Transcriptional Evaluation of Wheat Cultivars Under Drought and Salinity Stress: Identifying Potential Candidates for Stress-Resilient Breeding Programs

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ABSTRACT

Abiotic stresses such as drought and salinity significantly impact global wheat (Triticum aestivum) production, especially in arid and semi-arid regions. Understanding transcriptional responses to these stresses is vital for identifying resilient cultivars and enhancing crop performance through breeding programs. This study evaluated the expression of two key transcription factors, TaERF3 and TabZIP1, across fourteen Egyptian wheat cultivars under varying levels of drought (5%, 15%, and 25% PEG 6000) and salinity stress (50, 150, and 250 mM NaCl) using qPCR. Expression profiles were visualized using a correlation based heatmap, clustering cultivars by correlation without data transformation. The results revealed distinct transcriptional clusters, with Beni Suef 7 forming a unique single-sample cluster due to its high TaERF3 expression under salinity and drought conditions, making it a prime candidate for breeding programs. A second cluster grouped Gimmeza 12, Sakha 93, Misr 2, Misr 1, Sids 14, Sids 1, and Sohag 5, which showed balanced expression of both transcription factors, suggesting shared mechanisms of stress adaptation. Misr 3, Giza 186, and Sohag 4 formed a third cluster characterized by strong TabZIP1 expression under drought stress. Shandawel 1, Sakha 95, and Sids 12 clustered together, exhibiting the lowest transcriptional responses. This study highlights the diversity of transcriptional strategies among wheat cultivars, with Beni Suef 7 and Giza 186 emerging as key candidates for drought and salinity resilience. These findings provide valuable molecular insights for breeding programs targeting stress-tolerant wheat varieties, particularly for challenging environments such as Saudi Arabia.

Keywords: abiotic stress, gene expression markers, stress adaptation, breeding programs, climate resilience, wheat cultivars.

INTRODUCTION

biotic stresses, particularly drought and salinity, represent some of the most formidable challenges confronting global agriculture, especially in arid and semi-arid regions such as the Middle East. These stresses significantly impact crop productivity, leading to food insecurity and economic instability in these regions (Agarwal et al., 2019). In Saudi Arabia, where agricultural practices are heavily dependent on efficient water utilization and soil management, wheat (Triticum aestivum L.) emerges as a strategic crop due to its pivotal role in ensuring food security (Cevher-Keskin, 2023). The identification development of and wheat cultivars exhibiting enhanced tolerance to abiotic stresses are essential for fostering sustainable

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agricultural systems in this region (Zhang et al., 2019).

Among the various molecular approaches employed to investigate stress tolerance, the evaluation of gene expression in transcription factors (TFs) such as TaERF3 and TabZIP1 offers critical insights into the mechanisms that underpin wheat's adaptability to adverse environmental conditions (Nakayama et al., 2022). TaERF3, a member of the Ethylene-Responsive Factor (ERF) family, serves as a crucial regulator of abiotic stress responses in plants. This transcription factor modulates the expression of genes associated with ethylene signaling, which plays a significant role in stress acclimation processes, including reactive oxygen species (ROS) detoxification, proline biosynthesis, and osmotic adjustment (Sohail et al., 2022). In wheat, TaERF3 has been shown to respond promptly to both salt and drought stress, with studies indicating that its overexpression can lead to improved physiological and biochemical traits under these challenging conditions (Truong et al., 2021). The investigation of ERF transcription factors in related cereal species, such as rice, has further elucidated their roles in enhancing stress tolerance, particularly through mechanisms like the modification of root architecture (Rai et al., 2018).

Similarly, TabZIP1, a member of the Basic Leucine Zipper (bZIP) family, plays an integral role in abscisic acid (ABA)-mediated stress signaling pathways. ABA is a critical hormone that regulates plant various physiological responses to drought and salinity, including stomatal behavior and osmotic balance (Xu, 2023). Research in wheat has demonstrated that TabZIP1 expression is significantly induced under stress conditions, contributing to enhanced water-use efficiency and cellular homeostasis (Tyagi et al., 2021). Comparable findings in regarding particularly OsbZIP23. rice. which has been shown to enhance abiotic factors tolerance, underscore the conserved functional roles of bZIP transcription factors across cereal crops (Sun et al., 2021). Wheat varieties traditionally cultivated in Egypt have garnered attention for their adaptability to challenging environmental conditions, including water scarcity and salinity. These particularly relevant for cultivars are integration into progressive wheat breeding programs aimed at advancing Saudi Arabia's agricultural sector (Shamloo-Dashtpagerdi et al., 2023). By examining the gene expression patterns of *TaERF3* and *TabZIP1* in Egyptian wheat cultivars, this research aims to identify promising varieties capable of thriving under the specific environmental conditions as what prevalent in Saudi Arabia (Li, 2024). The evaluation of transcriptional activity of these TFs under controlled drought and salinity vield actionable stress conditions will molecular insights that can guide cultivar selection and breeding strategies (Gao et al., 2018).

