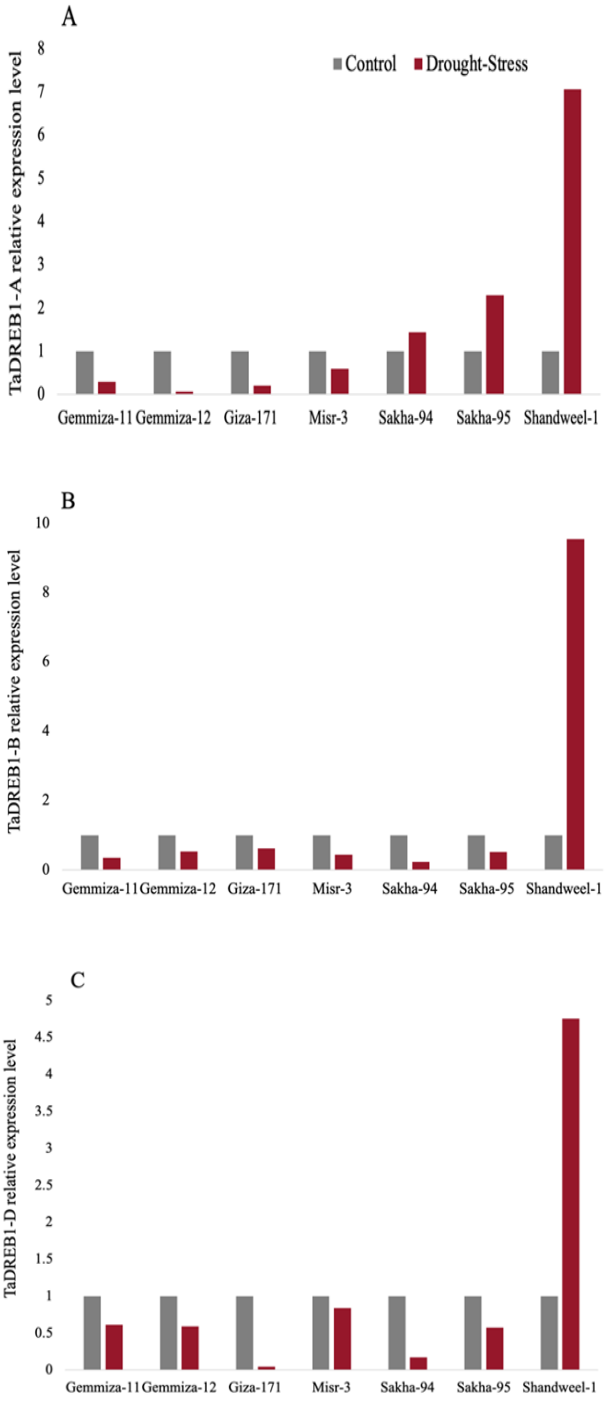
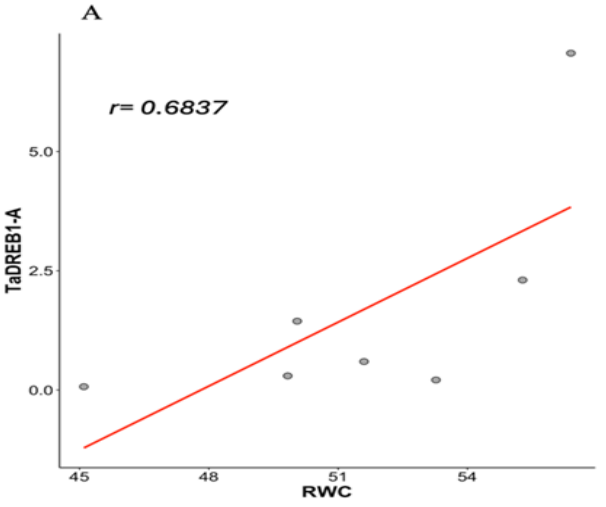


