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Diverse expression pattern of wheat transcription factors against abiotic stresses in wheat species

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ABSTRACT

Abiotic stress including drought and salinity affects quality and yield of wheat varieties used for the production of both bread and pasta flour. *bZIP*, *MBF1*, *WRKY*, *MYB* and *NAC* transcription factor (TF) genes are the largest transcriptional regulators which are involved in growth, development, physiological processes, and biotic/abiotic stress responses in plants. Identification of expression profiling of these TFs plays a crucial role to understand the response of different wheat species against severe environmental changes. In the current study, expression analysis of *TaWLIP19* (wheat version of bZIP), *TaMBF1*, *TaWRKY10*, *TaMYB33* and *TaNAC69* genes was examined under drought and salinity stress conditions in *Triticum aestivum* cv. (Yuregir-89), *Triticum turgidum* cv. (Kiziltan-91), and *Triticum monococcum* (Siyez). After drought stress application, all five selected genes in Kiziltan-91 were induced. However, *TaMBF1* and *TaWLIP19* were the only downregulated genes in Yuregir-89 and Siyez, respectively. Except *TaMYB33* in Siyez, expression level of the remaining genes increased under salt stress condition in all *Triticum* species. For determination of drought response to selected TF members, publicly available RNA-seq data were also analyzed in this study. *TaMBF1*, *TaWLIP19* and *TaNAC69* transcripts were detected through in silico analysis. This comprehensive gene expression analysis provides valuable information for understanding the roles of these TFs under abiotic stresses in modern wheat cultivars and ancient einkorn wheat. In addition, selected TFs might be used for determination of drought or salinity-tolerant and susceptible cultivars for molecular breeding studies.

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1. Introduction

Wheat is one of the three cereal grain crops, together with rice and maize, being used for human food and livestock feed for more than 5000 years (Peng et al., 2011). The world annual wheat production is about 671 million tons in 215 million ha of total cultivated area (FAO, 2012). It is estimated that the demand for wheat will increase to 60% by 2050. However abiotic and biotic stress factors may cause wheat production loss by 29% (Manickavelu et al., 2012). Therefore, new molecular genetic technology and plant gene sequence data have focused on improving tolerant cultivars against environmental stress factors.

The most widely cultivated wheat types in the modern world are hexaploid bread wheat (*Triticum aestivum*) and tetraploid pasta wheat (*Triticum durum*). Bread wheat ($2n = 6x = 42$, AABBDD) is believed to have emerged from two interspecies crossing events that took place between three distinct diploid species. It is believed that *Triticum urartu* (A genome donor) closely related to *Triticum monococcum* and *Aegilops speltoides* (B genome donor) participated in the first hybridization event that gave rise to the formation of *Triticum turgidum* ($2n = 4x = 28$, AABB). Bread wheat resulted from the second hybridization that took place between *T. turgidum*, cultivated in thousands of year as emmer wheat, and *Aegilops tauschii* providers of D genome (Dvorak and Akhunov, 2005). As a consequence of these hybridization events, bread wheat (*T. aestivum*) has three different genomes (A, B and D genomes) estimated to be 17 GB in size (Brenchley et al., 2012). Because of this genome formation, every bread wheat gene is potentially represented by at least six copies. Although bread wheat is believed to evolve from *T. urartu*, due to the extensive loss of genes, a genome of bread wheat has 6800 genes lesser than its progenitor (Hernandez et al., 2012; Ling et al., 2013).

Drought is one of the most important abiotic stress factors causing crop yield reduction together with soil salinity. Due to the increase in global temperature, drought stress or water shortage is projected to have a growing impact on plants and crop production (Fleury et al., 2010). Since plants are sessile organisms, they develop adaptation mechanism to cope with the detrimental effect of drought stress (Perez-Clemente et al., 2013). Cultivated wheat plants also have

Abbreviations: TF, transcription factor; qRT-PCR, quantitative reverse transcription PCR; SRA, Sequence Read Archive; NCBI, National Center for Biotechnology Information; IWGSP, International Wheat Genome Sequencing Consortium.

