Expression of Stress-Responsive Genes in Wheat (Triticum Aestivum) and its Progenitors Under Heat Stress

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ABSTRACT

Hexaploid wheat (Triticum aestivum, AABDDD) had developed from tetraploid and diploid progenitors with different genomic background. During evolution, changes in genome organization, gene expression, and genomic interactions occur. Real time PCR was employed to disclose changes in gene expression of three abiotic induced genes (DREB2, NAC6D, HSP17) in T. aestivum and four of its progenitors which had contributed in its development including Triticum turgidum (AABB), Triticum monococcum (AA), Aegilops speltoides (BB), and Aegilops tauschi (DD) with different ploidy levels. Results showed that the three genes presented wide variations between T. aestivum and its progenitors as well as among its progenitors. Results suggested that T. aestivum could have inherited the high expression pattern of DREB2 from its progenitor A. speltoids, inherited its expression pattern of NAC6D form T. turgidum and/or A. tauschi. The high expression pattern of HSP17 in T. aestivum could have been inherited from A. speltoides and/or T. turgidum. The obtained results support the notion that wheat progenitors have contributed in the gene expression profile of the hexaploid wheat. Results of this study will help detection of important genes in wheat progenitors that could be introduced into breeding programs for devoing new wheat varieties with desired characteristics.

Keywords: wheat, Triticum, Aegilops, DREB2, NAC6D, heat shock protein genes, wheat molecular evolution.

INTRODUCTION

Wheat (Triticum aestivum) is the main stable food crop for the world and is ranked third after maize and rice as source of food and feed (Elseehy and Atoibai, 2020). It belongs to the Triticeae tribe that contains barley (Hordeum vulgare), rye (Secale cereale), and other wheat species including diploid and tetraploid wheat progenitors (El-Shehawi el al, 2012). It has been accepted that tribe Triticeae had diverged at the diploid level from common ancestor (Cox, 1998).

Hexaploid T. aestivum carrying the hexaploid genome AABBDD was developed 8,000 years ago as a result of Triticum turgidum (AABB) and Aegilops tauschii (DD) hybridization (McFadden and Sears 1946, Kimber and Sears, 1987, Friebe and Gill, 1996). It is strongly accepted that the A genome had descended from T. urartu (AA), that is very close relative of T. monococcum (AA). Also, it was suggested that Aegilops speltoides (S genome) is the B genome donor for T. aestivum (Dvorak and Zhang, 1990; Tsunewaki and Ogihara, 1983) and the cytoplasmic genome donor of polyploid wheats (Wang et al, 1997; Gaut, 1998).

Evolutionary processes from wild to domesticated types are slow and involve morphological, biochemical, and molecular changes in plants (Evans, 1981). Artificial selection during breeding programs to maximize the end product of grain yield and its quality leads to the loss of some useful alleles involved in biotic and abiotic stress resistance (Shimshi et al, 1982). This leads to variations among the domesticated species and its progenitors.

Several studies reported variations between the hexaploid wheat T. aestivum and its progenitors. Tolerance to drought of wheat relatives was investigated using cell membrane stability, proline content, abscisic acid (ABA), sugar mobilization, and osmotic regulation (Peleg et al, 2005; Reynolds et al, 2007; Kurahashi et al, 2009). Hexaploid wheat (T. aestivum, T. compactum) showed lower antioxidant activity than tetraploid wheat (T. dicoccum, T. dicoccoides and T. durum) and diploid wheat (T. monococcum) (Zhang and Kirkham, 1994). Progenitors of bread wheat (T. aestivum) A. tauschi (DD) and T. dicoccoides (AABB) showed different antioxidant capacity in response to dehydration (Sunjea et al, 2017). Similarly, two different accession (XJ002 and XJ098) of A. tauschi (hexaploid wheat progenitor) showed differential gene expression in response to drought that was estimated by transcriptome analysis (Zhao et al, 2020). In this study, 6969 DEGs (differentially expressed genes) related to drought resistance were detected. Drought tolerance was based on various factors including –linolenic acid, starch, sucrose metabolism and other pathways which was parallel to physiological changes correlated with drought resistance (Zhao et al, 2020).