The primary objective of this study is to quantify the expression levels of *TaERF3* and *TabZIP1* in selected Egyptian wheat cultivars

subjected to drought and salinity stress, utilizing quantitative real-time PCR (qPCR) methodologies (Hussain et al., 2021). The findings from this research will contribute significantly to the identification of stress-resilient cultivars that are suitable for integration into the Saudi Arabian agricultural landscape (Magwanga et al., 2018). This research aligns with the broader objectives of progressive breeding programs that seek to enhance wheat resilience and productivity in arid regions, thereby ensuring the sustainability of this vital crop amidst escalating environmental challenges (Pailles et al., 2019).

MATERIAL AND METHODS

Plant Material

Fourteen wheat (Triticum *aestivum*) cultivars commonly grown in Egypt and obtained from Arish University were used in this study. These cultivars included Giza 186, Misr 1, Misr 2, Misr 3, Shandawel 1, Sohag 4, Sohag 5, Sakha 93, Sakha 95, Sids 1, Sids 12, Sids 14, Gimmeza 12, and Beni Suef 7. These varieties were selected due to their known adaptability to challenging environments, particularly drought and salinity, making them suitable candidates for testing their potential use in Saudi Arabian agricultural systems. This study is part of a larger effort to identify resilient wheat cultivars that could be incorporated into breeding programs for sustainable agriculture in arid regions.

Experimental Design

The experiment began by surface sterilizing wheat seeds with 70% ethanol for one minute, followed by a 10-minute treatment with 1% sodium hypochlorite, and thorough rinsing with sterile distilled water. The seeds were germinated in petri dishes lined with moistened filter paper. Once germinated, seedlings were transferred to hydroponic culture systems containing Hoagland's nutrient solution and maintained under controlled growth chamber conditions, including a 16-hour photoperiod, 25°C temperature, and 60% relative humidity. Plants were exposed to two abiotic stress treatments: drought and salinity. Drought stress was simulated by adding polyethylene glycol (PEG 6000) to the nutrient solution at concentrations of 5%, 15%, and 25%. Salt stress was induced using sodium chloride (NaCl) at concentrations of 50 mM, 150 mM, and 250 mM. Control plants were grown under the same conditions without the addition of PEG or NaCl. Stress treatments were applied for seven days at the two-leaf stage of seedling development. Leaf tissues were collected at the end of the treatment period, flash-frozen in liquid nitrogen, and stored at -80°C for subsequent RNA extraction.

RNA Extraction and cDNA Synthesis

Total RNA was extracted from frozen leaf tissues using TRIzol® reagent (Invitrogen, USA) following the manufacturer's protocol. RNA integrity was assessed on a 1.2% agarose gel, and RNA concentrations were measured using a NanoDrop spectrophotometer (Thermo Scientific, USA). To synthesize complementary DNA (cDNA), 1 µg of RNA was treated with DNase I to eliminate genomic DNA contamination and reversetranscribed using oligo (dT) primers with the RevertAid First Strand cDNA Synthesis Kit (Thermo Scientific, USA). The cDNA synthesis reaction was carried out in a total volume of 20 µL according to the manufacturer's instructions, and the resulting cDNA was diluted tenfold with nuclease-free water for downstream quantitative real-time PCR (qPCR) analysis.

Quantitative Real-Time PCR (qPCR)

Quantitative real-time PCR was performed to evaluate the expression levels of *TaERF3* and *TabZIP1* genes. Gene-specific primers were designed based on published sequences, and the β -actin gene was used as the housekeeping gene due to its stable expression under abiotic stress conditions. qPCR reactions were conducted using the QuantStudio 5 Real-Time PCR System (Applied Biosystems, USA) with the PowerUpTM SYBRTM Green Master Mix (Thermo Scientific, USA). Each reaction included 10 μ L of SYBR Green Master Mix, 2 μ L of cDNA, 0.5 μ L each of forward and reverse primers (10 μ M), and 7 μ L of nuclease-free water in a total volume of 20 μ L. The thermal cycling protocol consisted of an initial denaturation step at 95°C for 2 minutes, followed by 40 cycles of denaturation at 95°C for 15 seconds and annealing/extension at 60°C for 30 seconds. A melt curve analysis was performed after amplification to confirm the specificity of the PCR products.

