

EXPRESSION PROFILING OF TRANSCRIPTION FACTORS UNDER DROUGHT STRESS AT SEEDLING STAGE IN BREAD WHEAT (*TRITICUM AESTIVUM* L.)

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Abstract. This study sheds light on the regulatory mechanisms underlying drought stress tolerance at early development stages in bread wheat (*Triticum aestivum* L.). Two bread wheat cultivars with contrasting genotypes, namely Giza 168 as the high-yielding tolerant cultivar and Gemmiza 10 as the sensitive cultivar, were utilized in this study. RNA-Seq data were subjected to hierarchical clustering analysis and resulted in the occurrence of six differentially expressed (DE) patterns for each cultivar. Nine TFs were consistently regulated in the two cultivars of which three corresponding to bZIP protein isoform 1, ethylene-responsive (ER) erf053-like, and heat stress a-3-like were upregulated at 12 h time point, while three other TFs corresponding to asg4-like (two hits) and ER rap2-2-like were downregulated. The last three TFs corresponding to ER1-like isoform x3 and WRKY40-like (two hits) were downregulated at 2 and 12 h time points. There are three other TFs that are highly expressed in the drought-tolerant cultivar. Two of these TFs (e.g., bHLH49-like and bHLH135-like) were upregulated at 2 h time point, while the third (e.g., MYB39-like) was upregulated at 12 h time point. We speculate that sensitivity to drought stress in GM10 might be due to the absence of regulation of some bHLH, MYB and NAC transcription factors, besides the downregulation of three members of WRKY family, namely WRKY1-like (two hits), WRKY18-like and WRKY40-like (four hits). Further expression profiling influencing the differentially expressed TFs along with their concordantly expressed stress-related TFs are required to be studied further. Regulatory mechanisms for drought stress tolerance in this study can be utilized further in improving crop productivity under adverse conditions via metabolic engineering approaches.

Keywords: erf053, bZIP, NAC, bHLH, MYB, WRKY, auxin, suberization

Introduction

Hexaploid bread wheat (*Triticum aestivum*, 2n = 6x = 42, AABBDD) is among the most important cereal grain crops used for human consumption and livestock feed and stands as the third largest crop in production (Peng et al., 2011). It is originally emerged from two interspecies crossing events that took place between three distinct diploid species harboring genomes A, B and D (Dvorak and Akhunov, 2005) to recover an estimated genome size of ~17 GB (Brenchley et al., 2012). Due to the extensive gene loss during evolution in bread wheat genomes, it has fewer genes than its progenitor (Hernandez et al., 2012; Ling et al., 2013). The world annual wheat production is recently

estimated to be about 778.6 million tons cultivated in an area over 220 million ha (FAO, 2018). It is thought that the demand for wheat will increase to 60% by the year 2050 and can be higher due to the running wars. In addition, water deficit was also proven to reduce wheat production in terms of grain yield by over 30% mostly due to inadequate grain filling and reduced amount of the antioxidant polyphenols (Manickavelu et al., 2012; García et al., 2016; Mäkinen et al., 2018; Fuentealba-Sandoval et al., 2020). Therefore, new tools of molecular genetics have focused on understanding mechanisms of drought tolerance in wheat in order to improve its tolerance against drought and salt stresses.

Drought stress is considered lately as a severe environmental problem worldwide as it affects growth and development of plants (Bray, 1997; Memon et al., 2021). Among solutions to cope with this threat are mechanisms of drought escape, avoidance, and tolerance (Yıldırım and Kaya, 2017). In some cases, these mechanisms are concurrently involved in adapting the plant to withstand water deficit (Zhang, 2007). Therefore, it is very important to elucidate the mechanisms underlying drought stress responses and adaptation to water deficit in order to improve drought stress tolerance in domesticated crop plants. Plant survival under water deficit occurs at physiological, biochemical and molecular levels (Yamaguchi-Shinozaki and Shinozaki, 2006; França et al., 2012; Kabbadj et al., 2017). Cultivated bread wheats also have drought tolerance mechanisms including drought avoidance and dehydration tolerance (Ahmed et al., 2019). Understanding molecular mechanisms and gene regulation networks to confer tolerance to drought and salt stresses are mandatory for effective plant breeding programs (Farooq and Azam, 2001; Rampino et al., 2006; Bapela et al., 2022).

Transcription factors (TFs) are key proteins required in the regulation of almost all biosynthetic pathways in life (Latchman, 1997; Wang et al., 2015). TF is a protein that binds to specific DNA sequences, thus controls cellular transcription by either promoting or blocking the recruitment of RNA polymerase to its recognition site of a promoter region upstream its target gene(s) (Wang et al., 2015). TFs can bind either directly to target promoter region or to the RNA polymerase molecule. The actions of TFs are key determinants of whether the gene functions at a particular time or not. They are vital for the normal development of organisms and for all cellular functions and responses to biotic and abiotic stresses (Lindemose et al., 2013). Transcriptome approaches were utilized earlier to recover stress responsive genes in many plant species including rice, wheat, maize, *Arabidopsis* and *Ammopiptanthus mongolicus* (Rabbani et al., 2003; Aprile et al., 2009; Urano et al., 2009; Zheng et al., 2010; Liu et al., 2013). The data indicated that drought stress-responsive genes can regulate expression of downstream genes through signal transduction cascades. Some of the regulatory proteins that are activated in response to water stress include transcription factors (TFs) such as DREBs (dehydration-responsive element-binding proteins), AREBs (ABA-responsive element-binding proteins) and NAC proteins (Nakashima et al., 2009; Fujita et al., 2011). TFs also include bHLH, AP2, MYB, auxin-responsive, ethylene responsive, bZIP and WRKY (Rahaie et al., 2010; Liu et al., 2013; Baloglu et al., 2014).

In the present study, we conducted a drought stress experiment on two contrasting genotypes of bread wheat in terms of their ability to withstand drought stress in order to detect regulatory frameworks or TFs that are available in genomes of hexaploid bread wheat. Expression patterns of the drought-responsive TFs can be utilized further

as biomarkers for drought stress tolerance and to hunt downstream regulated stress-related genes under this adverse condition. The different expression patterns under stress may add to our understanding of the molecular mechanisms of drought stress responses and possible roles of TFs in driving concordant genes as a response to drought stress in bread wheat.

Materials and methods

Plant material

Two wheat cultivars grown in Egypt with contrasting genotypes in terms of their tolerance to drought stress were used in a drought stress experiment. These cultivars are the drought tolerant high-yielding Giza 168 (GZ168) and the drought sensitive Gemmiza 10 (GM10) cultivars (Al-Naggar et al., 2015; Abdelghany et al., 2016). Geographic locations and pedigrees of the two cultivars are shown in *Table A1*. The seeds were obtained from Field Crops Research Institute, Agricultural Research Center (ARC), Ministry of Agriculture, Egypt. Seeds were first surface sterilized with 10% NaOCl for 10 min, then washed three times with sterile distilled water. Then, seeds were put to germinate in half-strength Hoagland Solution (Hoagland and Arnon, 1950) for 9 days in a growth chamber at $22 \pm 2^\circ\text{C}$ with 16-h light and 8-h dark photoperiod and a light intensity of $450 \mu\text{mol m}^{-2}\text{s}^{-1}$. Then, seedlings of the two cultivars were treated with 20% PEG-6000 (Baloglu et al., 2014) for 0, 2 and 12 h. After leaf samples were taken, they were immediately frozen in liquid nitrogen for subsequent experiments.

RNA isolation

Total RNAs were extracted from flash-frozen emerged leaf samples of the two cultivars using Trizol (Invitrogen, Life Tech, Grand Island, NY, USA). Samples were treated with RNase-free DNase (Promega Corporation, Madison, WI, USA) in the presence of 1 U/ul of RNasin® Plus RNase Inhibitor (Promega Corp.) for 2 h at 37°C . Then, RNAs were quantified and the amount of 30 ug (400 ng/ul) was used for RNA-Seq. To test the presence of DNA contamination in RNA samples, the *actin* gene was amplified by PCR of the original RNA samples and results were negative (data provided upon request). Purified RNA samples of the two contracting genotypes were shipped to Beijing Genomics Institute (BGI), Shenzhen, China (in two replicates for each treatment) for deep sequencing and generation of transcriptomic datasets (at least 100 million reads per sample).

Next-generation mRNA sequencing

A number of 12 RNA-seq raw data was deposited in SRA database of the NCBI (<http://www.ncbi.nlm.nih.gov/sra>, SRA no. PRJNA306536, *Table 1*). Filtered reads were aligned to the *Triticum aestivum* cDNA plus ncRNA as the reference after downloading *Triticum aestivum* database from the ensemble plant database (https://plants.ensembl.org/Triticum_aestivum/Info/Index?db=core). Data were analyzed as previously described (Bahieldin et al., 2015) and differentially expressed (DE) transcripts were annotated using blast-2-GO software (version 2.3.5, <http://www.blast2go.org/>). Blastx was performed against the NCBI non-redundant protein database with an E-value cut off of $1e^{-5}$, then, GO terms were obtained with the default parameters. Identification of clusters with functional enrichment was done as

previously described (Bahieldin et al., 2015) and values of FPKM for the recovered TF transcripts were calculated based on their expression patterns under drought stress across different time points. Six expression patterns were resulted for each cultivar and recovered clusters were studied in order to extract data of drought-responsive TFs.

Table 1. Statistics of RNA-Seq numerical data analysis including three replicates of each of the two contrasting genotypes within treatment times (e.g., 0, 2 and 12 h). The 0 h time refer to control samples

IDs of replicated samples	Total no. reads	No. (%) reads aligned once	No. (%) reads aligned >1	% overall aligned reads	No. (%) unaligned reads
Giza168_0h_1	42,508,942	7,116,110 (16.74%)	27,402,981 (64.46%)	81.20%	7,989,851 (18.80%)
Giza168_0h_2	41,608,272	6,834,096 (16.42%)	24,555,192 (59.02%)	75.44%	10,218,984 (24.56%)
Giza168_2h_1	45,482,128	7,754,921 (17.05%)	29,950,029 (65.85%)	82.90%	7,777,178 (17.10%)
Giza168_2h_2	44,692,706	7,642,639 (17.10%)	29,211,342 (65.36%)	82.46%	7,838,725 (17.54%)
Giza168_12h_1	46,213,932	7,472,584 (16.17%)	29,742,611 (64.36%)	80.53%	8,998,737 (19.47%)
Giza168_12h_2	46,103,582	9,037,218 (19.60%)	31,303,119 (67.90%)	87.50%	5,763,245 (12.50%)
Gemmiza_0h_1	39,462,732	6,561,453 (16.63%)	24,439,459 (61.93%)	78.56%	8,461,820 (21.44%)
Gemmiza_0h_2	39,700,770	6,829,782 (17.20%)	24,878,178 (62.66%)	79.87%	7,992,810 (20.13%)
Gemmiza_2h_1	45,712,060	7,798,878 (17.06%)	30,218,320 (66.11%)	83.17%	7,694,862 (16.83%)
Gemmiza_2h_2	44,691,600	7,471,578 (16.72%)	29,528,523 (66.07%)	82.79%	7,691,499 (17.21%)
Gemmiza_12h_1	45,212,974	7,411,257 (16.39%)	28,933,119 (63.99%)	80.38%	8,868,598 (19.62%)
Gemmiza_12h_2	45,735,528	7,378,667 (16.13%)	28,280,105 (61.83%)	77.97%	10,076,756 (22.03%)

Validation of RNA-seq data by real time PCR

Nine regulated TF genes at 2 and/or 12 h time points were selected to validate RNA-Seq data by real time PCR with *actin* used as the reference house-keeping gene (Mackintosh et al., 2007). Primers were designed using Netprimer software (<http://www.premierbiosoft.com/netprimer/index.html>) with the sequences and amplicon size shown in Table A2 following standard criteria (Bahieldin et al., 2015). Total RNAs were extracted in three replicates from individual drought-stressed plants of the two cultivars and expression levels were detected by real time PCR as previously described (Bahieldin et al., 2015).