It has been shown that ancient wheat varieties look similar to modern varieties, yet close investigation of both at the molecular level revealed that ancient and heritage wheat varieties differ from modern wheat varieties in their antioxidant and anti-inflammatory capacities. This also was observed at the health impact...
of older varieties which is attributed to their constituents that modulate inflammation and tissue permeability (Spisni et al, 2019). A. tauschii showed wide natural variations in drought resistance and sensitivity to ABA. Drought tolerant A. taushii were less sensitive to ABA. These variations were not completely observed in synthetic wheats resulted from crossing Langdon cultivar and A. taushii. On the other hand, synthetic varieties from more drought tolerant A. taushii showed higher drought tolerance and ABA sensitivity. The more drought tolerant varieties had the higher expression level it showed of three transcription factors (Kurahashi et al, 2009).

Improving wheat tolerance to high temperature through breeding is based on the use of genetic variations in wheat progenitors which depends on the characterization of high temperature tolerance related genes. Exposure to high temperature induced the expression of heat-induced genes in the thermotolerant Aegilops umbellulata (Rampino et al, 2020). This was investigated by differential display PCR. Also, sequence and structural of heat shock protein26 (HSP26) showed variations among various wheat progenitor as well as non-progenitor species. Variations included chromosome locations, copy number, single nucleotide polymorphism (SNPs), indels (Suneja et al, 2019). Variations revealed that positive selection during wheat evolution and the common ancestor of HSP26 among plants (Suneja et al, 2019).

High genetic variations in salt tolerance were observed in the SBLs (synthetic backcross lines) and their parents. This was observed at different growth stages. Some SBLs showed higher tolerance than their parents which represent a valuable source for salt tolerant alleles for breeding (Dadshani et al, 2019).

Heat shock protein (HSPs) genes are induced by high temperature to protect plants from deleterious effects of exposure to elevated temperature (Wahid et al, 2007). HSPs are large protein family with various members including HSP90, HSP70, and HSP17 which are induced by high temperature (Basha et al, 2004, Alotaibi et al, 2020, Elseehy and Alotaibi, 2020). Many different transcription factors are induced by high temperature, such as DREB (Drought Responsive Element Binding) and NAC6D (Elseehy and Alotaibi, 2020, Alotaibi et al, 2020). DREB2 is induced by several abiotic stresses including drought, salinity, and elevated temperature (Du et al, 2019). NAC genes are involved in various biological processes in plants, such as cell division, development, flowering, and response to abiotic stresses (Guerin et al, 2019; Uauy et al, 2006; Nakashima et al, 2012).

The focus of this study was to investigate gene expression level of two abiotic responsive transcription factors and one heat shock protein gene to disclose the possible changes in gene expression in T. aestivum and its progenitors. This will help in understanding how gene expression changes during evolution and detect novel useful genes in progenitors for developing new wheat varieties by breeding programs.

**MATERIAL AND METHODS**

**Plant growth and treatments**

Wheat (T. aestivum) and 4 wheat progenitors (Table 1) were utilized in the current study. Wheat seeds were sterilized using 1% sodium hypochlorite for 20 min. Seeds were rinsed 3 times in distilled water, and the sterilized seeds were soaked in overnight. The imbibed seeds were germinated in plastic containers on 1% agar water; 2 containers for each wheat species. Germinated seeds were kept in the dark for 7 days at normal temperature. One container (about 100 plantlets) of each wheat lines was treated with high temperature at 40ºC for 1 h (Speakman and Krueger 1983), while the other containers were kept at normal temperature. Directly after high temperature exposure, plantlets were collected and freeze-dried (lyophilized) at -57 ºC for 48 h. Samples were ground in a coffee grinder and stored at -20 ºC or used directly for total RNA isolation.