Fig.1. Gene expression of TaDREB1-A (A), TaDREB1-B (B), and TaDREB1-D (C): Data represent the mean fold change in the relative expression of these genes versus the control sample, normalized to the reference gene, 18S rRNA

The correlation between the relative expression of the studied genes and RWC under drought stress was recorded. RWC was chosen as the most important parameter that can be used to calculate the correlation with the relative expression of tested genes because RWC has been reported as a good indicator of drought stress tolerance in leaves and could be used for the selection of drought-tolerant wheat genotypes (Bayoumi *et al.*, 2008; Baloch *et al.*, 2013 and Chowdhury *et al.*, 2021). In this study, the correlation between the relative expression of the studied genes and RWC under drought stress was calculated (Fig. 2). Notably, the correlation between the relative expression of TaDREB1-A and RWC (0.683) is higher than that of TaDREB1-B (0.555) and TaDREB1-D (0.518). The recorded correlation for all genes revealed intermediate values, whereas the correlation between the relative expression of TaDREB1-A and RWC is the highest compared to the other genes.

It is worth mentioning that the three tested genes upregulated in Shandweel-1 under severe drought stress showed the highest value of RWC and the other morpho-physiological traits compared to the other genotypes. Rustamova *et al.* (2021) found that the expression level of DREB1 genes increased in tolerant genotypes under drought stress conditions. DREB1 gene expression can be used as a gene marker for drought tolerance. Shandweel-1 can save water consumption without a significant reduction in wheat productivity (Seleiman and Abdel-Aal, 2018), and can be introduced in breeding programs to improve drought tolerance through gene pyramiding.



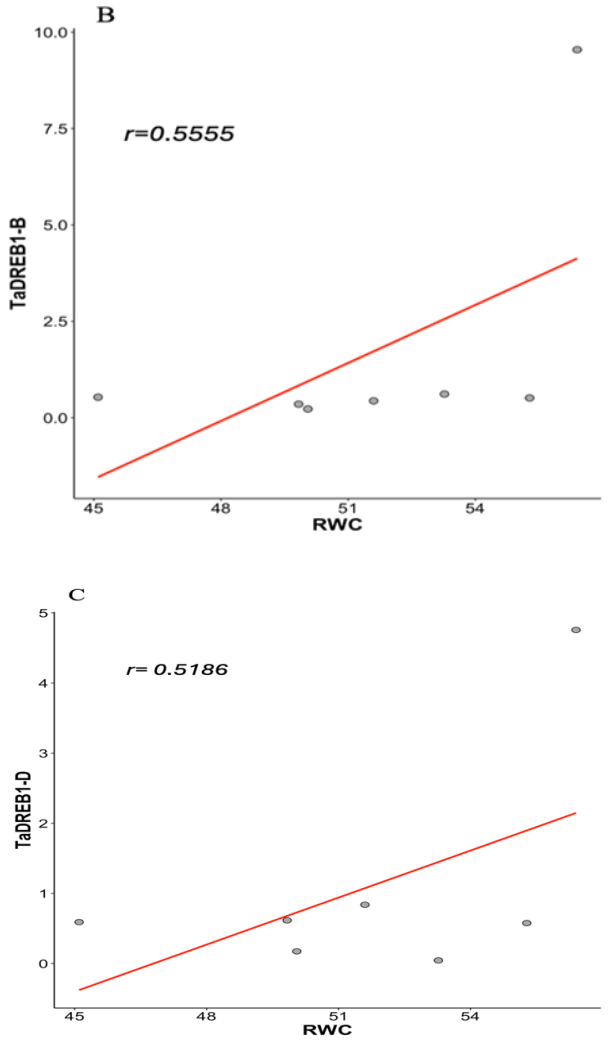


Fig.2. Correlation coefficient analysis between the relative expression of TADREB1-A (A), TADREB1-B (B), and TADREB1-D (C) genes and leaf RWC percentages under drought stress

CONCLUSION

This study revealed that drought stress induced the expression of the TDREB1-A gene in three (Sakha94, Sakha95, and Shandweel-1) out of seven Egyptian wheat cultivars, while repressing it in the remaining four genotypes. Interestingly, TaDREB1-B and TaDREB1-D showed induction only in one genotype (Shandweel-1), while being repressed in all six others. Thus, it is worth mentioning that all three genes upregulated in Shandweel-1 show the possibility of introducing it in breeding programs for drought tolerance improvement. Further sequence analysis of TaDREB1-A, TaDREB1-B, and TaDREB1-D is imperative to elucidate the variations in gene expression among these diverse drought-tolerant cultivars.