Results

Sequencing of cDNA samples of the two wheat cultivars yielded ~39 million reads corresponding to over 4 billion nucleotides per sample (Table 1). Between 75.44 and 87.5% of the raw sequencing reads were mapped to the reference wheat database. RNA-Seq data was used to detect the differential expression (DE) of the annotated *Triticum aestivum* transcripts encoding transcription factors (TFs) under drought stress. As these two cultivars differ in their tolerance to drought stress, we speculated that this differential performance under drought stress might be controlled by TFs driving drought stress-related genes.

To statistically obtain confirmation of the differences in gene expression across treatment time, FPKM-derived read counts were compared using a likelihood ratio test (Casella and Berger, 2021). Statistical analysis was reliable when applied to genes with

an FPKM value of ≥ 2 . To determine DE transcripts, a two-fold (or greater) change in expression and false discovery rate (FDR) of 10^{-3} or less was required. Expression profiles of the DE transcripts were determined by cluster analysis based on the *k*-means method using Pearson's correlation distance so that the similarity in relative change for each transcript or among transcripts across time of drought treatment was determined. These data were, then, subjected to hierarchical clustering also using the Pearson correlation as the distance metric (*Figure 1*). The data indicated the closer relationship between transcriptomes at 0 and 12 h time points for the drought tolerant cultivar GZ168, while between 0 and 2 h for the sensitive cultivar GM10. This might indicate the prompt response of the tolerant cultivar to drought stress, which reflects the possible involvement of transcription factors (TFs) in early regulation of drought stress. Later on, differential expression of TFs will be diminished so that expression levels at 12 h time point will come closer to those of the control levels. *Figure A2* refers to the results of real time PCR of nine transcripts under drought stress at different time points (0, 2 and 12 h) for the two contrasting genotypes GM10 and GZ168. These results were used to validate their analogues in RNA-Seq datasets for three randomly selected clusters (e.g., down/down/up, up/up/down and up/down/down) with *actin* used as an unregulated house-keeping gene. The results of real time PCR showed complete alignment with those of RNA-Seq analysis (*Figure A2*).

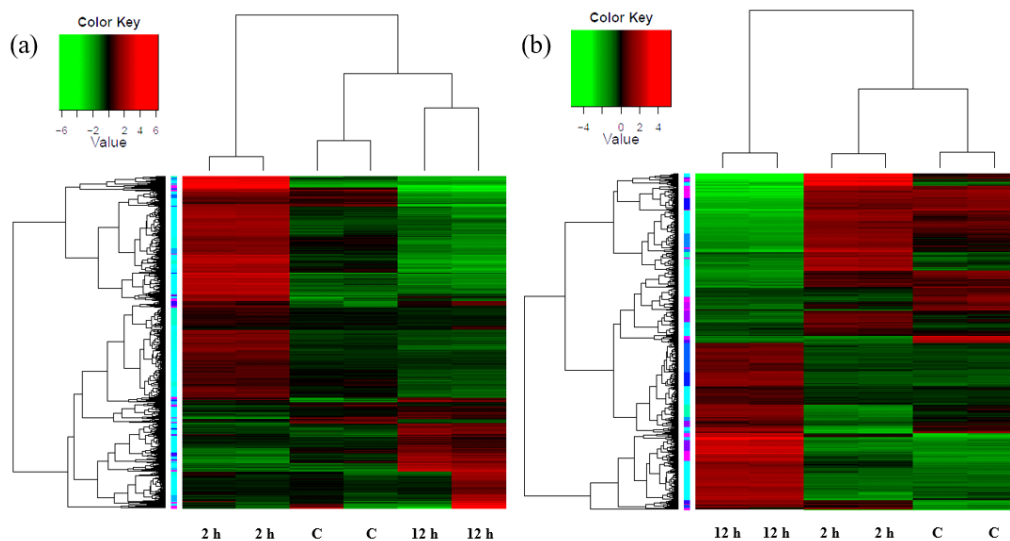


Figure 1. Hierarchical cluster analysis of gene expression based on log ratio RPKM data for transcriptome of the two wheat (*T. aestivum*) genotypes, e.g., GZ168 (a) and GM10 (b)

Differentially expressed genes were assigned to functional categories using blast2GO (<http://www.blast2go.org/>). The maximum numbers of functional groups for the three main categories “biological process”, “molecular function” and “cellular component” across the two genotypes either up (*Figure 2*) or downregulated (*Figure A1*), were 28, 15 and 7, respectively. The number of up or downregulated transcripts for the tolerant genotype (GZ168) was much higher than those of the sensitive genotype across the three categories. The most significant upregulated (*Figure 2a,b*) and downregulated (*Figure A1a,b*) biological process across genotypes and time points includes primary, organic substance, cellular, single-organism and nitrogen compound metabolic processes

as well as response to stress. The most significant upregulated (*Figure 2c,d*) and downregulated (*Figure A1c,d*) molecular function category includes ion binding as well as oxidoreductase, transferase and hydrolase activities. While the most significant upregulated (*Figure 2e,f*) and downregulated (*Figure A1e,f*) cellular component category includes cell, membrane-bounded organelles, vesicle and protein complex. There is no downregulated transcripts for the “cellular component” category at 12 h time point for the two cultivars GZ168 and GM10 (*Figure A1*).

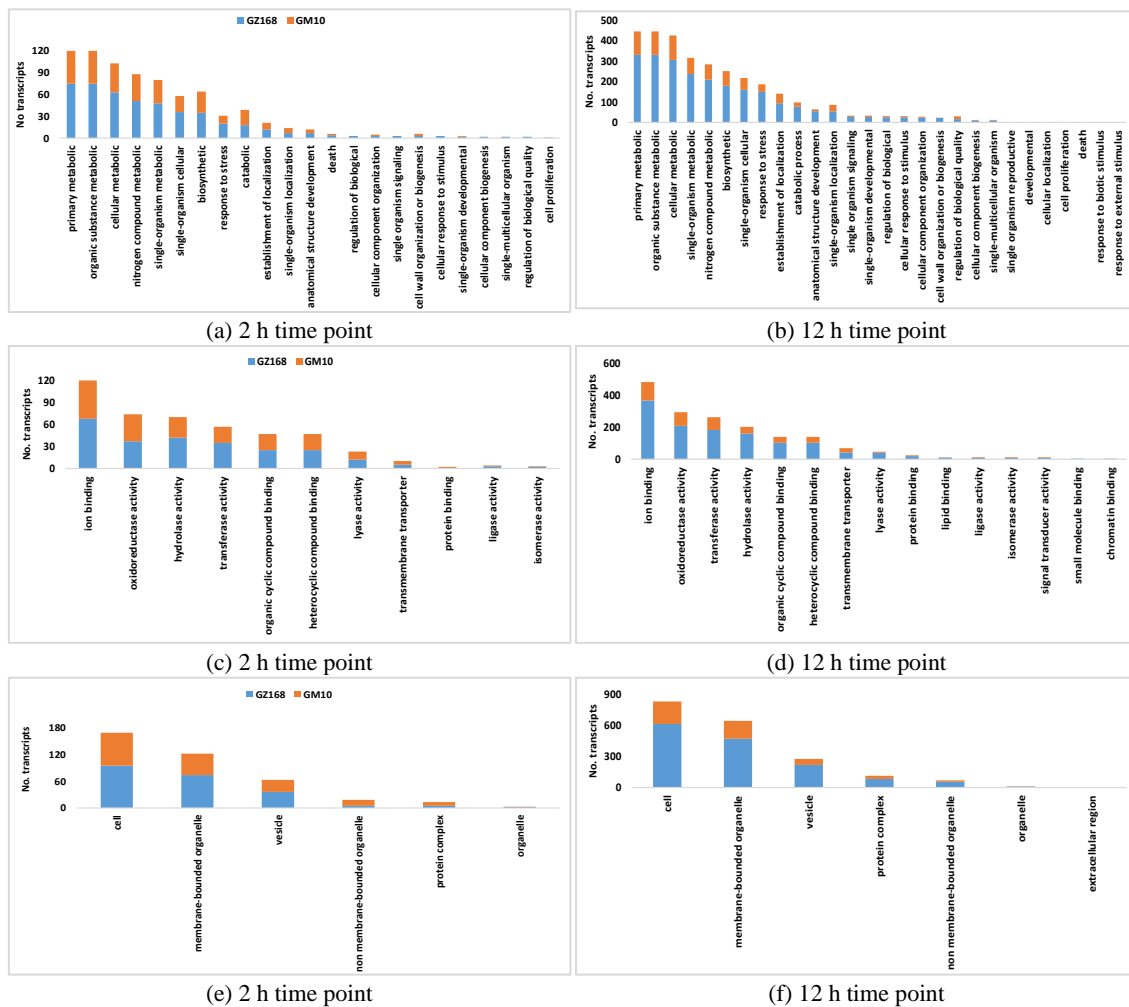


Figure 2. Gene ontology analysis describing the three main categories “biological process” (a & b), “molecular function” (c & d) and “cellular component” (e & f) for the two contrasting wheat genotypes GZ168 and GM10 based on the upregulated transcripts under drought stress conducted for 2 (a, c & e) and 12 h (b, d & f)

Two-dimensional clustering approach classified DE profiles into six expression patterns for either cultivar GZ168 (*Electronic Appendices 1-6*) or GM10 (*Electronic Appendices 7-12*) according to the similarity of their expression profiles. The resulting number of DE transcripts is 4898 across different expression patterns and genotypes (*Electronic Appendices 1-12*) of which 89 correspond to the TFs in five out of the six expression patterns for each of the two wheat cultivars (*Tables 2,3 and Figures 3,4*).

Table 2. List of transcription factors (TFs) along with their chromosome locations in different expression patterns under drought stress across time (0, 2 & 12 h) in GZ168. Genes between brackets were further used in validating RNA-Seq datasets

Chromosome arm	Transcription factor	Chromosome arm	Transcription factor
Down/Up/Down		Down/Down/Up	
Traes_3DS_81B8AB548.1	transcription factor bhlh35-like	Traes_5AL_5983E8F92.1	transcription factor bhlh93-like
Traes_2AL_411B944D6.1	transcription factor bhlh35-like isoform x1	Traes_5DS_DE2C70E4C.2	transcription factor myb108-like
Traes_2DS_FE62DB7F5.1	transcription factor bhlh47-like	Traes_3AS_5E49D47AF.1	transcription factor myb39-like
Traes_2AS_9AEA9BDEA.1	transcription factor bhlh47-like	Traes_1BL_1A885E733.1 (G1)	bzip transcription factor superfamily protein isoform 1
Traes_2BS_8A40B568C.1	transcription factor bhlh47-like	Traes_2AL_6B6086A7C.2	ethylene-responsive transcription factor erf110-like
Traes_5BL_34CEC5885.2	transcription factor bhlh49-like	Traes_2BL_E5F50498D.1	ethylene-responsive transcription factor erf110-like
Traes_5DL_A7B24A017.1	transcription factor bhlh49-like	Traes_2DL_2536A1C8C.2	ethylene-responsive transcription factor erf110-like
Traes_5BL_8C181ECA8.2	transcription factor bhlh49-like	Traes_6AL_A1A99719B.1 (G2)	ethylene-responsive transcription factor erf053-like
Traes_4DL_34826BB32.1	transcription factor bhlh61-like	Traes_3AS_855F86851.1	wrky transcription factor 6-like
Traes_7AS_15078CC8B.2	transcription factor bhlh62-like	Traes_3B_CDA5ADD75.1	wrky transcription factor 6-like
Traes_3B_843543612.2	transcription factor bhlh69-like isoform x2	Traes_3B_BB32DAA31.2	nac transcription factor nam-b2-like
Traes_7BS_D8A3A6004.2	transcription factor bhlh78- partial	Traes_2AS_53BFA14C7.2	heat stress transcription factor a-3-like
Traes_7DS_34DB6B305.2	transcription factor bhlh78- partial	Traes_2BS_1484A7516.1 (G3)	heat stress transcription factor a-3-like
Traes_3B_E1F2693CD.1	transcription factor bhlh87-like	Traes_1DS_34596D06E.1	trihelix transcription factor gt-3a-like
Traes_7DL_67FF12B6A.1	transcription factor bhlh93-like isoform 1	Traes_3B_F227310FD.1	transcription factor glk2-like
Traes_2BL_59B9199D9.1	transcription factor bhlh135-like	Up/Down/Up	
Traes_7AS_375BA0ECA.1	transcription factor bhlh135-like	Traes_4BL_F676F7705.1	transcription factor bhlh93-like
Traes_2AL_7FEF10153.1	transcription factor bhlh135-like	Up/Up/Down	
Traes_3DL_C73683B71.1	transcription factor org2-like	Traes_7AS_4FAB72487.2	transcription factor bhlh130-like
Traes_3B_FAAFF83D5.1	transcription factor org2-like	Traes_7AS_8ED861ED7.1	transcription factor bhlh137-like isoform x2
Traes_3B_2A12B8F93.2	transcription factor org2-like	Traes_3DL_30CF35BB3.1	transcription factor myb-like tcl2-like
Traes_3AL_B0A5AA3FD.1	transcription factor org2-like	Traes_1AS_1EE692FDC.1	transcription factor myb46-like
Traes_2BL_00608F8D6.1	transcription factor org2-like	Traes_1DS_5BAD8947E.1	transcription factor myb46-like
Traes_3B_089C0C2EB.2	transcription factor org2-like	Traes_6AL_362F82EAD.2 (G4)	transcription factor asg4-like