| Table 1. *Triticum* and *Aegilops* species that were used in this study |
|-----------------------|-----------------|------------------|
| **Species**            | **Description**  | **Ploidy level**  |
| *Triticum aestivum (Ta)* | Hexaploid wheat | AABBDD           |
| *Triticum turgidum (Ttu)* | wheat progenitor | ABB            |
| *Triticum monococcum monococcum (Tm)* | wheat progenitor | AA             |
| *Aegilops speltoides (Asp)* | wheat progenitor | SS             |
| *Aegilops taushii (At)* | wheat progenitor | DD             |
Primers
Specific primers were designed based on the respective gene accession from the gene bank. Table (2) summarizes the nucleotide sequence and primer information used in current study.

Isolation of RNA
RNA was isolated from ground shoot powder using QIAzol reagent (Qiagen, Hilden, Germany) (Elseehy and El-Shehawi 2020). One mL of QIAzol was transferred to 1.5 mL microfuge tube and 5 mg of lyophilized tissue was added and mixed thoroughly by vortexing. The mixture was kept at room temperature (RT) for 5 min. Chlororom, 0.2 mL, was added and mixed thoroughly, and then incubated at RT for 2 min. The mixture was centrifuged at 4°C for 16 min at 12000 xg. The supernatant was transferred to new tube. RNA was precipitated by the addition of one volume isopropanol and kept at RT for 15 min. The RNA pellet was recovered by centrifugation 12000 xg for 15 min at 4°C. The obtained RNA was rinsed in 0.5 mL of 70% ethanol, and air dried. The final RNA pellet was dissolved in DEPC-treated H2O. Quality and concentration of RNA were measured by the UV absorbance at 260 nm and the ration of A260/A280 respectively.

Real Time PCR (qPCR)
First strand synthesis of complementary DNA, cDNA, was carried out with the use of ImProm-II reverse transcriptase kit (Promega, Wisconsin, USA). The reaction was done in 20 μL total volume of 1X ImProm-II reaction buffer. Each reaction included 2 μg of total RNA, 0.5 μg of random hexmer, 8 mM MgCl2, 0.5 mM of dNTPs, and 1 μL ImProm-II reverse transcriptase. Real time PCR reaction, 20 μL, included 1X GoTaq qPCR master mix (Promega, Wisconsin, USA), 250 nM of forward and reverse primer (Table 2), 1 μL of cDNA and was conducted in the C1000 Thermal Cycler (BioRad, California, USA). DNA amplicon was amplified under the following PCR conditions; 95 °C for 3 min, 36 cycles of 95 °C for 20 s, 60 °C for 1 min. The gene expression level of targeted genes (DREB2, NAG6D, HSP17) was normalized to the expression level of actin and was estimated by the 2−ΔΔCt method (Livak and Schmittgen 2001, Shukry et al 2020). Real time PCR data were analyzed using SPSS program (13.3 versions) and Duncan multiple range test (Heinisch, 1962) was applied to separate the means (P ≤ 0.05). Data are represented as means ± SE (n=3).

RESULTS AND DISCUSSION
Results
The impact of high temperature on the expression of two stress-induced transcription factors (DREB2, NAC6D) and one heat shock protein gene (HSP17) was investigated in the current study. Gene expression was estimated using real time PCR utilizing the cDNA generated from RNA as template. Five wheat species were used representing the three genomes AA, BB, and DD in various genomic contexts. The hexaploid wheat T. aestivum has the three genomes (AABBDD), its progenitor can disclose how gene expression changes among T. aestivum and its progenitors as well as the interaction of various genomes during evolution.