Data Analysis

Relative gene expression levels were calculated using the $2^{-}\Delta\Delta Ct$ method (Livak and Schmittgen, 2001), with untreated control samples as the calibrator. Fold-change values were computed for each cultivar under both PEG and NaCl treatments relative to the control conditions, allowing the identification of stress-responsive patterns of *TaERF3* and *TabZIP1* gene expression.

The expression data both transcription factors across the fourteen wheat cultivars under drought (PEG) and salinity (NaCl) stress conditions were visualized using a heatmap generated by the ClustalVis webtool (https://biit.cs.ut.ee/clustvis/). The input data consisted of raw fold-change values, and no transformation or normalization was applied. Hierarchical clustering was performed using Pearson correlation for rows (varieties).

RESULTS AND DISCUSSION

Drought Stress (PEG-Induced Osmotic Stress)

Out of the fourteen wheat cultivars evaluated, nine cultivars demonstrated the ability to successfully survive the full duration of the drought experiment induced by PEG treatment. These cultivars included Giza 186, Misr 2, Shandawel 1, Misr 1, Sids 12, Misr 3, Sakha 95, Beni Suef 7, and Sohag 4. These varieties exhibited resilience under increasing osmotic stress levels (5%, 15%, and 25% PEG). The expression patterns of *TaERF3* and *TabZIP1* transcription factors varied significantly among the fourteen wheat cultivars under PEG-induced osmotic stress. For *TaERF3*, fold change levels were generally low across all PEG treatments (5%, 15%, and 25%). However, Beni Suef 7 demonstrated notable induction of *TaERF3* at both 15% and 25% PEG concentrations, with

fold change values of ~ 1 and 2, respectively, making it the cultivar with the highest *TaERF3* expression under drought stress. In contrast, Giza 186, Misr 2, Shandawel 1, and Sids 12 showed marginal upregulation of *TaERF3* at the 5% PEG concentration, but their expression decreased at higher stress levels (Figure 1).



Figure 1. Expression of *TaERF3*-QF in Wheat Cultivars under PEG-Induced Osmotic Stress. Fold-change values of *TaERF3*-QF expression in nine wheat cultivars subjected to three concentrations of PEG (5%, 15%, and 25%) to simulate osmotic stress.

For *TabZIP1*, the expression patterns were more pronounced under PEG-induced stress. Giza 186 showed consistent upregulation of *TabZIP1* at 5% and 15% PEG concentrations (fold changes of ~ 3 and 4, respectively), but its expression diminished at 25% PEG. Sids 12 also showed significant induction of *TabZIP1* at 5% PEG (fold change of 2), while Beni Suef 7 exhibited upregulation at 25% PEG (fold change of 1). Other cultivars, including Misr 3, Shandawel 1, and Sakha 95, displayed minimal changes in *TabZIP1* expression across all PEG concentrations (Figure 2).



Figure 2. Expression of *TabZIP1* in Wheat Cultivars under PEG-Induced Osmotic Stress. Fold-change values of *TabZIP1* expression in nine wheat cultivars subjected to three concentrations of PEG (5%, 15%, and 25%) to simulate osmotic stress.

Beni Suef 7 emerged as the bestperforming cultivar for *TaERF3*, given its ability to sustain and increase expression at higher PEG concentrations, which aligns with its potential role in regulating drought tolerance mechanisms such as reactive oxygen species (ROS) scavenging. For *TabZIP1*, Giza 186 demonstrated superior performance under mild drought stress (5% and 15% PEG), suggesting its involvement in maintaining osmotic balance and ABAmediated responses. These cultivars could be strong candidates for breeding programs focused on improving drought resilience.

Salinity Stress (NaCl-Induced Stress)

Interestingly, all fourteen cultivars survived through the salinity experiment, which involved exposure to NaCl concentrations of 50 mM, 150 mM, and 250 mM. This indicates a baseline tolerance to saline conditions across the tested varieties. The response of wheat cultivars to NaClinduced salinity stress revealed distinct patterns of TaERF3 and TabZIP1 expression across the three stress levels. For TaERF3, Beni Suef 7 stood out with markedly high expression at 50 mM and 150 mM NaCl (fold changes of 7 and 6, respectively). Similarly, Gimmeza 12 exhibited strong induction of TaERF3 at 50 mM and 150 mM NaCl (fold changes of 2), although its expression was suppressed at 250 mM. Most other cultivars, including Giza 186, Misr 2, Shandawel 1, and Sohag 4, exhibited modest upregulation of TaERF3 at 50 mM NaCl, but the expression diminished at higher salinity levels (Figure 3).