Chromosome arm	Transcription factor	Chromosome arm	Transcription factor
Traes_2DL_865346DA2.2	transcription factor org2-like	Traes_6BL_7E9C131F4.2 (G5)	transcription factor asg4-like
Traes_2AL_ACBA6A31.1	transcription factor org2-like	Traes_4AL_D00AB8CFA.2	transcription factor asg4-like
Traes_3DL_886A895A4.1	transcription factor org2-like	Traes_4AS_8A64DBE8E.1 (G6)	ethylene-responsive transcription factor rap2-2-like
Traes_3AL_95403ABBA.1	transcription factor org2-like	Traes_2BL_94E5996F7.1	bzip transcription factor superfamily protein
Traes_6AS_93815D497.1	transcription factor myb44-like	Up/Down/Down	
Traes_3DL_F1DB969A8.1	transcription factor myb1r1-like	Traes_4BL_78C84E85A.1 (G7)	ethylene-responsive TFr 1-like isoform x3
Traes_6DL_31D678054.1	ethylene-responsive transcription factor erf003-like	Traes_5AL_1B215E169.1 (G8)	wrky transcription factor 40-like
Traes_3AS_9151583B9.1	nuclear transcription factor y subunit b-3-like	Traes_5BL_8BEF7F9CD.1 (G9)	wrky transcription factor 40-like
Traes_3DS_833AD0EE2.1	nuclear transcription factor y subunit b-3-like		
Traes_6DL_BD5DCDC4E.1	heat stress transcription factor b-2c-like		
Traes_5BL_054434680.2	transcription factor pif1-like isoform x1		
Traes_5DS_8F3BD4450.2	transcription factor as1-like		
Traes_5BS_E635B0A281.2	transcription factor as1-like		

TFs in green and blue colors refer to those upregulated at 2 h or at 12 h (blue color) time point, respectively, with fold change of ≥ 5

Table 3. List of transcription factors (TFs) along with their chromosome locations in different expression patterns under drought stress across time (0, 2 & 12 h) in GM10. TFs between brackets were further analyzed and validated

Chromosome arm	Transcription factor
Down/Up/Down	
Traes_4BL_AA4929712.1	ethylene-responsive transcription factor 1-like
Down/Down/Up	
Traes_1BL_1A885E733.1 (GM1)	bzip TF superfamily protein isoform 1
Traes_6AL_A1A99719B.1 (GM2)	ethylene-responsive transcription factor erf053-like
Traes_3B_E408A7B56.1	wrky transcription factor 50-like
Traes_2BS_1484A7516.1 (GM3)	heat stress transcription factor a-3-like
Traes_3B_5BE5A6676.1	heat stress transcription factor c-1b-like
Up/Down/Up	
Traes_7AS_067BEC7B5.1	nuclear transcription factor y subunit b-9-like
Up/Up/Down	
Traes_6AL_362F82EAD.2 (GM4)	transcription factor asg4-like
Traes_6BL_7E9C131F4.2 (GM5)	transcription factor asg4-like
Traes_6DL_30F715B53.2	transcription factor asg4-like
Traes_4AS_8A64DBE8E.1 (GM6)	ethylene-responsive transcription factor rap2-2-like
Up/Down/Down	
Traes_4BL_78C84E85A.1 (GM7)	ethylene-responsive TF 1-like isoform x3
Traes_5BL_175E7FC38.1	wrky transcription factor 1-like
Traes_5DL_09F1F8F79.1	wrky transcription factor 1-like
Traes_5AL_7164FEAC3.1	wrky transcription factor 18-like
Traes_5AL_1B215E169.1 (GM8)	wrky transcription factor 40-like
Traes_5BL_8BEF7F9CD.1 (GM9)	wrky transcription factor 40-like
Traes_5BL_90757F0CC.1	wrky transcription factor 40-like
Traes_5DL_32D78D06A.1	wrky transcription factor 40-like

The six expression patterns of GZ168 or GM10 are down/up/down (*Electronic Appendices 1* and *7*, respectively), down/down/up (*Electronic Appendices 2* and *8*, respectively), down/up/up (*Electronic Appendices 3* and *9*, respectively), up/down/up (*Electronic Appendices 4* and *10*, respectively), up/up/down (*Electronic Appendices 5* and *11*, respectively) and up/down/down (*Electronic Appendices 6* and *12*, respectively). There was no DE TFs in the two cultivars that are upregulated at both 2 and 12 h (expression pattern down/up/up) from the onset of drought stress.

The results indicated that nine TFs in three, out of the five, expression patterns were consistently regulated in the two cultivars. Three of them corresponding to ethylene-responsive transcription factor (or ER) erf053-like whose encoding gene is located on chromosome arm 6AL, bzip transcription factor (or bZIP) superfamily protein isoform 1 (whose gene located on chromosome arm 1BL) and heat stress transcription factor (HS) a-3-like (whose gene located on chromosome arm 2BS) were upregulated only at 12 h time point in the two wheat cultivars (*Tables 2, 3*). On the other hands, three other TFs were down-regulated only at the same time point in the two cultivars. Two of them correspond to transcription factor asg4-like and located on chromosome arms 6AL and 6BL. The third TF is again an ER rap2-2-like (whose gene is located on chromosome arm

4AS) (Tables 2, 3). The last three TFs were downregulated at 2 and 12 h time points. One of these three TFs also corresponds to ER 1-like isoform x3. The other two TFs correspond to WRKY40-like transcription factor (whose encoding genes are located on chromosome arms 5AL and 5BL, respectively) (Tables 2, 3). These nine TFs were successfully utilized in validating RNA-Seq data analysis via real time PCR (Figure A2).

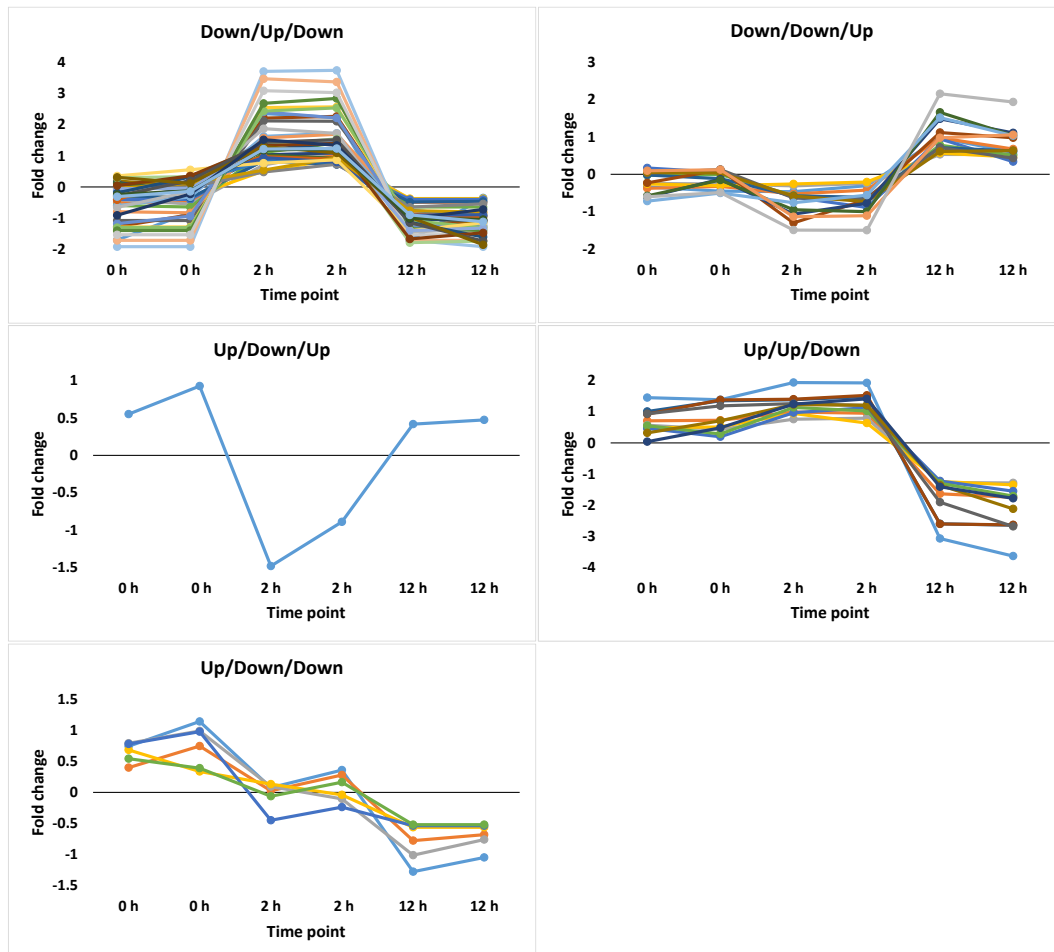


Figure 3. Expression patterns of transcription factors under drought stress across time (0, 2 & 12 h) in GZ168. Transcription factors in these five expression patterns were extracted from Electronic Appendices 1, 2, 4, 5 and 6, respectively. Transcripts of this figure for the different expression patterns are shown in yellow boxes in the different tables

Transcripts selected from the datasets of wheat leaves for the two cultivars that showed up or downregulation with fold change (FC) of ≥ 5 are shown in Table A3. Of which, three TFs were shown to be highly expressed in the drought tolerant cultivar GZ168 at 2 h (expression pattern down/up/down) or 12 h (expression pattern down/down/up) (Electronic Appendices 1, 2, respectively). These TFs are transcription factor bHLH49-like and transcription factor bHLH135-like for the first expression pattern, while only transcription factor MYB39-like for the second (Figure 5 and Tables 2, Electronic Appendix 1). The three TFs are not regulated in GM10 (Electronic Appendix 1).