Conflict of Interests

The authors declare that there is no conflict of interest regarding the publication of this paper.

REFERENCES

Abd El-Moneim, D., M.M. Alqahtani, M.A. Abdein and M.O. Germoush. 2020. Drought and salinity stress response in wheat: physiological and TaNAC gene expression analysis in contrasting Egyptian wheat genotypes. J. Plant Biotechnol. 47: 1–14.

Ahmed, A.A.M., M.F.A. Dawood, A. Elfarash, E.A. Mohamed, M.Y. Hussein, A. Börner and A. Sallam. 2022. Genetic and morpho-physiological analyses of the tolerance and recovery mechanisms in seedling stage spring wheat under drought stress. Front. Genet. 13, 272.

Allen, M.D., K. Yamasaki, M. Ohme-Takagi, M. Tateno and M. Suzuki. 1998. A novel mode of DNA recognition by a beta-sheet revealed by the solution structure of the GCC-box binding domain in complex with DNA. EMBO J. 17: 5484–5496.

Baloch, M.J., J. Dunwell, N.U. Khan, W.A. Jatoi, A.A. Khakhwani, N.F. Vessar and S. Gul. 2013. Morpho-physiological characterization of spring wheat genotypes under drought stress. Int. J. Agric. Biol. 15: 945–950.

Bayoumi, T.Y., M.H. Eid and E.M. Metwali. 2008. Application of physiological and biochemical indices as a screening technique for drought tolerance in wheat genotypes. Afr. J. Biotechnol. 7: 2341–2352.

Chowdhury, M.K., M.A. Hasan, M.M. Bahadur, M.R. Islam, M.A. Hakim, M.A. Iqbal, T. Javed, A. Raza, R. Shabbir, S. Sorour and N.E. Elsanafawy. 2021. Evaluation of drought tolerance of some wheat (*Triticum aestivum* L.) genotypes through phenology, growth, and physiological indices. Agron. 11, 1792.

Dietz, K.J., M.O. Vogel and A. Viehhauser. 2010. AP2/EREBP transcription factors are part of gene regulatory networks and integrate metabolic, hormonal and environmental signals in stress acclimation and retrograde signalling. Protoplasma 245: 3-14.

Elseehy, M.M. and A.M. El-Shehawi. 2018. Expression profile of wheat DNA methyltransferases genes in Egyptian wheat (*Triticum Aestivum*) varieties under PEG induced dehydration. Alex. Sci. Exch. J. 39: 695-701.

Ghanem, H.E. and M.O. Al-Farouk. 2024. Wheat drought tolerance: morpho- physiological criteria, stress indexes, and yield responses in newly sand soils. J. Plant Growth Regul. 1-17.

Golldack, D., I. Lüking and O. Yang. 2011. Plant tolerance to drought and salinity: stress regulating transcription factors and their functional significance in the cellular transcriptional network. Plant Cell Rep. 30: 1383–1391.

González, L. and M. González-Vilar. 2001. Determination of relative water content. In: Reigosa Roger, M.J. (Ed). Handbook of Plant Ecophysiology Techniques, Springer, Dordrecht, 207-212.

Ismail, E., R.B. Ahmed, A.A. Aboelkassem, R. Ashraf, S. Elghaity, R. Abdo and H.M.A. Elsayed. 2023. Response of early seedling inbred lines of wheat against 20% PEG6000 induced condition. MOJ. Biol. Med. 8: 45-48.

