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Organ-Specific Gene Expression Profiles of Bread Wheat (*Triticum aestivum* L.) in Response to Combined Abiotic Stress Factors*

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Abstract: This study investigated the regulation of gene expression in root, leaf, and grain tissues of bread wheat (*Triticum aestivum* L.) in response to drought and heat stresses at the grain-filling stage for the first time by transcriptome analysis. The sequencing result, obtained on a Roche 454 GS FLX+, yielded a total of 117,790,028 base reads and 8,351 unigenes with an average length of 461 bp. Through transcriptome analysis, numerous transcripts have been identified to be involved in maintaining osmotic and ionic balance, detecting and transmitting signals, modifying protein structure and function, ensuring membrane integrity and stability, and are associated with energy and carbohydrate metabolism. Against drought and high-temperature stresses, tolerance mechanisms in the root, leaf, and grain tissues differentially regulated many specific transcription factors identified. *Betaine aldehyde dehydrogenase, callose synthase, cell wall-associated hydrolase, MYB33,* and *NAC69* transcription factors expression levels were measured with qRT-PCR. Transcriptome analysis revealed that the transcripts identified were related to osmotic and ionic balance, signal detection and transduction, modification of structural and functional proteins, cell membrane structure and stability, energy and carbohydrate metabolism, and their expression level varied according to the tissue or drought and high-temperature stress applied.

Keywords: Drought, grain, heat, leaf, root, transcriptome

1. Introduction

Increasing agricultural production is important to meet food demand and ensure food security for a growing global population (Fischer et al., 2014; Miralles et al., 2021). Contamination in agricultural resources, including fresh water and soil, limited plant nutrition resources, and climate change due to global warming are major threats to food security. Climate change, driven by global warming, changes in precipitation patterns, and an increase in the frequency and intensity of climatic extremes, has diminished food security and affected water security. These challenges make achieving the Sustainable Development Goals more difficult (Anonymous, 2021). Among various adverse weather conditions and climatic extremes, drought and heat are major abiotic stresses that limit crop production (Fahad et al., 2017; Senapati et al., 2019; Sabagh et al., 2021). Future projections of climate trends indicate that the frequency and intensity of drought and heat stresses will increase, with some regions of the world being more affected. Regions impacted by hot extremes and agricultural and ecological drought include Mediterranean countries, where wheat is widely grown as a staple crop.

Hexaploid (2n= 42) bread wheat (*Triticum aestivum* L.) is an important source of people's daily calories with high carbohydrate content as well as protein, vitamins and minerals. The most widely grown crop in the world in terms of meeting 20% of its consumption (Xi et al., 2023). Although generally, wheat is an indispensable product of droughty and semi-droughty regions, high

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temperature and drought conditions lead to significant decreases in yield and quality (Pequeno et al., 2021). Drought stress is characterized by reduced water content, limited leaf water potential, and loss of turgor, closure of stomata, decreased cell growth and development (Pour-Aboughadareh et al., 2020). Temperature stress is generally defined as the temperature rising above the threshold level that causes irreversible damage to plant growth and development for a certain period (Lal et al., 2021).

Plants have several adaptations to survive under different stress conditions. These adaptations can be at the physical, morphological or molecular levels. Tolerance mechanism against high temperature and drought stress factor; signal transduction and regulatory pathways (Saidi et al., 2023) or stress genes encoding proteins that provide resistance (Wang et al., 2004) or functional and involves the regulation of enzymes responsible for the synthesis of structural metabolites (Rontein et al., 2002; Park et al., 2004). Perception of stress and signals to appropriate responses conversion is the key step leading to stress tolerance in the plant (Shiriga et al., 2014). The combined effect of high temperature and drought stress reduces plant photosynthetic capacity through chloroplast membrane, thylakoid lamellar damage and metabolic limitation (Chaidee and Pfeiffer, 2006; Wang et al., 2010; Pradhan et al., 2012). In addition, in a study of the tobacco (*Nicotiana tabacum* L.) plant, the effects of heat stress and drought stress on gene expression were examined together (Rizhsky et al., 2002; Wang et al., 2003). The effect of drought and temperature together was like drought stress alone in terms of suppressing photosynthesis and similar to temperature stress in terms of increased respiration. Therefore, the plant is exposed to drought and high-temperature stress; it undergoes morphological, physiological, biochemical, and molecular changes regulated by many genes (Rahaie et al., 2010). The importance of the transcriptome analysis technique, which provides tolerance to abiotic stress factors such as drought, high temperature, etc. and enables the regulation of gene expression as part of the plant stress response and the examination of all transcript levels of plant genes, has been rapidly increasing in recent years. The new generation sequencing technology has caused dramatic changes in genome-level studies because it provides reliable results in all organisms regardless of genome size, requires less sequencing, lower sequencing cost per base, and is fast, autonomous, and easily applicable (Hawkins et al., 2010).