Investigation of DREB2 expression showed differences among the five wheat species in response to high temperature for 1 hour. A. speltoides was the highest responder to high temperature with DREB2 expression of 4.9 fold compared to normal condition. Also, T. aestivum showed 4.5 fold of DREB2 expression compared to the control (Fig.1). The other three species T. turgidum, T. monococcum, and A. taushii showed lower response to high temperature with fold change 2.8, 2.4, and 2.3 compared to the normal conditions consecutively (Fig. 1). For each wheat

Table 2. Nucleotide sequence and information of primers used in the current study

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primer</th>
<th>Nucleotide sequence 5’ to 3’</th>
<th>Tm</th>
<th>Accession #</th>
<th>PCR product, bp</th>
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<td>DREB2</td>
<td>TADREB2m-1F</td>
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<tr>
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</table>
species the DREB2 was significantly induced by high temperature compared to normal condition. Tm and At did not show significant induction of DREB2, whereas Ttu was induced significantly compared to Tm and At. Asp and Ta were significantly induced compared to other species (Fig.1). Based on their response to high temperature, the five species can be ranked as Asp>Ta>Ttu>Tm>AT.

Expression of NAC6D was widely differed among the five wheat species investigated in this study. All five species had significant level of NAC6D induction in response to high temperature compared to normal condition (Fig.2). Different species differed in their response to high temperature. T. aestivum responded highest to high temperature in NAC6D expression with 5.1 fold change increase. T. turgidum and A. taushii had very close level of expression of NAC6D at 4.8 and 4.7 fold respectively. The top three species (Ta, Ttu, At) showed insignificant variations in NAC6D expression in response to high temperature. T. monococcum and A. speltoides showed close level of expression with 2.3 and 2 fold changes respectively. T. monococcum and A. taushii did not represent significant difference in NAC6D expression (Fig. 2). The five species can be ranked as Asp>Ta>Ttu>AT based on their expression level induced by high temperature.

Under high temperature, wheat species presented significant upregulation of HSP17 gene expression compared to its expression under normal condition. A. speltoides showed the highest level of HSP17 induction in response to high temperature with 4.6 folds change increase (Fig.3). T. aestivum and T. turgidum presented 4.3 and 4.1 fold of induction of HSP17 gene with insignificant difference. T. monococcum and A. taushii showed 2.3 and 2 fold change of induction with insignificant differences (Fig.3). The five species can be ranked as Asp>Ta>Ttu>Tm>AT based on their expression level induced by high temperature.

Fig. 1. Real time PCR of DREB2 transcription factor in the five wheat species used in this study. Ta: T. aestivum, Ttu: T. turgidum, Tm: T. monococcum, Asp: A. speltoides, At: A. tauschii. Different letters means significant different (p≤0.05)

Fig. 2. Real time PCR of NAC6D transcription factor in the five wheat species used in this study. Ta: T. aestivum, Ttu: T. turgidum, Tm: T. monococcum, Asp: A. speltoides, At: A. tauschii. Different letters means significant different (p≤0.05)

Fig. 3. Real time PCR of HSP17 transcription factor in the five wheat species used in this study. Ta: T. aestivum, Ttu: T. turgidum, Tm: T. monococcum, Asp: A. speltoides, At: A. tauschii. Different letters means significant different (p≤0.05)
Discussion

The expression pattern of 3 genes (2 abiotic stress response transcription factors, 1 HSP gene) was employed to investigate the changes in expression pattern through evolution. Five Triticum and Aegilops species representing different ploidy levels (2n-6n) as well as different evolutionary stages of the hexaploid wheat (T. aestivum) were used. The three used genes presented significant variations among the five Triticum and Aegilops species in response to high temperature.

A. tauschii (DD) had contributed the DD genome to T. aestivum (AABBDD) and T. turgidum (AABB) has contributed the AA and BB genomes (McFadden and Sears, 1946; Kimber and Sears, 1987; Friebe and Gill, 1996). The AA genome was derived from T. monococcum (AA) which is close relative to T. urartu (AA), whereas the BB genome was derived from A. speltoids and contributed to T. aestivum through T. turgidum (Sasanuma et al, 1996).