Kurahashi, Y., A. Terashima and S. Takumi. 2009. Variation in dehydration tolerance, ABA sensitivity and related gene expression patterns in D-genome progenitor and synthetic hexaploid wheat lines. Int. J. Mo. Sci. 10: 2733–2751.

Lata, C. and M. Prasad. 2011. Role of DREBs in regulation of abiotic stress responses in plants. J. Exp. Bot. 62: 4731–4748.

Liu, M., Z. Wang, H. Xiao and Y. Yang. 2018. Characterization of TaDREB1 in wheat genotypes with different seed germination under osmotic stress. Hereditas 155, 26.

Liu, Q., M. Kasuga, Y. Sakuma, H. Abe, S. Miura, K. Yamaguchi-Shinozaki and K. Shinozaki. 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. Plant Cell 10: 391–1406.

Livak, K.J. and T.D. Schmittgen. 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2−ΔΔCT method. Methods 25: 402–408.

Menglin, L., M. Xinguo, C. Xiaoping, L. Xia and J. Ruilian. 2019. Functional marker mapping and expression characteristics analysis of wheat TaDREB1. J. Agric. Biotechnol. 27: 229–236.

Noor, S., S. Ali, H. Rahman, F. Farhatullah and G.M. Ali. 2018. Comparative study of transgenic (DREB1A) and non-transgenic wheat lines on relative water content, sugar, proline and chlorophyll under drought and salt stresses. Sarhad J. Agric. 34: 986-993.

Okay, S., E. Derelli and T. Unver. 2014. Transcriptome-wide identification of bread wheat WRKY transcription factors in response to drought stress. Mol. Genet. Genomic. 289: 765–781.

Rustamova, S.M., A. Shrestha, A.H. Naz and I.M. Huseynova. 2021. Expression profiling of DREB1 and evaluation of vegetation indices in contrasting wheat genotypes exposed to drought stress. Plant Gene 25, 100266.

Sakuma, Y., Q. Liu, J. G. Dubouzet, H. Abe, K.Shinozaki and K.Yamaguchi-Shinozaki. 2002. DNA-Binding specificity of the ERF/AP2 domain of arabidopsis DREBs, transcription factors involved in dehydration- and cold-inducible gene expression. Biochem. Biophys. Res. Commun. 290: 998–100.

Seleiman, M.F. and M.S.M. Abdel-Aal. 2018. Response of growth, productivity, and quality of some Egyptian wheat cultivars to different irrigation regimes. Egypt. J. Agron. 40: 313-330

Sharma, V., A. Kumar, A. Chaudhary, A. Mishra, S. Rawat, V. Shami and P. Kaushik. 2022. Response of wheat genotypes to drought stress stimulated by PEG. Stresses 2: 26–51.

Shen, Y.G., W.K. Zhang, S.J. He, J.S. Zhang, Q. Liu and S.Y. Chen. 2003. An EREBP/AP2-type protein in *Triticum aestivum* was a DRE-binding transcription factor induced by cold, dehydration, and ABA stress. Theor. Appl. Gene. 106: 923–930.

Sokal, R. and F. Rohlf. 1981. Biometry. 2nd ed. New York, N.Y: W. H. Freeman.

Vinocur, B. and A. Altman. 2005. Recent advances in engineering plant tolerance to abiotic stress: achievements and limitations. Curr. Opin. Biotechnol. 16: 123–132.

Vuković, R., I.S. Čamagajevac, A.Vuković, K. Šunić, L. Begović, S. Mlinarić, R. Sekulić, N. Sabo and V. Španić. 2022. Physiological, biochemical and molecular response of different winter wheat varieties under drought stress at germination and seedling growth stage. Antioxid. 11, 693.

Wang, C., P. Deng, L. Chen, X. Wang, H. Ma, W. Hu, N. Yao, Y. Feng, R. Chai, G. Yang and G. He. 2013. A wheat WRKY transcription factor TaWRKY10 confers tolerance to multiple abiotic stresses in transgenic tobacco. PLoS One 8, e65120.