This study aimed to determine the gene expression regulation in the root, leaf and grain tissues of bread wheat (*T. aestivum*) in response to drought and high-temperature stresses treatments at the whole transcriptome level.

2. Materials and Methods

2.1. Plant material

The bread wheat (*T. aestivum*) cultivar, cv. Zubkov, was used as the plant material in this study. The seeds of the plant material were kindly provided by the International Maize and Wheat Improvement Center (CIMMYT) Türkiye office. The cultivar, originating from Kyrgyzstan, is a winter-type bread wheat with a high yield under drought conditions (Mursalova et al., 2015).

2.2. Growth conditions

Seeds of wheat cultivar were treated with fungicide (Dividend 2 DS, Syngenta) against seed-borne disease. Fourteen seeds were sown by hand at a depth of 3 cm in pots (pot diameter at the top and bottom was 28 and 26 cm, respectively; pot depth was 28 cm) filled with field soil in early November. There were 16 pots for the experiment. After sowing, the pots were transferred to the field and buried at ground level to maintain them in field conditions and prevent frost damage to the root zones. Before the tillering stage, the plants were tinned to seven plants per pot. Plants were grown under natural conditions until flowering.

2.3. Stress treatments

Two growth chambers (Model PG42 and PG36, Digitech, Ankara, Türkiye) were used for stress treatments. The stress treatments (drought, heat and drought with heat stresses) were exposed to plants as described by Pradhan et al. (2012) with some modifications. Four pots per stress treatments were randomly transferred from the field to growth chambers. Both growth chambers are maintained at a day/night temperature regime of 24/14 °C with a 16/8 h photoperiod. After 50% flowering was observed in the plants, one growth chamber's temperature was increased gradually at 34/24 °C (day/night) with a 3 day transition period. To avoid the negative effect of high temperature on pollen viability and fertilization, stress treatments were applied after fertilization. At the same time half of the plants (four pots) in each temperature regime were subjected to drought stress by withholding water for 15 days, and the other half was fully irrigated. In the drought stress treatment, all pots were filled with water up to the field capacity during the flowering stage and no water was given to the pots to be subjected to drought stress from this point onwards. After stress treatments, all plants were transferred from growth chambers to field

conditions. The experiment was performed in three biological replicates.

2.4. Sample collection

Root, leaf and grain samples were collected randomly from 3 different pots containing both control and stress-treated plants at the end of the 15th day of stress treatments. The collected samples were placed in separate transparent bags and rapidly preserved in liquid nitrogen and the materials were placed in a -80 °C deep freezer for RNA isolation studies.

2.5. RNA isolation, transcriptome cDNA library preparation and sequencing

The TRIzol (Ambion, TX, USA) method was used for total RNA isolation from root and leaf tissue. Since the TRIzol method was insufficient to isolate RNA from cereal samples due to their high starch content, the Ma and Yang (MMY) method was used (Mornkham et al., 2013). The quality and concentration of the total RNA samples were examined using the NanoDrop spectrophotometer (ND-2100c, Thermo Scientific). DNA was removed by digestion with RNase-free DNase (Qiagen), and RNA was purified and concentrated using an RNeasy column (Qiagen). RNA quality was evaluated by gel electrophoresis, spectrophotometer analysis and an Agilent 2100 High Sensitivity bioanalyzer. The cDNA library of 12 samples (control root, control leaf, control grain, drought root, drought leaf, drought grain, high-temperature root, high-temperature leaf, high temperature grain, high-temperature and drought root, high temperature and drought leaf, high temperature and drought grain) selected for RNA sequencing was prepared in according to the Roche 454 GS FLX+ protocol (Trachtenberg and Holcomb, 2013). The prepared cDNA libraries were sequenced using the Roche 454 GS FLX+ Titanium Sequencing Kit XL+ (Roche 454 Life Science, Mannheim, Germany) according to the protocol.