DREB2 showed significant induction in T. aestivum compared to the other two Triticum species (T. turgidum, T. monococcum) although T. monococcum is the AA genome donor to T. aestivum through T. turgidum and T. turgidum (AADD). A. speltoids had higher level of DREB2 compared to the other Aegilops species A. taushi (DD). It seems T. aestivum inherited the expression pattern of DREB2 from A. speltoids through its BB genome not from AA from T. monococcum or DD genome from A. taushi. Interestingly, T. turgidum had lower level of DREB2 expression although it supposed to inherit the BB genome form the A. speltoids. This could be explained by DREB2 was suppressed in T. turgidum after it was combined with AA genome from T. monococcum, while domestication of T. aestivum led to the induction of DREB2 in the new genomic context AABBDD.

This explanation agrees with the accepted notion that during evolution from wild species to domesticated ones through biochemical and loss of alleles and finally leading to variations among the domesticated species and their progenitors (Evans, 1981, Shimshi et al, 1982). For example, antioxidant activity of hexaploid wheat (T. aestivum and T. compactum) was decreased in compared to tetraploid wheat (T. dicoccum, T. dicoccoides and T. durum) as well as the T. monococcum (diploid wheat) indicating a decrease of antioxidant capacity through evolution (Zhang and Kirkham, 1994). Also, progenitors of T. aestivum (A. tauschii (DD) and T. dicoccoides (AABB)) differed in their antioxidant activity after their exposure to drought (Suneja et al, 2017). In addition, upon exposure to drought, two accessions of A. tauschii (DD genome contributor) represented difference in gene expression of 6969 genes related to drought tolerance which was parallel to physiological changes correlated with drought resistance (Zhao et al, 2020). Interestingly, ancient and modern wheat varieties revealed various molecular differences including antioxidant and anti-inflammatory capacities. Also, they differ in their health benefits attributed to modulation of inflammation and tissue permeability (Spisni et al, 2019). Synthetic backcross lines (SBLs) and their parents showed high genetic variations in salt tolerance. Some SBLs showed higher tolerance than their parents which represent a valuable source for salt tolerance alleles for breeding (Dadshahi et al, 2019).

Wide natural variations were observed in A. taushi regarding drought tolerance and ABA sensitivity compared to synthetic wheat varieties produced by crossing Langdon cultivar and A. taushi. On the contrary, varieties produced by drought tolerant A. taushi were more drought-tolerant and more sensitive to ABA. It is noteworthy that, the more drought tolerant varieties had higher expression level of three transcription factors (Kurahashi et al, 2009). Aegilops umbellulata showed induction of high temperature-induced genes after exposure to elevated temperature (Rampino et al, 2020).

Variations of heat shock protein26 (HSP26) structure were detected among wheat progenitors species including chromosome locations, copy number, single nucleotide polymorphism (SNPs), indels (Suneja et al, 2019). Based on the detected Variations, it was suggested that positive selection had occurred during wheat evolution common ancestor of HSP26 among plants (Suneja et al, 2019).

The induction of the three studies genes agrees with their induction in previous reports. Heat shock protein (HSPs) genes are induced by high temperature to protect plants from deleterious effects of exposure to elevated temperature (Wahid et al, 2007, Alotaibi et al, 2020). HSP17 was induced by high temperature in Egyptian wheat varieties (Elseehy and Alotaibi, 2020) and its induction was regulated by methylation of its promoter (Alotaibi et al, 2020). DREB2 was induced by high temperature and several abiotic stresses including salinity and drought (Du et al, 2018, Elseehy and Alotaibi 2020). NAC6D was induced by high temperature in Egyptian wheat varieties (Elseehy and Alotaibi, 2020). The hexaploid wheat (T. aestivum) and its tetraploid as well as diploid progenitors showed variations in the organization and expression of the mitochondrial gene orf256 which is associated with cytoplasmic male sterility (Hedgcoth et al 2002, El-Shehawi et al 2003, El-Shehawi et al, 2012).

From the obtained results, there was a change in gene expression response to high temperature during wheat evolution. This was indicated in differences in
gene expression level among hexaploid wheat (T. aestivum) and its tetraploid and diploid progenitors.

REFERENCES


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