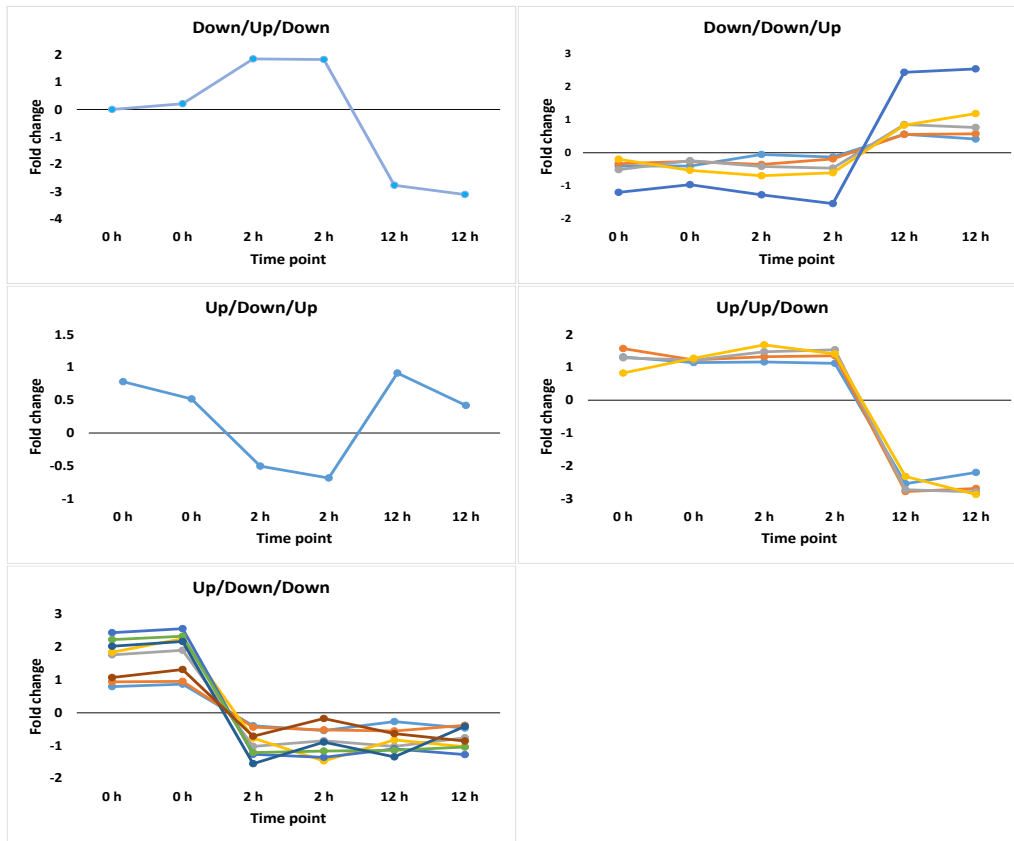


Figure 4. Expression patterns of transcription factors under drought stress across time (0, 2 & 12 h) in GM10. Transcription factors in these five expression patterns were extracted from Electronic Appendices 7, 8, 10, 11 and 12, respectively. Transcripts of this figure for the different expression patterns are shown in yellow boxes in the different tables

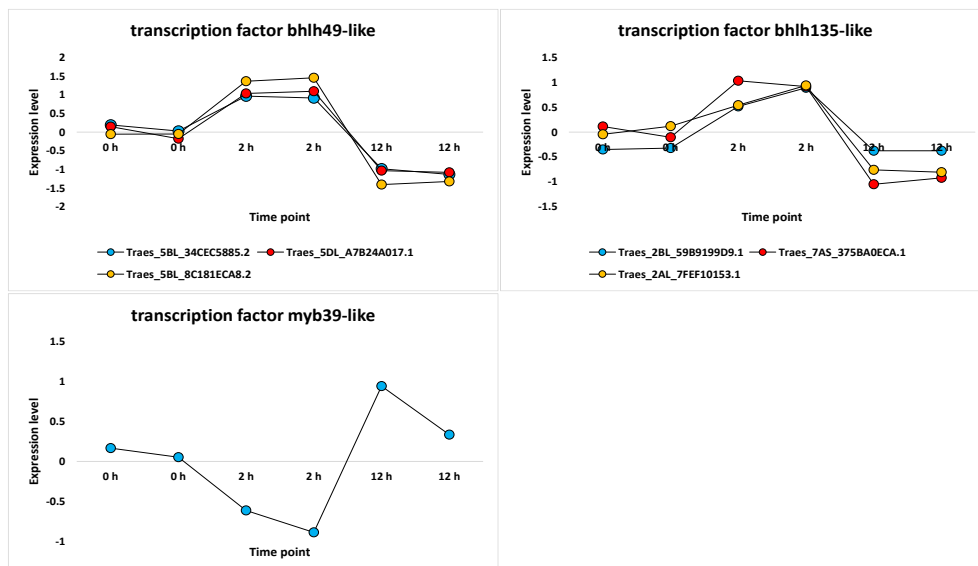


Figure 5. Expression patterns of transcription factors under drought stress across time (0, 2 & 12 h) in GZ168 with fold change of ≥ 5 . Transcription factors *bhlh49*-like and *bhlh135*-like exist in expression patterns down/up/down (Table A1) and transcription factor *myb39*-like exists in expression pattern down/down/up (Table A2)

Discussion

Transcription factors (TFs) provide a possibility for plants to respond to environmental stresses and are also involved in modulating developmental processes (Mitsuda and Ohme-Takagi, 2009; Zhang et al., 2012). It is proven that a single TF can control the expression of a cascade of genes within a given pathway or regulon as it binds to the *cis*-acting elements in their promoter regions (Nakashima et al., 2009). Three families of TFs with known action under drought stress are exclusively upregulated only at 2 h time point in the drought-tolerant wheat cultivar (GZ168). These drought-related TFs include members of bHLH (and its homologue org2, <http://www.uniprot.org/uniprot/Q9M1K1>), MYB and nuclear TF Y subunit b-3 gene families (Table 2). The first two families of TFs were recently reported to function under drought and salt stresses in wheat (Baloglu et al., 2014), while the third family of TF was reported to act under drought stress in maize (Nelson et al., 2007). Other members of bHLH (e.g., bHLH93 whose gene located on chromosome arm 5AL) and MYB (e.g., MYB44 and MYB1r1 whose genes are located on chromosome arms 6AS and 3DL, respectively) as well as three ER erf110 (whose genes are located on chromosome arms 2AL, 2BL and 2DL), two WRKY6 (whose genes are located on chromosome arm 3AS and chromosome 3B) and one NAC transcription factor nam-b2-like (whose gene is located on chromosome 3B) are upregulated under drought stress only at 12 h time point in GZ168 cultivar (Table 2). None of these TFs were upregulated in the drought-sensitive GM10 cultivar (Table 3). However, a unique type of WRKY50 (whose gene is located on chromosome 3B) was upregulated at 12 h time point only in GM10 (Table 3). A number of five WRKY of members 1, 18 and 40, in addition to the two mentioned earlier, were downregulated under drought stress only in the sensitive cultivar GM10 (Table 3). In addition to the well-known TFs MYB, WRKY, NAC, nuclear TF b for their action under drought stress, we can speculate that bHLH genes in GZ168 might also contribute to its ability to tolerate drought stress. We can also speculate that sensitivity to drought stress in GM10 might partially be due the absence of several members of bHLH, MYB and NAC families, which likely promote upregulation of drought-related genes, as well as due to down-regulation of several members of WRKY family.

We investigated the possible influence of high expression level of the three transcription factors (Table 2). They are bHLH49-like (whose gene analogs are located on chromosome arms 5BL and 5DL), bHLH135-like (whose gene analogs are located on chromosome arms 2AL, 2BL and 7AS) and MYB39-like (whose gene is located on chromosome arm 3AS). The first two TFs are upregulated at 2 h time point, while the third is regulated at 12 h time point (Table 2). As these three highly expressed TFs are not regulated in the drought sensitive cultivar GM10, we assume that they might contribute to the ability of GZ168 to tolerate drought stress (Figure 5 and Tables 2, 3). Recent research on TF bHLH49-like indicated its participation in balancing hormonal (auxin) production during flowering and embryo identity or formation of bamboo orchid (*Arundina graminifolia*) (Ahmad et al., 2014; Radoeva, 2019) and Arabidopsis (Lockhart, 2019) and in cell elongation in burflower (*Neolamarckia cadamba*) [published online, <https://doi.org/10.3390/ijms231911842>]. For example, auxin namely indole acetic acid (IAA) acts as an intermediate step between the two KEGG (Kyoto Encyclopedia of Genes and Genomes) cross-talking pathways “Tryptophan metabolism” and “Plant hormone signal transduction”. IAA auxin is considered as a limiting step in initiating high expression of genes participating at this important stage

of plant development to promote cell enlargement and plant growth and to mediate plant's ability to complete life cycle. In terms of bHLH135-like, recent studies indicated that this TF also participates in promoting plant growth and cell elongation as well as in light signaling pathway in *Arabidopsis* (Castelain et al., 2012; Zhao et al., 2012; Min et al., 2017; Lockhart, 2019; Hao et al., 2021; Liang et al., 2022). The latter pathway attenuates the rate of plant photosynthesis and production of energy compounds required by plant, especially under adverse condition. In terms of MYB39-like, it was proven that it has a regulatory role in the production of the lipophilic cell-wall macromolecule suberin (e.g., suberization) in *Arabidopsis* (Wang et al., 2020; Cohen et al., 2020; Geng et al., 2020) and tobacco (*Nicotiana benthamiana*) (Shukla et al., 2021).

This macromolecule accumulates in periderm of plant cells to provide insulation and protection against the surrounding adverse environmental conditions like drought and salt stresses. Periderm acts on sealing around secondary stems after wounding and on abscission of root, stem and leaf, where MYB39-like promotes pathway SHR (or short-root)/MYB36 and ABA-triggered response (Graça, 2015; Wang et al., 2020; Cohen et al., 2020). This TF also participates in modulating anthocyanin biosynthesis during fruit maturation in peach (*Prunus persica*) (Khan et al., 2022). Color formation is an important factor in fruit maturity process and in commercialization of peach. This characteristic was further confirmed in pepper (*Capsicum annuum* L.) (Wang et al., 2018). Spatiotemporal studies of MYB39-like expression indicated that it is originally localized in plant root (Wang et al., 2020), however, in the present study, this TF was proven to be highly expressed in plant leaf under drought stress (Figure 5).

The other information available for basic helix-loop-helix (bHLH) indicates that it represents a superfamily of transcriptional regulators existing in eukaryotes with a diversity of functions (Moore et al., 2000; Toledo-Ortiz et al., 2003; Heim et al., 2003). Different bHLH protein structures are available in the Protein Data Bank (PDB) (<http://www.pdb.org/>) and signature is constituted by an α -helix of ~ 16 amino acids at the N terminus that binds DNA to the canonical E-box (CANNTG) and a helix-loop-helix (HLH) domain at the C terminus for homo- and/or heterodimerization (Murre et al., 1989). The class or function of a given type of bHLH transcription factor (TF) is determined by the tissue where it exists, by DNA-binding specificity and by dimerization potentiality (Murre et al., 1989; Toledo-Ortiz et al., 2003; Jones, 2004). bHLH proteins in eukaryotes were shown to be involved in the regulation of many transcriptional events (Toledo-Ortiz et al., 2003). A marked divergence in protein sequence outside of the conserved bHLH domain was also observed (Atchley et al., 1999; Toledo-Ortiz et al., 2003). Diversity of this sequence might be a result of extensive domain shuffling after the duplication events (Atchley et al., 1999) and might be responsible for the different molecular functions of bHLH domain such as the detection of incoming regulatory signals from various cellular pathways and the direction of the transcriptional activity of a target gene (Toledo-Ortiz et al., 2003). More recently, gene expression analysis indicated the involvement of bHLH94 in conferring salt stress tolerance in wheat (Rahaie et al., 2011). This particular bHLH was not regulated in wheat under drought stress in the present study. However, its homologue in *Arabidopsis*, namely bHLH3, in addition to MY39 that is regulated in the present study (Table 2), are involved in the regulation of ABA signaling and has a major role in drought tolerance and ABA treatment response (Li et al., 2007; Rahaie et al., 2011).

MYB is one of the largest families of transcription factors in plants. They contain one to four imperfect repeats of 50-53 amino acids in their DNA-binding domain near to the

N terminus and are classified into four subfamilies (Mitsuda and Ohme-Takagi, 2009; Rahaie et al., 2010; Casella, 2021). MYB repeat contains three regularly-distributed tryptophan (or phenylalanine) residues, which can together form a hydrophobic core. Each repeat forms three α -helices: the two that are located at the C terminus adopt a variation of the helix–turn–helix (HLH) conformation that recognizes and binds to the DNA major groove at the specific recognition site such as C/TAACG/TG (Ogata, 1998). Roles of MYB genes in a number of physiological and biochemical processes were identified in different plant species (Wilkins et al., 2009). They include the control of cell development, signal transduction and the response to various biotic and abiotic stresses (Mitsuda and Ohme-Takagi, 2009). However, a little is known about this TF family in bread wheat albeit we have recognized that MYB39 indirectly participates in the response to abiotic stress by regulating ABA production in GZ168. More recently, a new gene namely MYBsdul is upregulated in leaves and roots of wheat plants exposed to prolonged drought stress treatment. It is suggested that this gene participates in the adaptation to both salt and drought stresses in wheat (Wang et al., 2015). Qin et al. (2012) also indicated that a new MYB, namely MYB33, in wheat was induced by ABA, NaCl, and PEG treatments due to the presence of the promoter element ABRE. This gene induced P5CS (for inducing proline biosynthesis) and ZAT12 (for regulating ascorbate peroxidase expression) in transgenic Arabidopsis. This indicates that this gene might be involved in ROS detoxification and osmotic balance reconstruction (Rahaie et al., 2010). Another MYB gene in wheat, namely MYB3R1, is potentially involved in wheat response to drought, stress. PEG treatment induced expression of this gene up to 6 h but expression was decreased afterward (Baloglu et al., 2014).