2.6. Bioinformatics analysis

Adapter regions and low-quality reads were discarded from the reads generated after sequencing, and these reads of libraries were assembled separately using the GS De novo Assembler program on the computer of the Roche 454 GS FLX+ device. Sequencing results were mapped onto the genome of haploid bread wheat (*T. aestivum*) published by the International Wheat Genome Sequencing Consortium-IWGSC (Anonymous, 2015a) which is approximately 6.6 Gb in size. Reads Per Kilobase per Million mapped reads (RPKM) calculations were completed for each library using CLC Genomics Workbench v6.0.4, CLC Bio software (CLC Inc, Denmark). After RPKM calculations, all nucleotides and Expressed Sequence Tags (ESTs) were downloaded from the National Center of Biotechnology Information (NCBI) database to identify the transcripts of each library that showed changes in expression level compared to the control group, and BlastN 2.2.28 algorithm was performed with each transcript sequence according to the E-value 1e-10 parameter with Bio-Linux 8 software (Field et al., 2006). In addition, for each library of root, leaf and grain samples, transcription factors were downloaded from the Plant Transcription Factor Database (PlantTFDB v3.0; Anonymous, 2015b) to obtain the relevant transcription factors and BlastX 2.2.28 was performed with Bio-Linux 8 software with an E-value of 1e-5.

2.7. Quantitative real-time polymerase chain reaction analysis

To verify the transcriptome data, the expression level of the selected five genes (betaine aldehyde dehydrogenase, cell wall-associated hydrolase, callose synthase, MYB33 and NAC69) were measured via quantitative Real-Time Polymerase Chain Reaction (qRT-PCR) system. Using a LightCycler 480 Instrument II (Roche, Germany) and the SYBR Green I Master Kit (Roche Germany), qRT-PCR was carried out as previously described (Turktas et al., 2013). The qRT-PCR was carried out in 96-well optical plates, and PCR reactions were performed in a total volume of 20 µl containing 0.1 µl each of the primers (100 pmol), 2 µl of cDNA, 10 µl FastStart SYBR Green I Master Mix and nuclease-free water was added up to 20 µl. The 18S rRNA gene was used as the normalizer gene, and five gene-specific PCR primers were designed using the Primer3Plus software. The qRT-PCR conditions were set up as follows: preheating at 95 °C for 5 min followed by 50 cycles of 95 °C for 10 s; 53 °C or 55 °C for 20 s; and 72 °C for 10 s. The melting curves were adjusted to 95 °C for 5 s and 55 °C for 1 min and then cooled to 40 °C for 30 s. A list of the primers used in qRT-PCR is presented in Table 1. For each biological replicate, the reaction was repeated three times. The mean-signal intensity throughout the three replicates was used to compute the expression levels.

3. Results

3.1. Transcriptome sequencing and assembly

Analysis of the transcriptome sequencing obtained from the Roche 454 GS FLX+ revealed that the 12 libraries, consisting of root, leaf, and grain samples from both control and stress treatment groups, produced clean reads ranging from 14.430 to 32.293, with read lengths between

Table 1. Selected genes and their primer sequences used for the validation of transcriptome results

Description	Tm (°C)	Product size (bp)	Forward primer (5′ ->3 ′)	Reverse primer (5′ ->3 ′)
Callose synthase	57	124	TGATCGTTCCTTAGCCGTGT	TCCGAGTTCTTAGCACACGT
Cell wall hydrolase	59	115	AGCAGGACATAGTGATCCGG	CCACGGAAGATAGGGACCAA
Betaine aldehite dehydrogenase	56	128	TCATCACTGACATCAACACATCA	TCGATGGCTTCCTCTTCAGT
NAC69	57	119	GGCTACGTGAACATCGACAC	TGGTTCTTCACATGTGCAGC
MYB33	56	127	GACGAGATGGACTTCTGGGT	GGATGAACACCGCTACGAAC