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drought tolerance mechanism including drought avoidance and dehydration tolerance (Nevo and Chen, 2010). However, genetic diversity was negatively affected from domestication and breeding programs resulted in decreased tolerance to environmental stresses (Nevo, 2007; Tanksley and McCouch, 1997). In this context, *A. speltoides* and *A. tauschii*, diploid progenitors of bread and durum wheat, have higher genetic diversity in terms of drought tolerance capacity than that of both cultivated wheat types (Farooq and Farooq-E-Azam, 2001; Rampino et al., 2006).

Abiotic stresses such as drought and high salinity induce biochemical, physiological, morphological and molecular responses in plants. Removal of reactive oxygen species (ROS) is achieved by enzymes as biochemical responses. During physiological response, many different strategies including stomatal closure, deep root systems and production of osmoprotectants were developed by drought tolerant plants to cope with water shortage stress (Blum, 2005; Bohnert et al., 1995). Plants can also adapt to stress conditions by changing the expression of stress responsive genes. Diverse set of genes related with drought stress response has been identified (Ingram and Bartels, 1996). Among them many families of transcription factors (TFs) that regulate the expression of many other downstream genes and gene clusters, have been shown to have important role in drought and salinity tolerance in wheat plants. Many families of TFs have been demonstrated to play a role in stress responses in plants. Among them, NAC (Manickavelu et al., 2012; Xue et al., 2006), C_2H_2 zinc finger (Kam et al., 2008), bZIP (Cao et al., 2012), and WRKY (Okay et al., 2014) families comprise a high proportion of abiotic stress responsive members.

The most detrimental stresses, drought and salinity adversely affect the growth and development of wheat, resulting in crop loss and yield reduction. Identification of expression profiling of TFs plays a crucial role to understand the response of different wheat cultivars against severe environmental changes. In this study, the effects of drought and salinity stresses on expression profiles of *TaWLIP19* (wheat version of bZIP), *TaMBF1*, *TaWRKY10*, *TaMYB33* and *TaNAC69* genes were investigated in three *Triticum* species [*T. aestivum* cv. (Yuregir-89), *T. turgidum* durum cv. (Kiziltan-91), *T. monococcum* (Siyez)]. Similar expression pattern was observed between *TaMBF1*, *TaWRKY10* and *TaWLIP19* genes under both drought and salt stress conditions in all three *Triticum* species. Genotypic variation in response of the *TaMYB33* and *TaWLIP19* genes to salt and drought stressed was also observed in this study. Monitoring expression changes may provide important information for understanding roles of these TFs under abiotic stresses in modern wheat varieties and ancient einkorn wheat. Selected TFs might be potential targets for determination of drought or salinity-tolerant and susceptible cultivars for molecular breeding studies.

2. Materials and Methods

2.1. Growth of Plants and Stress Applications

In this study, drought-tolerant wheat cultivar Kiziltan-91 (*T. turgidum* ssp. durum.), medium-drought tolerant wheat cultivar Yuregir-89 (*T. aestivum*) and low-drought tolerant einkorn wheat Siyez (*Tiriticum monococcum*) were used for gene expression studies. The seeds were obtained from Central Research Institute for Field Crops, Ankara (Turkey) and Ihsangazi Municipality, Kastamonu (Turkey). Seeds were surface sterilized with 10% NaOCl for 10 min and then washed three times with sterile distilled water. They were germinated and hydroponically grown in half-strength Hoagland's Solution (Hoagland and Arnon, 1950) for 10 days in a growth chamber at 24 ± 2 °C with 16-h light and 8-h dark photoperiod at a light intensity of 400 μmol m^{-2} s^{-1}.