Recent studies showed that WRKY family acts mainly in regulating plant growth, development, and biotic stress, whereas their precise functions in abiotic stress remain to be elucidated (Cai et al., 2011). WRKY genes were shown to respond fast to abiotic stresses. Wu and colleagues conducted an expression analysis of a number of wheat WRKY genes and found that several of them are responsive to low temperature, high temperature, NaCl or PEG treatment (Wu et al., 2008). Niu and colleagues characterized 43 putative wheat WRKY genes and found that two of them are induced by drought and salt stresses (Niu et al., 2012), while Wang and colleagues scored upregulation of WRKY10 in wheat under polyethylene glycol, NaCl, cold and H₂O₂ treatment (Wang et al., 2013). Overexpression of the wheat WRKY2, WRKY19 (Niu et al., 2012) and WRKY10 (Wang et al., 2013) in Arabidopsis and tobacco significantly improved tolerance to drought and salt stresses, respectively. Baloglu and colleagues scored the upregulation of the wheat WRKY10 genes 3 and 12 h after drought stress (Baloglu et al., 2014). In the present study, we scored upregulation of WRKY6 at 12 h time point, while down-regulation of WRKY40 under drought stress in the tolerant cultivar GZ168. In GM10, WRKY50 is upregulated at 2 h time point, while two WRKY1, one WRKY18 and four WRKY40 are down-regulated (*Table 3*).

Recently, bZIP and NAC families were reported to regulate plant development, physiological processes as well as biotic/abiotic stress responses (Baloglu et al., 2014; Liu et al., 2014). Regulation of bZIP takes place at the post-translational level via the formation of heterodimers (Llorca et al., 2014). Kobayashi and colleagues determined the positive role of Wlip19 in transgenic tobacco under drought stress, especially during 6-12 h of drought treatment (Kobayashi et al., 2008). The wheat version of this bZIP also exhibited upregulation at 12-24 h under drought stress in wheat (Baloglu et

al., 2014). NAC constitutes one of the largest TF families in plant that also regulates plant development and biotic/abiotic stress responses. Baloglu and colleagues also found that transcription the wheat NAC69 is upregulated at 3 h and peaked at 12 h under drought treatment (Baloglu et al., 2014). A newly identified NAC gene, NAC67, in common wheat also gave response to drought stress conditions (Mao et al., 2014). In the present study, NAC nam-b2-like also peaked at 12 h time point of drought stress (Table 2). This TF was previously reported in wheat to participate in accelerating senescence and increasing grain protein concentration (GPC) with no prior known influence or response under drought stress (Distelfeld et al., 2012; Avni et al., 2014; Cormier et al., 2015).

Conclusion

In this work, we have detected a number of drought-stress responsive genes that might contribute to the tolerance mechanisms in wheat. In addition, we have detected the possible contribution of members of bHLH superfamily in conferring drought tolerance in bread wheat. Regulation patterns of some members of MYB, WRKY, bZIP and NAC families in the present study align with their known functions. It seems that wheat has several regulatory frameworks that are consulted upon exposure to any environmental stress. From the discussion of the present work, we can say that these frameworks are genotype-dependent. Some of the newly detected TFs can further be studied via knockdown and overexpression in order to confirm their contribution to drought stress tolerance in wheat. This study sheds light on the possible use of the newly recognized regulatory frameworks via metabolic engineering in conferring drought stress tolerance in wheat.

Data Availability Statement. Raw data of RNA-Seq is available in SRA database of the NCBI (<http://www.ncbi.nlm.nih.gov/sra>, SRA no. PRJNA306536).

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Conflicts of Interests. The authors declare no conflict of interests.

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APPENDIX

Table A1. Geographic locations and pedigrees of the two Egyptian wheat cultivars

Name	Geographic location	Pedigree
Giza 168	Delta, Egypt	MRL/BUC//SERT
Gemmiza 10	Delta, Egypt	MAYA74”S”/ON//1160-147/3/BB/GLL/4/CHAT”S”/5/CROW”S”

Table A2. TF code, ID and description and primer sequences along with the expected amplicon size (bp) utilized in validating RNA-Seq datasets via real time PCR

Code	ID	Description	Primer sequence (5’-3’)	Amplicon size (bp)
GZ1/G M1	Traes_1BL_1A8 85E733.1	bzip transcription factor superfamily protein isoform 1	F: CAATGGCAACTCTGAGGTC R: CTCACTGATTGCCCTTTCG	300
GZ2/G M2	Traes_6AL_A1 A99719B.1	ethylene-responsive transcription factor erf053-like	F: TTACTIONCAGTCTCGCTTCG R: AGAAAAGGTCATGAGATGTTCC	280
GZ3/G M3	Traes_2BS_148 4A7516.1	heat stress transcription factor a-3-like	F: ACAACTTCTCCAGCTTCGTCAG R: ATTGTGCTCCTCCTTCAGCCTG	300
GZ4/G M4	Traes_6AL_362 F82EAD.2	transcription factor asg4-like	F: CCCACAATTCTATCCCACCAG R: GATTTCTCATCAACAACAGTGC	300
GZ5/G M5	Traes_6BL_7E9 C131F4.2	transcription factor asg4-like	F: AAAGCGTAAAGCTGCCAC R: TCTGGCATAATGCGGAGTG	300
GZ6/G M6	Traes_4AS_8A6 4DBE8E.1	ethylene-responsive transcription factor rap2-2-like	F: GATAAGCTCCGAATCCAGCG R: TCGATCTTCTCAGCATCGGC	300
GZ7/G M7	Traes_4BL_78C 84E85A.1	ethylene-responsive transcription factor 1-like isoform x3	F: CCATTTTTTGACGTTTCCGTG R: ATGACACAAGATAACCAGGTTT	299
GZ8/G M8	Traes_5AL_1B2 15E169.1	wrky transcription factor 40-like	F: GTGCTCCATCTCCATCAAC R: TTTCTGAAGTTTCGCGTAGTC	298
GZ9/G M9	Traes_5BL_8BE F7F9CD.1	wrky transcription factor 40-like	F: TATGGAGGCGAAGTTCACG R: TTGACCTTGATTCTCCGGC	294
House-keeping		<i>actin</i>	F: GCCACACTGTTCCAATCTATGA R: TGATGGAATTGTATGTCGCTTC	369