437 bp and 507 bp. The reads from 12 libraries were de novo assembled using the GS De Novo Assembler program, resulting in the creation of contigs. Since the reference genome information for the bread wheat plant is publicly available, transcript identification and expression level determination were performed using the chromosome-separated genome information. Using the whole genome information of approximately 6.6 Gb in size haploid bread wheat published by the IWGSC, the sequenced reads were mapped onto the haploid bread wheat (*T. aestivum*) genome using the GS Reference Mapper program, and contigs were obtained. After mapping to the reference genome, we detected 8.351 unigenes. The mapped total read and base number values indicate that 90-95% of all library reads were aligned to the reference genome. Among the libraries, the lowest numbers of unigene number, total contig number and total base number were observed in the control grain library with 157, 256, 34.456 and the highest numbers were observed in the high temperature leaf library with 967, 1127, 611.665. In addition to the number of transcripts obtained, the size of the transcripts consisting of short sequences is also important. The parameter N50, which indicates the relationship between transcript length and the success of transcriptome analysis, ranged between 720 and 1756 among the samples. The highest N50 value was determined in the high-temperature leaf sample by the results obtained from reference genome mapping (Table 2).

3.2. Regulation of transcripts in response to stress treatment

Each stress treatment group was evaluated in terms of gene expression level with the control group, and a total of 9 different transcriptome profiles were obtained as a result of these analyses. Among the 2148 transcripts with altered expression levels, 824, 735, and 589 were differently regulated in response to high temperature, drought, and a combination of drought and high-temperature stresses, respectively (Figure 1). When comparing different tissues, grain samples exhibited a higher number of differentially regulated transcripts in response to stress factors, whereas root samples exhibited fewer such responses. The drought root sample had the fewest regulated expressions, with 171 transcripts, while the drought grain sample had the most changes, with 390 transcripts.

The expression level of 286 transcripts out of 390 transcripts increased in the grain (drought grain) sample under drought stress compared to the control group, while the expression level of 104 transcripts decreased. Among the differentially

Table 2. Summary of *T. aestivum* transcriptome sequences analyzed

Sample	Total number of readings mapped	Total number of bases mapped	Number of all mappings	Number of fragmented mappings	Number of not mapped	N50 contig length	Unigene number	Total number of contigs	Total number of bases
Control root	21.929	9.293.965	13.400	2.899	769	1195	671	770	441.573
Control leaf	18.902	7.802.759	14.706	2.084	48	1217	867	968	565.964
Control grain	22.105	9.893.373	20.986	1.223	36	720	157	256	34.456
Drought root	22.105	9.609.853	15.467	2.478	307	1409	748	874	466.410
Drought leaf	23.422	10.844.006	17.757	2.822	86	1456	672	797	506.550
Drought grain	8.660	3.112.866	3.587	12.922	761	835	882	1013	521.818
High temperature root	14.868	5.740.835	6.737	2.953	710	1104	836	970	510.124
High temperature leaf	19.407	8.766.288	14.161	2.484	139	1756	967	1127	611.665
High temperature grain	20.548	8.988.402	17.196	10.079	480	960	383	558	268.468
High temperature + drought root	13.917	5.312.627	6.792	2.729	503	1185	771	902	475.942
High temperature + drought leaf	16.889	7.285.293	11.241	2.419	106	1465	715	808	484.486
High temperature + drought grain	21.631	10.025.680	16.076	2.217	67	1654	682	865	480.860