All stress treatments were initiated after 10 days of normal growth. Stress treatments were applied using half-strength Hoagland's Solution containing 20% polyethylene glycol 6000 (PEG-6000) and 250 mM of sodium chloride (NaCl) for drought and salinity, respectively (Baloglu et al., 2012). Both treated (stress) and non-treated (control) plants were kept in the growth chamber with the same growth conditions. Leaf samples from treated and control plants were harvested after 0, 3, 12, and 24 h of stress application and immediately frozen in liquid nitrogen. Time point zero (0 h) was used as a control. Each set of experiment was repeated three times and samples from each set were used as biological replicates.

2.2. RNA Extraction and Characterization

Total RNA extraction was performed with TRIzol reagent (Life Technologies Corporation, Grand Island, NY, USA). DNA contamination in samples was removed with DNase I (Fermentas, Vilnius, Lithuania) according to the manufacturer's instructions. The quality and integrity of the total RNA was checked with agarose gel electrophoresis and the NanoDrop 2000D (NanoDrop Technologies, Wilmington, DE, USA).

2.3. cDNA Synthesis and Quantitative Real-Time RT PCR

cDNA synthesis was done from the RNase-free DNase treated total RNAs from three *Triticum* species [(*T. aestivum* cv. (Yuregir-89), *T. turgidum* durum cv. (Kiziltan-91), *T. monococcum* (Siyez)] that exposed both salt and drought stress. For qRT-PCR, we carried out cDNA synthesis using a 2 μg DNase-treated aliquot of the total RNAs as described in a Fermentas First Strand cDNA Synthesis Kit (Fermentas, Vilnius, Lithuania). PCR primers for real-time PCR were designed with the parameters of optimum primer GC content and 18–30 nucleotide length, and also an expected amplicon size of genes which was 135–151 bp (Table S1). To perform the expression level of five transcripts (*TaWRKY10*, *TaWLIP19*, *TaMBF1*, *TaMYB33* and *TaNAC69*) between three *Triticum* species, the qRT-PCR was carried out in 96-well plates, on the LightCycler480 II Real-Time PCR (Roche, Madison, USA). Experiments were performed as previously reported (Turktas et al., 2013). According to this reference, 2 μl of five times diluted cDNA was amplified with 1 pmol of the specific forward and reverse primers in a total volume of 18 μl, using 10 μl of 2× SYBR Green I Master (Roche Applied Science, Penzberg, Germany). All qPCR reactions were run in three independent biological and technical triplicates with a no-template control to check the contaminations. PCR program was set as 5 min at 95 °C, 55 cycles each of 10 s at 95 °C, 20 s at 54 °C and 72 °C at 10 s. Lastly, a melting curve analysis was performed to validate the presence of a single product and absence of primer-dimers. 18S rRNA was used as an internal control to normalize the variations in cDNA populations. Fold changes were calculated by the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001).

2.4. Expression Profiling of Transcription Factor Genes Using Transcriptome Data

To calculate the expression level of candidate genes, RNA-seq data were downloaded from the SRA database following accession numbers; ERX101742 (normalized cDNA from seedling leaves in a circadian time-course) and ERX101745 (normalized cDNA from various tissues, drought-stressed and senescent leaves, and leaves over a circadian time-course). NCBI SRA Toolkit's fastq-dump was used to convert sra files to fastq files. Low-quality reads ($Q < 20$) and adapter sequences were removed from raw RNA-seq data by using CLC Genomics Workbench 7. The quality of RNA-seq data was evaluated with FastQC in terms of per-base sequence qualities, per-sequence quality scores, per-base nucleotide content and sequence duplication levels before and after trimming. Clean reads were mapped to IWGSP genome assembly to reconstruct the transcript structures by using a CLC Genomics Workbench RNA-seq analysis tool with default parameters. The *T. aestivum* IWGSP annotated reference genome, chromosome survey sequence for *T. aestivum* cv. Chinese Spring generated by the International Wheat Genome Sequencing Consortium, was downloaded from

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