<p>chlorophyll a-b binding protein of lhci type 1-like chlorophyll a-b binding protein of lhci type 1-like chorismate chloroplast chorismate chloroplast cinnamoyl- reductase 1-like cinnamoyl- reductase 1-like cinnamoyl- reductase 1-like cinnamoyl- reductase 1-like coupled to transmembrane movement of substances curcuminoid synthase-like curcuminoid synthase-like cysteine proteinase inhibitor 4-like cysteine proteinase inhibitor 4-like cysteine-rich receptor-like protein kinase 20-like cysteine-rich receptor-like protein kinase 26-like cysteine-rich receptor-like protein kinase 26-like cytochrome b6 cytochrome b6 f complex subunit iv cytochrome c oxidase subunit 1 cytochrome c oxidase subunit 1 cytochrome c oxidase subunit 1 cytochrome c oxidase subunit 1 dehydration-responsive element-binding protein dehydrin dhn3-like dehydrin dhn3-like dehydrin dhn3-like dehydrin dhn3-like dehydrin dhn3-like dehydrin dhn3-like dehydrin dhn3-like dehydrin dhn3-like dehydrin dhn3-like dehydrin dhn3-like dehydrin dhn3-like dehydrin dhn3-like dehydrin dhn3-like dehydrin dhn3-like dehydrin dhn3-like dehydrin dhn3-like delta-1-pyrroline-5-carboxylate synthase-like delta-1-pyrroline-5-carboxylate synthase-like delta-1-pyrroline-5-carboxylate synthase-like delta-1-pyrroline-5-carboxylate synthase-like delta-1-pyrroline-5-carboxylate synthase-like delta-1-pyrroline-5-carboxylate synthase-like dentin sialophospho dentin sialophospho dentin sialophospho dentin sialophospho dentin sialophospho isoform x4 epoxide hydrolase 3-like esterase pir7b-like esterase pir7b-like eukaryotic peptide chain release factor subunit 1-2 eukaryotic peptide chain release factor subunit 1-2 eukaryotic peptide chain release factor subunit 1-2 eukaryotic peptide chain release factor subunit 1-2 eukaryotic peptide chain release factor subunit 1-2 eukaryotic peptide chain release factor subunit 1-2 eukaryotic peptide chain release factor subunit 1-2 exoglucanase xynx-like exoglucanase xynx-like exoglucanase xynx-like exoglucanase xynx-like exoglucanase xynx-like exoglucanase xynx-like expansin-a2-like isoform 2 expansin-a2-like isoform 2 expansin-a2-like isoform 2 fasciclin-like arabinogalactan protein 10-like fasciclin-like arabinogalactan protein 10-like</p>	<p>cytochrome p450 86b1-like cytochrome p450 86b1-like cytochrome p450 86b1-like cytochrome p450 86b1-like cytochrome p450 86b1-like cytochrome p450 86b1-like cytochrome p450 86b1-like cytochrome p450 86b1-like cytochrome p450 86b1-like cytochrome p450 86b1-like cytochrome p450 86b1-like cytochrome p450 86b1-like cytochrome p450 86b1-like cytochrome p450 86b1-like cytochrome p450 86b1-like cytochrome p450 86b1-like cytochrome p450 86b1-like cytochrome p450 87a3-like cytochrome p450 89a2-like cytokinin dehydrogenase 9-like death-inducer obliterator 1-like defensin-like protein p322-like defensin-like protein p322-like defensin-like protein p322-like defensin-like protein p322-like dehydration-responsive element-binding protein dentin sialophospho isoform x4 dentin sialophospho isoform x4 dentin sialophospho isoform x4 dentin sialophospho isoform x4 dentin sialophospho isoform x4 dentin sialophospho isoform x4 dihydrolipoyllysine-residue acetyltransferase component of acetoin cleaving system-like dirigent protein 11-like dof zinc finger dof zinc finger dof zinc finger dof zinc finger dof zinc finger dof zinc finger dof zinc finger dof zinc finger dof zinc finger dof zinc finger dof zinc finger dolichyl-diphosphooligosaccharide--protein glycosyltransferase subunit 1-like dof246 domain-containing protein at1g04910-like e3 ubiquitin-protein ligase xb3-like e3 ubiquitin-protein ligase xb3-like e3 ubiquitin-protein ligase xb3-like ec protein i ii-like ec protein i ii-like elongation factor 2-like isoform x2 embryonic protein dc-8 precursor embryonic protein dc-8 precursor ent-cassadiene c2-hydroxylase-like ent-isokaurene c2-hydroxylase-like ent-isokaurene c2-hydroxylase-like isoform x2 epoxide hydrolase 3-like fatty acyl- reductase 1-like fatty acyl- reductase 1-like fatty acyl- reductase 1-like fatty acyl- reductase 1-like fe(2+) transport protein 1-like fe(2+) transport protein 1-like gdsl esterase lipase at1g28600-like gdsl esterase lipase at1g28600-like gdsl esterase lipase at2g23540-like gdsl esterase lipase at2g23540-like gdsl esterase lipase at2g23540-like gdsl esterase lipase at2g23540-like gdsl esterase lipase at4g16230-like gdsl esterase lipase at4g16230-like gdsl esterase lipase ex11-like gdsl esterase lipase ex13-like gdsl esterase lipase ex13-like gdsl esterase lipase ex13-like gdsl esterase lipase ex13-like gdsl esterase lipase ex13-like gdsl esterase lipase ex13-like</p>
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<p>monoglyceride lipase-like isoform 1 monoglyceride lipase-like isoform 1 myb-like transcription factor tc12-like nadh dehydrogenase subunit 6 nadh dehydrogenase subunit 6 nadp-dependent malic enzyme-like nadp-dependent malic enzyme-like nitrate reductase nitrate reductase non-cyanogenic beta-glucosidase non-specific lipid-transfer protein at5g64080-like nuclease harbi1-like nuclease s1-like nuclease s1-like nucleobase-ascorbate transporter lpe1-like nucleobase-ascorbate transporter lpe1-like nucleobase-ascorbate transporter lpe1-like nucleotide binding protein nucleotide binding protein oleosin 16 kda- partial oleosin 16 kda- partial oligopeptide transporter 7-like oligopeptide transporter 7-like oligopeptide transporter 7-like omega-hydroxypalmitate o-feruloyl transferase-like omega-hydroxypalmitate o-feruloyl transferase-like omega-hydroxypalmitate o-feruloyl transferase-like omega-hydroxypalmitate o-feruloyl transferase-like omega-hydroxypalmitate o-feruloyl transferase-like peroxidase p7- partial peroxidase p7- partial peroxidase p7- partial phosphomethylpyrimidine chloroplastic isoform x1 phosphomethylpyrimidine chloroplastic isoform x1 phosphomethylpyrimidine chloroplastic isoform x1 phosphomethylpyrimidine chloroplastic isoform x1 phosphomethylpyrimidine chloroplastic isoform x1 phosphomethylpyrimidine chloroplastic isoform x1 photosystem i assembly protein ycf4 photosystem ii 47 kda protein photosystem ii protein 1 phytoene chloroplastic-like phytoene chloroplastic-like phytoene chloroplastic-like pleiotropic drug resistance protein 6-like pleiotropic drug resistance protein 6-like pollen-specific leucine-rich repeat extensin protein 1 pollen-specific leucine-rich repeat extensin protein 1 pollen-specific leucine-rich repeat extensin protein 1 poly polymerase-like polygalacturonase inhibitor-like polygalacturonase inhibitor-like PREDICTED: beta-amylase-like PREDICTED: beta-amylase-like PREDICTED: beta-amylase-like PREDICTED: chemocyanin-like PREDICTED: chemocyanin-like PREDICTED: chemocyanin-like PREDICTED: chemocyanin-like PREDICTED: chemocyanin-like PREDICTED: chemocyanin-like PREDICTED: putative glutaredoxin-C14-like PREDICTED: remorin-like PREDICTED: remorin-like PREDICTED: titin-like probable adp-ribosylation factor gtpase-activating protein agd11-like probable aquaporin tip4-1-like probable aquaporin tip4-1-like probable aquaporin tip4-1-like probable aquaporin tip4-1-like probable calcium-binding protein cml10-like probable calcium-binding protein cml31-like probable carboxylesterase 5-like</p>	<p>mannan endo- -beta-mannosidase 1-like metal tolerance protein 7-like o-methyltransferase zrp4-like oryzain beta chain-like oryzain beta chain-like outer envelope pore protein 16- chloroplastic-like outer envelope pore protein 16- chloroplastic-like outer envelope pore protein 16- chloroplastic-like outer envelope pore protein 16- chloroplastic-like patatin group a-3-like patatin group a-3-like peptide transporter ptr2-like peptide transporter ptr2-like peroxidase 1-like peroxidase 1-like peroxidase 1-like peroxidase 1-like peroxidase 1-like peroxidase 2-like peroxidase 2-like peroxidase 2-like isoform 2 peroxidase 43-like peroxidase 43-like peroxidase 43-like peroxidase 52-like peroxidase 52-like peroxidase 72-like peroxidase 72-like peroxidase 72-like peroxidase 72-like peroxidase 72-like peroxidase 72-like peroxidase 72-like peroxidase p7- partial PREDICTED: chemocyanin-like PREDICTED: expansin-B3-like PREDICTED: expansin-B3-like PREDICTED: expansin-B3-like PREDICTED: expansin-B3-like PREDICTED: expansin-B3-like PREDICTED: glutaredoxin-C15-like PREDICTED: glutaredoxin-C15-like PREDICTED: glutaredoxin-C15-like PREDICTED: glutaredoxin-C15-like PREDICTED: glutaredoxin-C1-like PREDICTED: glutaredoxin-C1-like PREDICTED: glutaredoxin-C1-like PREDICTED: glutaredoxin-C1-like PREDICTED: glutaredoxin-C1-like PREDICTED: laccase-17-like PREDICTED: laccase-3-like PREDICTED: laccase-3-like PREDICTED: laccase-3-like PREDICTED: laccase-4-like PREDICTED: laccase-4-like PREDICTED: periaxin-like PREDICTED: periaxin-like PREDICTED: periaxin-like PREDICTED: periaxin-like PREDICTED: periaxin-like PREDICTED: periaxin-like PREDICTED: periaxin-like PREDICTED: periaxin-like PREDICTED: periaxin-like PREDICTED: periaxin-like inactive receptor kinase at5g10020-like isoform x2 probable indole-3-acetic acid-amido synthetase -like probable mixed-linked glucan synthase 3-like probable mixed-linked glucan synthase 3-like probable mixed-linked glucan synthase 3-like probable mixed-linked glucan synthase 6-like probable nad h-dependent oxidoreductase 1-like probable nad h-dependent oxidoreductase 1-like probable non-specific lipid-transfer protein akcs9-like probable non-specific lipid-transfer protein akcs9-like</p>
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<p>probable carboxylesterase 5-like probable carboxylesterase 5-like probable carboxylesterase 5-like probable carboxylesterase 5-like probable disease resistance protein at1g58602-like probable disease resistance protein at1g58602-like probable e3 ubiquitin-protein ligase herc2 isoform 2 probable e3 ubiquitin-protein ligase herc2 isoform 2 probable e3 ubiquitin-protein ligase herc2 isoform 2 probable e3 ubiquitin-protein ligase herc2 isoform 2 probable flavin-containing monooxygenase 1-like probable glucuronosyltransferase os03g0287800-like probable glutamate carboxypeptidase 2-like isoform 2 probable glutamate carboxypeptidase 2-like isoform 2 probable glutathione s-transferase gstu6-like probable glutathione s-transferase gstu6-like glycerophosphoryl diester phosphodiesterase 1 inactive receptor kinase at5g10020-like isoform x2 probable sulfate transporter -like probable thiol methyltransferase 2-like probable thiol methyltransferase 2-like probable wrky transcription factor 40-like probable wrky transcription factor protein 1-like proline-rich protein 4-like proline-rich receptor-like protein kinase perk10-like proline-rich receptor-like protein kinase perk2-like protein argonaute 7-like protein aspartic protease in guard cell 2-like protein hothead-like protein hothead-like protein hothead-like protein hothead-like isoform 1 protein hothead-like isoform 1 protein lhy-like protein lhy-like protein lhy-like protein lhy-like protein lhy-like protein lhy-like protein lhy-like protein mother of ft and tf 1-like protein mother of ft and tf 1-like probable mother of ft and tf 1-like probable mother of ft and tf 1-like protein notum homolog protein odorant1-like protein radialis-like 3-like protein srg1-like protein srg1-like purple acid phosphatase 4-like purple acid phosphatase 4-like purple acid phosphatase 4-like purple acid phosphatase 4-like purple acid phosphatase 4-like receptor-like serine threonine-protein kinase ale2-like retinitis pigmentosa 1-like 1 retinitis pigmentosa 1-like 1 retinitis pigmentosa 1-like 1 retinitis pigmentosa 1-like 1 retinitis pigmentosa 1-like 1 rho gdp-dissociation inhibitor 1-like ribosomal protein s18 ribose -bisphosphate carboxylase oxygenase large uni ring fyve phd zinc finger superfamily isoform 1 ring-h2 finger protein at10-like ring-h2 finger protein at10-like rna polymerase sigma factor chloroplastic mitochondrial- partial root phototropism protein 2-like root phototropism protein 2-like root phototropism protein 2-like root phototropism protein 2-like salt tolerance-like salt tolerance-like</p>	<p>probable non-specific lipid-transfer protein akcs9-like probable non-specific lipid-transfer protein akcs9-like probable non-specific lipid-transfer protein akcs9-like probable non-specific lipid-transfer protein akcs9-like probable non-specific lipid-transfer protein akcs9-like probable non-specific lipid-transfer protein akcs9-like probable non-specific lipid-transfer protein akcs9-like probable non-specific lipid-transfer protein akcs9-like probable non-specific lipid-transfer protein akcs9-like probable o-methyltransferase 2-like probable o-methyltransferase 2-like probable o-methyltransferase 2-like probable o-methyltransferase 2-like probable protein phosphatase 2c 8-like probable protein phosphatase 2c 8-like probable receptor-like protein kinase at1g80640-like probable salt tolerance-like protein at1g78600-like probable serine threonine-protein kinase at1g01540 probable serine threonine-protein kinase at1g01540 probable sulfate transporter -like protein srg1-like protein srg1-like protein tpx2-like protein tpx2-like protein tpx2-like protein transparent testa 12-like protein wax2-like protein wax2-like protein yls7-like protein yls7-like protein yls7-like protochlorophyllide reductase chloroplastic-like protochlorophyllide reductase chloroplastic-like protochlorophyllide reductase chloroplastic-like protochlorophyllide reductase chloroplastic-like protochlorophyllide reductase chloroplastic isoform 2 protochlorophyllide reductase chloroplastic isoform 2 protochlorophyllide reductase1 protochlorophyllide reductase1 protodermal factor 1-like protodermal factor 1-like pto-interacting protein 1-like pumilio homolog chloroplastic-like pumilio homolog chloroplastic-like isoform x2 pumilio homolog chloroplastic-like isoform x2 purine permease 3-like purine permease 3-like purine permease 3-like purine permease 3-like purple acid phosphatase 23- partial secologanin synthase-like secologanin synthase-like secologanin synthase-like secologanin synthase-like secologanin synthase-like seed maturation protein pm41 seed maturation protein pm41 serine threonine-protein kinase at5g01020-like serine threonine-protein kinase pbs1-like snf1-related protein kinase regulatory subunit gamma-1 soluble starch synthase chloroplastic amyloplastic-like soluble starch synthase chloroplastic amyloplastic-like s-type anion channel slah3-like sulfotransferase 16-like sulfotransferase 16-like sulfotransferase 16-like sulfotransferase 16-like tonoplast dicarboxylate transporter-like tonoplast dicarboxylate transporter-like tonoplast dicarboxylate transporter-like tonoplast dicarboxylate transporter-like</p>
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<p>salt tolerance-like salt tolerance-like salt tolerance-like salt tolerance-like sarcoplasmic reticulum histidine-rich calcium-binding sarcoplasmic reticulum histidine-rich calcium-binding transcription factor bhlh135-like* transcription factor bhlh49-like* transcription factor bhlh49-like* transcription factor bhlh49-like* transcription factor myb39-like* transcription factor myb39-like* transcription factor myb39-like* transcription factor myb39-like* transcription factor myb39-like* transcription factor myb39-like* transcription factor myb39-like* transcription factor pcf3-like transcription factor pcf3-like transmembrane protein 184c-like transmembrane protein 184c-like transport and golgi organization 2 homolog transport and golgi organization 2 homolog two-component response regulator-like prr1-like two-component response regulator-like prr1-like two-component response regulator-like prr1-like two-component response regulator-like prr1-like two-component response regulator-like prr1-like two-component response regulator-like prr1-like udp-glycosyltransferase 85a2-like udp-glycosyltransferase 85a2-like udp-glycosyltransferase 91c1-like udp-glycosyltransferase 91c1-like ultraviolet-b receptor uvr8-like ultraviolet-b receptor uvr8-like</p>	<p>tonoplast dicarboxylate transporter-like transcription factor asg4-like transcription factor asg4-like transcription factor asg4-like transcription factor asg4-like transcription factor asg4-like transcription factor asg4-like transcription factor bhlh135-like* uncharacterized abhydrolase domain-containing protein uncharacterized abhydrolase domain-containing protein uncharacterized acetyltransferase at3g50280-like uncharacterized acetyltransferase at3g50280-like upf0098 protein mth_273-like upf0098 protein mth_273-like vacuolar amino acid transporter 1-like vacuolar amino acid transporter 1-like vamp protein sec22 wall-associated receptor kinase 2-like wat1-related protein at1g21890-like wuschel-related homeobox 11-like wuschel-related homeobox 11-like xylem cysteine proteinase 1-like zf-hd homeobox protein at5g65410-like zinc finger protein constans-like 9-like zinc finger protein constans-like 9-like zinc finger protein constans-like 9-like zinc finger protein constans-like 9-like zinc finger protein constans-like 9-like zinc finger protein constans-like 9-like zinc finger protein zat11-like zinc finger protein zat11-like zinc finger protein zat11-like zinc finger protein zat8-like zinc finger protein zat8-like zinc finger protein zat8-like zinc finger protein zat8-like</p>
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(b) *GM10*