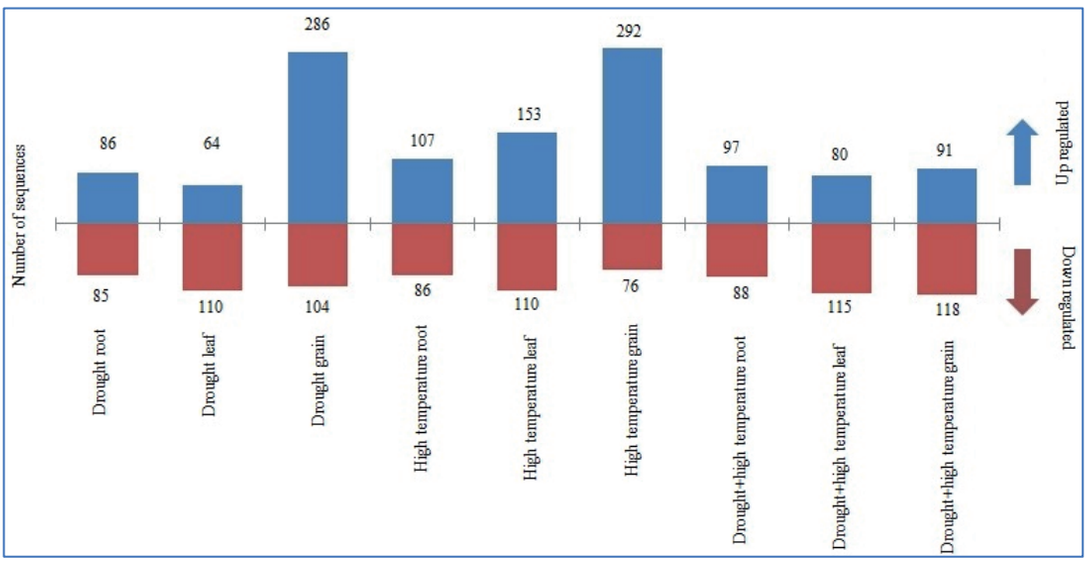

Figure 1. Number of transcripts which expression changed according to stress treatments in root, leaf and grain samples*

*: p≤ 0.05 and 1.5-fold change was used to consider differential expression of genes

expressed transcripts, the expression levels of β-glucosidase (+4.88-fold), glutamate synthase (+2.8-fold), and serine/threonine kinase (+1.9-fold) were significantly upregulated. Additionally, in drought-stressed leaves, 110 out of 174 transcripts related to photosynthesis were downregulated, while 64 were upregulated in response to drought. In Photosystem II activity, a decrease was detected in the expression level of and ribulose 1,5-bisphosphate carboxylase/oxygenase gene (-3.38 fold) where the genes significantly upregulated in leaves under drought stress. In root tissue subjected to drought stress (drought root), 85 out of 171 transcripts exhibited decreased expression levels, while 86 transcripts showed increased expression levels. Notably, there was an up-regulation of transcripts encoding calcium-dependent protein kinase, aldose reductase, auxin response protein, and apocytochrome b. Conversely, the expression levels of *cytochrome c oxidase* and *ATP sulfurylase* genes decreased. As a result of high-temperature stress in grain samples (high-temperature grain), 292 out of 368 transcripts exhibited increased expression levels, while 76 transcripts showed decreased expression levels compared to those in control samples. Notably, the expression levels of *Acetyl CoA dehydrogenase* and *glutamate synthase* increased by 5.77-fold and 2.84-fold, respectively, whereas the expression level of the *uridine diphosphate-glycosyltransferase* gene decreased by 2.04-fold. Among 263 transcripts differentially regulated in leaves, 110 exhibited decreased expression levels, and 153 showed increased expression levels in response to high-temperature stress. In leaf tissue, a decrease in the expression level of ribulose 1,5-bisphosphate carboxylase/oxygenase (-4.82 fold), indole-2-monooxygenase (-2.58 fold) transcripts were observed, while the expression level of *glucose-6-phosphate dehydrogenase* and *peroxidase* antioxidant enzyme and *heat-shock protein* genes increased. In root tissue, the expression level of 86 of 193 transcripts decreased and 107 of 193 transcripts increased; the expression level of *catalase*, *prolyl hydroxylase* (+3.45 fold), *calmodulin* (CaM) genes increased, while the expression level of *cytochrome c oxidase* (COX) and *ATP synthase* gene decreased. Combined high temperature and drought stress resulted in the differential regulation of 185, 195, and 209 transcripts in root, leaf, and grain tissues, respectively. Among these, 79 transcripts in root, 57 transcripts in leaf, and 67 transcripts in grain were uniquely regulated in response to the combined high temperature and drought stress. In the grain sample subjected to combined high temperature and drought stress, 91 out of 209 transcripts showed increased expression levels, while 118 transcripts exhibited decreased expression levels. Notably, the expression level of the g*lucose-6-phosphate isomerase* gene which plays a crucial role in ammonium assimilation and can be down-regulated due to stress factors such as high temperature or drought, decreased by 6.06-fold. Conversely, there was an increase in the expression levels of callose synthase, *serine/threonine protein kinase* (by 1.12-fold), and *glutathione synthetase* (by 4.53-fold). These genes are involved in the mechanical strengthening of the cell wall through the accumulation of lignin and cellulose. The increase in glutathione biosynthesis in grain under combined high temperature and drought stress enhances the