Wei, B., R. Jing, C. Wang, J. Chen, X. Mao, X. Chang and J. Jia. 2009. Dreb1 genes in wheat (*Triticum aestivum* L.): development of functional markers and gene mapping based on SNPs. Mol. Breed. 23: 13–22.

Xu, Y., F.Y. Sun, C. Ji, Q.W. Hu, C.Y. Wang, D.X. Wu and G. Sun. 2019. Nucleotide diversity patterns at the DREB1 transcriptional factor gene in the genome donor species of wheat (*Triticum aestivum* L). PLOS One 14, e0217081.

Xu, Z., M. Chen, L. Li and Y. Ma. 2011. Functions and application of the AP2/ERF transcription factor family in crop improvement[F]. J. Integr. Plant Biol. 53: 570–585.

Yousfi, S., A. J. Márquez, M. Betti, J.L. Araus and M.D. Serret. 2016. Gene expression and physiological responses to salinity and water stress of contrasting durum wheat genotypes. J. Integr. Plant Biol. 58: 48–66.

Zhou, Y., M. Chen, J. Guo, Y. Wang, D. Min, Q. Jiang, H. Ji, C. Huang, W. Wei, H. Xu, X. Chen, L. Li, Z. Xu, X. Cheng, C. Wang, C. Wang and Y. Ma. 2020. Overexpression of soybean DREB1 enhances drought stress tolerance of transgenic wheat in the field. J. Exp. Bot. 71: 1842–1857.

الملخص العربي

تحليل التعبير الجيني لجينات TaDREB1 في أصناف مصرية لقمح الخبز تحت ظروف اجهاد الجفاف

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تواجه النباتات إجهادات حيوية وغير حيوية مختلفة، بما في ذلك الملوحة والجفاف ومسببات الأمراض، والتي تؤدي في النهاية إلى تقليل إنتاجية المحاصيل على مستوى العالم. يعد فهم آليات تحمل الجفاف في النباتات أمرًا بالغ الأهمية لتعزيز النمو وتحسين إنتاجية المحاصيل. تعمل بروتينات DREB، وهي عوامل للنسخ في النباتات، على تنظيم تعبير الجينات الضرورية لتحمل للجفاف والملوحة العالية واستجابات الإجهاد عند درجات الحرارة المنخفضة من خلال الارتباط بعناصر DRE/CRT. وقد تم استخدام هذه العوامل لتعزيز قدرة القمح على تحمل الجفاف، مما أدى إلى تحسين النمو والإنتاج والصفات الفسيولوجية. في هذا السياق، تهدف الدراسة الحالية إلى معرفة كيفية تأثير الجفاف الناجم عن PEG على مستويات التعبير الجيني لجينات TaDREB1-A، وTaDREB1-B، وTaDREB1-D في سبعة أصناف من القمح المصري، والتي تظهر درجات متفاوتة من القدرة على مقاومة الجفاف. تكشف النتائج التي توصلنا إليها أن التعبير الجيني لـ TDREB1-A اعطى مستوى متزايد للنسخ بالمقارنة بالكنترول في ثلاثة من أصناف القمح السبعة (Sakha94، Sakha95، وShandweel-1)، مما يدل على مستويات متميزة من تحمل الجفاف، في حين في الأنماط الجينية الأربعة المتبقية اظهرت مستوى منخفض من النسخ. والجدير بالذكر أن TaDREB1-B وTaDREB1-D أظهرا تزايد في التعبير الجيني لصنف واحد (Shandweel-1) لكن انخفض التعبير الجيني في باقي الاصناف المنزرعة. وبالتالي، تجدر الإشارة إلى أن Shandweel-1 هو الصنف الواعد الذي سيتم إدخاله في برامج التربية لتحسين تحمل الجفاف، وتم تسجيل أعلى ارتباط بين التعبير النسبي لجينات DREB1 والمحتوى المائي النسبي (RWC) لجين TaDREB1-A. تؤكد هذه النتائج على التنظيم المعقد للتعبير الجيني لـ DREB1 داخل أصناف القمح السداسي تحت اجهاد الجفاف.