<p>11-beta-hydroxysteroid dehydrogenase 1b-like 12s seed storage globulin 1-like 18 kda seed maturation 18 kda seed maturation 18 kda seed maturation 18 kda seed maturation 2-hydroxy-6-oxo-6-phenylhexa- -dienoate hydrolase-like 3beta-hydroxysteroid-dehydrogenase decarboxylase isoform 1 3-hydroxybenzoate 6-hydroxylase 1-like 9-cis-epoxycarotenoid dioxygenase chloroplastic-like 9-cis-epoxycarotenoid dioxygenase chloroplastic-like 9-cis-epoxycarotenoid dioxygenase chloroplastic-like abc transporter g family member 5-like abc transporter g family member 5-like achilleol b synthase-like actin-depolymerizing factor 3-like actin-depolymerizing factor 3-like actin-depolymerizing factor 3-like adagio-like protein 3-like adenyl-sulfate chloroplastic-like alpha-amylase isozyme 3a-like alpha-amylase isozyme 3a-like alpha-amylase isozyme 3a-like alpha-amylase isozyme 3a-like alpha-amylase isozyme 3a-like alpha-amylase isozyme 3d-like alpha-amylase isozyme 3d-like antimicrobial peptide mbp-1 precursor chlorophyll a-b binding protein of lhci type 1-like chlorophyll a-b binding protein of lhci type 1-like</p>	<p>asparagine synthetase aspartic proteinase asp1-like aspartic proteinase nepenthesin-2-like auxin-induced in root cultures protein 12-like auxin-induced protein x10a-like auxin-induced protein x10a-like auxin-induced protein x10a-like auxin-induced protein x10a-like bidirectional sugar transporter sweet2b-like bidirectional sugar transporter sweet2b-like bidirectional sugar transporter sweet2b-like bidirectional sugar transporter sweet2b-like bifunctional epoxide hydrolase 2-like bz1p transcription factor superfamily protein isoform 1 casp-like protein os07g0442900-like chitinase 1-like chlorophyll a-b binding chloroplastic-like isoform 3 chlorophyll a-b binding chloroplastic-like isoform 3 chlorophyll a-b binding chloroplastic-like isoform 3 chlorophyll a-b binding protein chloroplastic-like chlorophyll a-b binding protein chloroplastic-like chlorophyll a-b binding protein chloroplastic-like isoform 3 chlorophyll a-b binding protein chloroplastic-like isoform 3 chlorophyll a-b binding protein chloroplastic-like isoform 3 chlorophyll a-b binding protein chloroplastic-like isoform 4 chlorophyll a-b binding protein chloroplastic-like isoform 5 chlorophyll a-b binding protein chloroplastic-like isoform 5 chlorophyll a-b binding protein chloroplastic-like isoform 6 chlorophyll a-b binding protein chloroplastic-like isoform 6 dehydrin dh3-like dehydrin dh3-like dehydrin dh3-like</p>
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<p>chlorophyll a-b binding protein of lhci type 1-like chlorophyll a-b binding protein of lhci type 1-like cinnamoyl- reductase 1-like cobra-like protein 2-like cold-regulated 413 plasma membrane protein 1-like cold-regulated 413 plasma membrane protein 1-like cortical cell-delineating cortical cell-delineating cysteine proteinase inhibitor 4-like cytochrome p450 71c4-like cytochrome p450 71d7-like cytochrome p450 734a6-like cytochrome p450 86b1-like cytochrome p450 86b1-like cytochrome p450 86b1-like cytochrome p450 86b1-like cytochrome p450 86b1-like cytochrome p450 93a1-like cytochrome p450 93a1-like cytochrome p450 94a1-like cytochrome p450 99a2-like cytochrome p450 99a2-like cytochrome p450 99a2-like defensin-like protein p322-like defensin-like protein p322-like defensin-like protein p322-like dehydration-responsive element-binding protein dehydrin dhnl ent-kaur-16-ene chloroplastic-like ent-kaur-16-ene chloroplastic-like esterase pir7b-like eukaryotic peptide chain release factor subunit 1-2-isoform 2 eukaryotic peptide chain release factor subunit 1-2-isoform 2 exoglucanase xynx-like fasciclin-like arabinogalactan protein 7-like fasciclin-like arabinogalactan protein 8-like flavanone 3-dioxygenase-like flavonol synthase flavanone 3-hydroxylase-like flavoprotein wrba-like flowering-promoting factor 1-like protein 5-like gamma-aminobutyrate transaminase mitochondrial-like gamma-aminobutyrate transaminase mitochondrial-like gdsl esterase lipase appg-like gdsl esterase lipase at1g28600-like gdsl esterase lipase at5g45910-like germin-like protein 8-11-like germin-like protein 8-11-like germin-like protein 8-4-like germin-like protein 8-4-like gibberellin 20 oxidase 2-like glutamate decarboxylase-like isoform 1 glutamate decarboxylase-like isoform 1 glutathione s-transferase chloroplastic-like glutathione transferase gst 23-like glycerophosphodiester phosphodiesterase gde1-like g-type lectin s-receptor-like serine threonine-protein kinase at2g19130-like heat shock factor protein 1 heat shock factor protein 1 lipid-transfer protein dir1-like lipid-transfer protein dir1-like l-lactate dehydrogenase a-like low quality protein: 5-pentadecatrienyl resorcinol o-methyltransferase-like low quality protein: 5-pentadecatrienyl resorcinol o-methyltransferase-like low quality protein: lysine-specific demethylase 8-like low quality protein: probable protein phosphatase 2c 37-like low temperature-induced</p>	<p>dehydrin dhnl-like dehydrin dhnl-like dehydrin dhnl-like dehydrin dhnl-like dehydrin dhnl-like dentin sialophospho isoform x4 dentin sialophospho isoform x4 dentin sialophospho isoform x4 dof zinc finger dof zinc finger dof zinc finger e3 ubiquitin-protein ligase e15- partial ec protein i ii-like elongation factor 1- elongation factor 1- elongation factor 1- elongation factor 1-alpha elongation factor 1-alpha embryonic protein dc-8 precursor endo- -beta-d-glucanase-like ent-copalyl diphosphate synthase 2-like isoform x1 ent-copalyl diphosphate synthase 2-like isoform x1 ent-copalyl diphosphate synthase 2-like isoform x2 ent-kaur-16-ene chloroplastic-like ent-kaur-16-ene chloroplastic-like ent-kaur-16-ene chloroplastic-like ent-kaur-16-ene chloroplastic-like homeobox protein bell1 homolog high molecular mass early light-inducible protein chloroplastic-like homeobox-leucine zipper protein hox24-like isoform 2 indole-3-glycerol phosphate chloroplastic-like indole-3-glycerol phosphate chloroplastic-like invertase inhibitor-like invertase inhibitor-like kda class i heat shock protein 1-like kda class i heat shock protein 1-like kda class i heat shock protein 1-like kda class i heat shock protein 3-like kda class i heat shock protein 3-like kda class i heat shock protein 3-like kda class i heat shock protein 3-like kda class i heat shock protein 3-like kda heat shock chloroplastic-like kda proline-rich kda proline-rich kda proline-rich protein precursor late embryogenesis abundant group 3-like late embryogenesis abundant group 3-like late embryogenesis abundant group 3-like late embryogenesis abundant protein d-34-like late embryogenesis abundant protein d-34-like late embryogenesis abundant protein d-34-like leucine-rich repeat extensin-like protein 2-like light-inducible protein cprf2-like isoform 1 lipid binding protein precursor lipid binding protein precursor lipid-transfer protein dir1-like lipid-transfer protein dir1-like pathogenesis-related protein 1-like pectinesterase inhibitor domain containing protein precursor peptide transporter ptr2-like peroxidase n-like phenolic glucoside malonyltransferase 1-like isoform x2 phosphomethylpyrimidine chloroplastic-like isoform x1 phosphomethylpyrimidine chloroplastic-like isoform x1 phosphomethylpyrimidine chloroplastic-like isoform x1 phytosulfokine receptor 2-like PREDICTED: serpin-ZX-like probable 1-deoxy-d-xylulose-5-phosphate chloroplastic-like probable aldo-keto reductase 2-like probable aldo-keto reductase 2-like probable e3 ubiquitin-protein ligase herc2-like isoform 2</p>
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<p>low temperature-induced lrr receptor-like serine threonine-protein kinase gso1-like lysine-specific demethylase 8-like lysine-specific demethylase 8-like lysine-specific demethylase 8-like lysine-specific demethylase 8-like lysine-specific demethylase 8-like lysine-specific demethylase 8-like nadp-dependent malic enzyme-like non-lysosomal glucosylceramidase-like obtusifoliol 14-alpha demethylase-like obtusifoliol 14-alpha demethylase-like o-methyltransferase zrp4-like o-methyltransferase zrp4-like o-methyltransferase zrp4-like isoform 4 oryzain beta chain-like otu domain-containing protein at3g57810-like outer envelope pore protein 16- chloroplastic-like outer envelope pore protein 16- chloroplastic-like oxalate oxidase gf- -like isoform 2 patatin group a-3-like isoform 1 probable protein phosphatase 2c 43-like probable protein phosphatase 2c 68-like probable protein phosphatase 2c 68-like probable protein phosphatase 2c 8-like probable protein phosphatase 2c 8-like probable protein phosphatase 2c 9-like probable wrky transcription factor 40-like probable wrky transcription factor 40-like probable wrky transcription factor protein 1-like protein lhy-like protein lhy-like protein lhy-like protein mother of ft and tf 1-like protein mother of ft and tf 1-like protein mother of ft and tf 1-like protein phosphatase 1 regulatory subunit 12c-like protein srg1-like protein transparent testa 12-like pumilio homolog chloroplastic-like pumilio homolog chloroplastic-like pumilio homolog chloroplastic-like isoform x2 pyrophosphate--fructose 6-phosphate 1-phosphotransferase subunit alpha-like respiratory burst oxidase homolog protein b-like retinitis pigmentosa 1-like 1 ring fyve phd zinc finger superfamily isoform 1 ring-h2 finger protein atl32-like salt tolerance-like salt tolerance-like sec14 cytosolic factor-like secologanin synthase-like two-component response regulator-like prr1-like u-box domain-containing protein 19-like ultraviolet-b receptor uvr8-like</p>	<p>probable e3 ubiquitin-protein ligase herc2-like isoform 2 probable glutathione s-transferase gstf1-like isoform 3 probable glutathione s-transferase gstu6-like monogalactosyldiacylglycerol synthase chloroplastic probable non-specific lipid-transfer protein 2-like probable non-specific lipid-transfer protein akcs9-like probable non-specific lipid-transfer protein akcs9-like probable non-specific lipid-transfer protein akcs9-like probable non-specific lipid-transfer protein akcs9-like probable non-specific lipid-transfer protein akcs9-like probable non-specific lipid-transfer protein akcs9-like probable non-specific lipid-transfer protein akcs9-like probable o-methyltransferase 2-like probable o-methyltransferase 2-like probable protein phosphatase 2c 30-like probable protein phosphatase 2c 30-like secologanin synthase-like seed maturation protein pm41 serine threonine-protein kinase at5g01020-like serine threonine-protein kinase sapk3-like sex determination protein tasselseed-2-like snf1-related protein kinase regulatory subunit gamma-1-like snf1-related protein kinase regulatory subunit gamma-1-like snf1-related protein kinase regulatory subunit gamma-like pv42a-like soluble starch synthase chloroplastic amyloplastic-like soluble starch synthase chloroplastic amyloplastic-like spx domain-containing membrane protein os02g45520-like s-type anion channel slah3-like subtilisin-like protease-like subtilisin-like protease-like sugar transport protein 1-like sugar transport protein 1-like sulfotransferase 16-like tetrahydrocannabinolic acid synthase-like tetrahydrocannabinolic acid synthase-like tetrahydrocannabinolic acid synthase-like tetrahydrocannabinolic acid synthase-like trans-cinnamate 4-monoxygenase-like transcription factor asg4-like transcription factor asg4-like transcription factor asg4-like transcription factor bhlh69-like transcription factor bhlh69-like transcription factor bhlh69-like transcription factor bhlh69-like transmembrane protein 184c-like zinc finger protein constans-like 3- partial zinc finger protein constans-like 3- partial zinc finger protein constans-like 9-like zinc finger protein constans-like 9-like zinc finger protein constans-like 9-like zinc finger protein constans-like 9-like zinc finger protein zat8-like zinc finger protein zat8-like upf0098 protein mth_273-like upf0098 protein mth_273-like wrky transcription factor 18-like wrky transcription factor 18-like</p>
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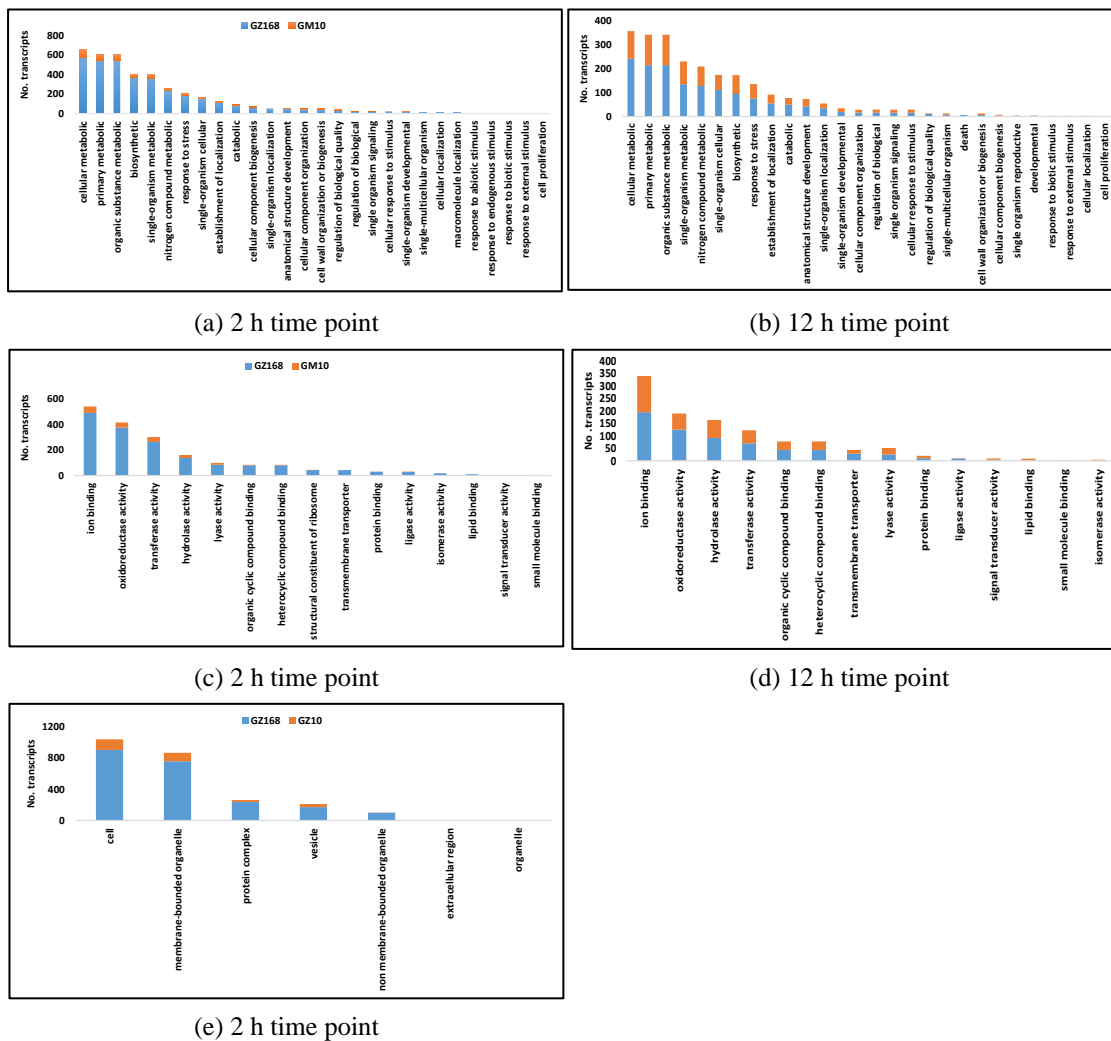