cellular defense mechanism against oxidative stress and increases the metal-binding capacity.

3.3. Transcription factors in response to high temperature and/or drought stress

We found 920 transcripts encoded a transcription factor (TF) were found to be distributed into 37 TF families. The TF families with the highest number of transcripts were; myeloblastosis (MYB, 132 transcripts), Lateral Boundary Domain (LBD, 125 transcripts), WRKY transcription factor family (56 transcripts), zinc finger C2H2 (zfC2H2, 55 transcripts), NAM, ATAF1,2, CUC domain protein (NAC, 54 transcripts), Auxin Response Factor (ARF, 48 transcripts), GRAS transcription factor family (zfGRAS, 48 transcripts), basic-leucine zipper transcription factor family (bZIP, 47 transcripts), Far-Red Impaired Response1 transcription factor family (FAR1, 46 transcripts), helix-loop-helix (bHLH, 42 transcripts), ethylene response factor (ERF, 33 transcripts), single-finger DNA-binding protein (Dof, 23 transcripts) and GATA transcription factor family (zfGATA, 19 transcripts). The results show that transcription factor families containing a large number of transcripts are functional and highly expressed in drought, high temperature, combined high temperature and drought stress treatment groups as abiotic stress response compared to control groups. NAM, ATAF1,2, CUC (NAC) domain proteins, which are plant-specific transcriptional regulators, have important roles such as lateral root formation, defense and abiotic stress responses, and their expression increased in root, leaf and grain samples as a result of drought, high temperature and combined high temperature and drought stress compared to the control group. Myeloblastosis (MYB) is one of the transcription factors that play an important role in drought stress response and responds to stress in an abscisic acid-dependent manner.

3.4. Confirmation of transcriptome results by qRT-PCR

To verify the results obtained after determining the genes whose expression levels changed as a result of stress treatments by transcriptome analysis, the expression changes of *betaine aldehyde dehydrogenase, cell wall-associated hydrolase, callose synthase* genes and *NAC69* and *MYB33* transcription factors in the root, leaf and grain of Zubkov bread wheat variety tolerant to drought and high-temperature stress were determined by qRT-PCR using three different biological replicates (Figure 2). *Betaine aldehyde dehydrogenase* increases the amount of the osmotic protector glycine betaine. In agreement with the transcriptome data, gene expression was significantly increased in root and leaf tissue, especially after both stresses were applied together. The expression of the *cell wall-associated hydrolase* gene increased in leaf and grain as a result of high-temperature stress compared to the control

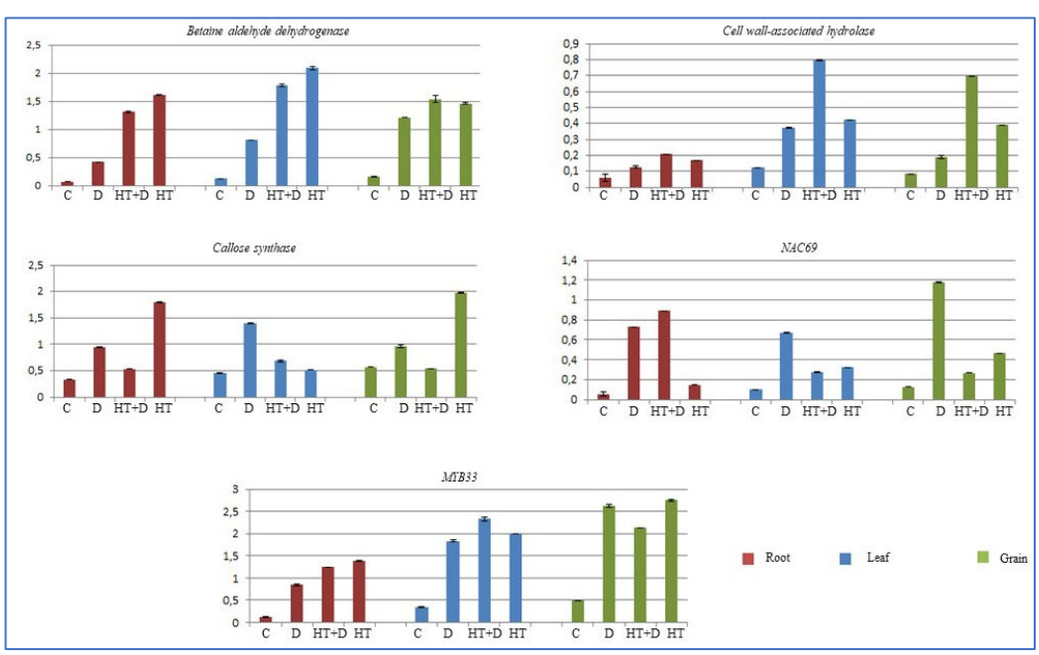

Figure 2. Determination of expression levels of some genes and transcription factors responding to stress applications by qRT-PCR

C: Control, D: Drought, HT+D: High temperature + drought, HT: High temperature

groups, while lower expression level was detected in root. The expression of *callose synthase* gene increased in grain and root as a result of drought and high-temperature stresses, and in leaf tissue as a result of drought stress. *NAC69* transcription factor has an important role in abiotic stress response. It exhibited a profile in which its expression level increased in all three groups, root, leaf and grain, as a result of drought and high-temperature **s**tress. The results are in agreement with the transcriptome data, with increased expression levels in grain as a result of drought stress and in root as a result of high-temperature stress. MYB33 transcription factor expression, which is involved in abscisic acid-dependent stress response, was in parallel with the transcriptome data.

4. Discussion and Conclusion

The increased frequency and intensity of drought and heat stress due to climate change will adversely affect crops grown mainly under natural conditions in arid and semi-arid regions. Bread wheat, which has a broad adaptive capacity, is generally essential for arid and semi-arid regions, but is significantly affected by stresses such as drought and high temperature (Sareen et al., 2023). Understanding the molecular, cellular and physiological adaptation to drought and high-temperature conditions is possible by elucidating the signal transduction mechanisms and perception of stress signals in plants, the activation of a large number of transcription factors and other regulators, and the expression products of genes (Nouraei et al., 2022). In this study, using Roche 454 GS FLX+ technology, RNA-sequencing was performed for root, leaf tissue and grain after drought, high temperature, high temperature + drought stresses were applied in the Zubkov wheat variety, which is known to be tolerant to drought and high temperature. In this way, changes in gene expression were determined and genes, and transcription factors thought to be involved in the stress resistance mechanism were identified. In addition, explanatory information on the role of the identified transcripts in molecular and biological processes and their relationship with metabolic pathways was obtained. According to the results of the study, transcripts showing expression changes after stress treatments were determined to be associated with stress response. Depending on the expression change of transcripts, responses such as antioxidant activity, production of metabolites involved in maintaining osmotic and ionic balance, photosynthesis, cell membrane and protein modification, as well as a response to hormones such as abscisic acid, ethylene or auxin, which are closely related to stress tolerance, were observed (Mikołajczak et al., 2023). The expression of glutamate synthase, which participates in nitrogen metabolism and osmotic balance in the grain, increases due to drought stress. In a study conducted on maize, Lu et al. (2020) reported that the expression of glutamate synthase increased as a result of drought stress, leading to an increase in the protein content of grains.