Figure A1. Gene ontology analysis describing the three main categories “biological process” (a & b), “molecular function” (c & d) and “cellular component” (e) for the two contrasting wheat genotypes GZ168 and GM10 based on the downregulated transcripts under drought stress conducted for 2 (a, c & e) and 12 h (b & d)

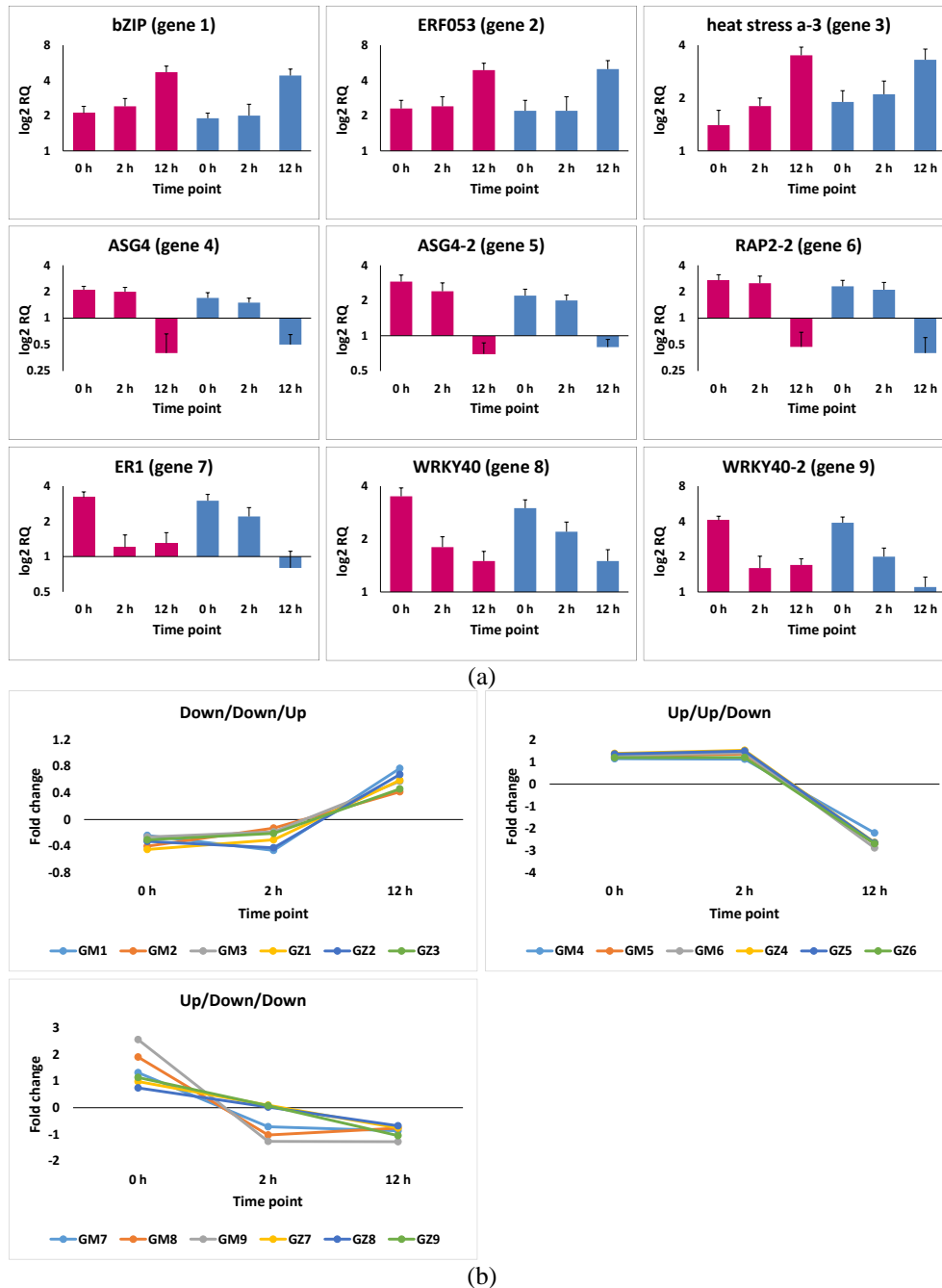


Figure A2. Real time PCR (a) and profiles (b) of fold change values of nine transcripts (1-9) resulted from RNA-Seq analysis for selected clusters (e.g., down/down/up, up/up/down and up/down/down) used for validating RNA-Seq data of leaves collected under drought stress at different time points (0, 2 and 12 h) for two contrasting genotypes GM10 (red columns) and GZ168 (blue column). Serial numbers GM1-9 and G1-9 refer to genes described in Tables 2 & 3. The “actin” gene was used as the unregulated house-keeping gene (Table A2)

Electronic Appendices

Electronic Appendix 1. Fold change values of assembled transcripts for RNA-Seq dataset of *T. aestivum* cultivar GZ168 showing the clusters of the expression pattern down/up/down at different time points (0, 2 and 12 h) of drought stress. (Part 1)

Electronic Appendix 2. Fold change values of assembled transcripts for RNA-Seq dataset of *T. aestivum* cultivar GZ168 showing the clusters of the expression pattern down/down/up at different time points (0, 2 and 12 h) of drought stress. (Part 2)

Electronic Appendix 3. Fold change values of assembled transcripts for RNA-Seq dataset of *T. aestivum* cultivar GZ168 showing the clusters of the expression pattern down/up/up at different time (Part 3)

Electronic Appendix 4. Fold change values of assembled transcripts for RNA-Seq dataset of *T. aestivum* cultivar GZ168 showing the clusters of the expression pattern up/down/up at different time points (0, 2 and 12 h) of drought stress. (Part 4)

Electronic Appendix 5. Fold change values of assembled transcripts for RNA-Seq dataset of *T. aestivum* cultivar GZ168 showing the clusters of the expression pattern up/up/down at different time points (0, 2 and 12 h) of drought stress. (Part 5)

Electronic Appendix 6. Fold change values of assembled transcripts for RNA-Seq dataset of *T. aestivum* cultivar GZ168 showing the clusters of the expression pattern up/down/down at different time points (0, 2 and 12 h) of drought stress. (Part 6)

Electronic Appendix 7. Fold change values of assembled transcripts for RNA-Seq dataset of *T. aestivum* cultivar GM10 showing the clusters of the expression pattern down/up/down at different time points (0, 2 and 12 h) of drought stress. Differentially expressed transcription factors are shown in the yellow rows. (Part 1)

Electronic Appendix 8. Fold change values of assembled transcripts for RNA-Seq dataset of *T. aestivum* cultivar GM10 showing the clusters of the expression pattern down/down/up at different time points (0, 2 and 12 h) of drought stress. (Part 2)

Electronic Appendix 9. Fold change values of assembled transcripts for RNA-Seq dataset of *T. aestivum* cultivar GM10 showing the clusters of the expression pattern down/up/up at different time points (0, 2 and 12 h) of drought stress. (Part 3)

Electronic Appendix 10. Fold change values of assembled transcripts for RNA-Seq dataset of *T. aestivum* cultivar GM10 showing the clusters of the expression pattern up/down/up at different time points (0, 2 and 12 h) of drought stress. (Part 4)

Electronic Appendix 11. Fold change values of assembled transcripts for RNA-Seq dataset of *T. aestivum* cultivar GM10 showing the clusters of the expression pattern up/up/down at different time points (0, 2 and 12 h) of drought stress (Part 5)

Electronic Appendix 12. Fold change values of assembled transcripts for RNA-Seq dataset of *T. aestivum* cultivar GM10 showing the clusters of the expression pattern up/down/down at different time points (0, 2 and 12 h) of drought stress. (Part 6)