Transcription factors are regulatory proteins responsible for the regulation of gene expression. They perform their cell type-specific functions by binding specifically to cis-acting elements in the promoter regions of target genes (Nakashima et al., 2014). In our study, it was observed that NAC domain protein expression increased in root, leaf tissue and grain samples due to drought and high-temperature stress. Nakashima et al. (2012) reported that OsNAC5 and OsNAC6 expression in rice (*Oryza sativa* L.) increased significantly under drought and high-temperature conditions. Myeloblastosis transcription factors are transcription factors that play an important role in the regulation of developmental processes and stress in plants (Adel and Carels, 2023). The expression of the MYB transcription factor, which is involved in abscisic acid-dependent stress response, was significantly increased especially in the grain stress treatment groups. Chunhua et al. (2014) showed that MYB transcription factors play a role in plant development, metabolism and stress responses in rice. At the same time, it was reported that cellulose synthase genes were stimulated and cellulose content increased in grain due to increased MYB TF expression (Qin et al., 2008). It was observed that GRAS transcription factor expression increased due to the increase in antioxidant activity in leaf tissue subjected to drought and high-temperature stress. Heat-shock factors (HSF) have a central role in the stress-dependent and developmental stage-dependent expression of heat-shock proteins (HSPs) in plants. In our study, Rubisco activase expression, which is involved in Rubisco catalytic metabolism, increased due to high-temperature stress application compared to the control group and the transcript associated with heat-shock factors was detected in root, leaf and grain samples in high temperature and drought + high-temperature stress groups. Plants exposed to stress factors may respond to stress through a wide range of stress-related proteins, metabolites and epigenetic regulation (Vranić et al., 2023; Chinnusamy and Zhu, 2009). Through transcriptome analysis, it was determined that betaine aldehyde dehydrogenase expression increased in leaf and root tissue as a result of high temperature + drought stress and the results were confirmed by qRT-PCR. Wang et al. (2010) reported

that betaine aldehyde dehydrogenase activity increased in transgenic bread wheat lines as a result of drought and heat stress. With betaine aldehyde dehydrogenase activation, the amount of glycine betaine increases, contributing to the stress tolerance mechanism, glycine betaine is localized in chloroplasts and plays an important role in the protection of chloroplast structure, and thylakoid membranes and maintains photosynthetic activity and plasma membrane integrity. As in grain, an increase in the expression level of the callose synthase gene, which provides lignin and cellulose accumulation in root tissue due to high temperature + drought stress, was determined and confirmed by qRT-PCR. In addition, the expression of kinase activity, serine/threonine protein kinase and leucine-rich repeat receptor kinase increased in leaves.

In conclusion, the transcriptome profile of root, leaf tissue and grain samples of the Zubkov variety, which is known to be tolerant to drought and high-temperature stress, after drought, high temperature, combined high temperature and drought stress were applied together, were analyzed using Roche 454 GS FLX+ technology in this study. Depending on the stress conditions, it was determined that cellular, physiological and molecular regulations and resistance mechanisms against stress factors exist in the plant. Transcriptome analysis revealed that the transcripts identified were related to osmotic and ionic balance, signal detection and transduction, modification of structural and functional proteins, cell membrane structure and stability, energy and carbohydrate metabolism, and their expression level varied according to the tissue or drought and high-temperature stress applied. In addition, many transcription factors specific to root, leaf and grain samples were identified in the tolerance mechanism against drought and high-temperature stress. Evaluation of this information together with metabolic data and transcriptome profile of sensitive cultivars is thought to provide important contributions in terms of determining the molecular mechanism of abiotic stress tolerance, identifying related genes and molecular markers and using these markers in further plant breeding studies.

Ethical Statement

The authors declare that ethical approval is not required for this research.

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Declaration of Author Contributions

Material, Methodology, Investigation, Data Curation, Formal Analysis, Writing-Original Draft Preparation, Writing-Review & Editing, *E. DERELLİ TÜFEKÇİ*; Material, Methodology, Investigation, Writing-Original Draft Preparation, *G. AKDOĞAN*; Methodology, Formal Analysis, *M. TÜRKTAŞ*; Methodology, Investigation, *S. URANBEY*. All authors declare that they have seen/read and approved the final version of the article ready for publication.

Declaration of Conflicts of Interest

All authors declare that there is no conflict of interest related to this article.

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