Figure 3. Expression of *TaERF3*-QF in Wheat Cultivars under NaCl-Induced Salinity Stress. Fold-change values of *TaERF3*-QF expression in fourteen wheat cultivars subjected to three salinity stress levels (50 mM, 150 mM, and 250 mM NaCl).

For *TabZIP1*, Giza 186 was the most consistent performer, with significant upregulation at all three NaCl concentrations (fold changes of 3, 6, and 1 for 50 mM, 150 mM, and 250 mM, respectively). Sids 12 displayed remarkable upregulation of *TabZIP1* at 50 mM NaCl (fold change of 8), but its expression sharply declined at higher concentrations. Gimmeza 12 and Beni Suef 7 also exhibited moderate increases in *TabZIP1* expression at 50 mM and 150 mM NaCl. Other cultivars, such as Misr 3, Sohag 5, and Sakha 93, showed minimal or no significant change in *TabZIP1* expression under salinity stress (Figure 4).



Figure 4. Expression of *TabZIP1* in Wheat Cultivars under NaCl-Induced Salinity Stress. Fold-change values of *TabZIP1* expression in fourteen wheat cultivars subjected to three salinity stress levels (50 mM, 150 mM, and 250 mM NaCl).

From these results, Beni Suef 7 demonstrated the highest expression of TaERF3 under mild to moderate salinity stress (50 mM and 150 mM NaCl), suggesting its role in enhancing ethylene signaling and ROS detoxification. Similarly, Giza 186 emerged as the best candidate for TabZIP1 expression across all salinity levels, indicating its potential involvement in ABA signaling and osmotic adjustment. These results position Beni Suef 7 and Giza 186 as top-performing cultivars for salinity tolerance and ideal candidates for integration into breeding programs aimed at improving wheat resilience in saline environments.

Expression patterns and potential candidates The heatmap provided a comprehensive visualization of the expression profiles of TaERF3 and TabZIP1 across fourteen wheat cultivars under drought and salinity stress conditions (Figure 5). The clustering of wheat cultivars based on their transcriptional responses under drought and salinity stress conditions revealed four distinct groups. Beni Suef 7 formed a single-sample cluster, unique transcriptional emphasizing its behavior across stress conditions. This cultivar displayed the highest expression of TaERF3, particularly under salinity stress, and moderate expression under severe drought conditions. Its singular clustering highlights its potential as a standout candidate for breeding programs aiming develop highly stress-tolerant wheat to cultivars.



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Figure 5. Heatmap of TaERF3 and TabZIP1 Expression in Wheat Cultivars under Drought and Salinity Stress.
The heatmap illustrates the expression levels of TaERF3 and TabZIP1 transcription factors in fourteen wheat cultivars under drought (PEG: 5, 15 and 25%) and salinity (NaCl: 50, 150 and 250 mM) stress conditions.
Cultivars are clustered based on correlation, revealing groups with similar transcriptional responses.

The second cluster grouped Gimmeza 12 and Sakha 93 with Misr 2, Misr 1, Sids 14, Sids 1, and Sohag 5. These cultivars exhibited moderate to high expression levels of both TaERF3 and TabZIP1 under various stress conditions. The transcriptional responses within this cluster were wellcorrelated, indicating shared mechanisms of adaptation, particularly in balancing ethylene and ABA-mediated pathways. The presence of Gimmeza 12 and Sakha 93 in this group suggests their ability to cope effectively with salinity stress, while the other cultivars displayed a more balanced response to both stress types.

The third cluster included Misr 3, Giza 186, and Sohag 4, which showed moderate expression of *TabZIP1*, particularly under

drought stress, and lower expression of *TaERF3* under salinity stress. The clustering of these cultivars reflects their reliance on ABA-mediated pathways for drought adaptation, with Giza 186 showing the strongest transcriptional response among the group. This cluster's correlation suggests shared but distinct strategies for handling osmotic and ionic imbalances caused by abiotic stress.

The fourth cluster consisted of Shandawel 1, Sakha 95, and Sids 12, which exhibited low to moderate expression of both transcription factors across all conditions. The cultivars in this group demonstrated the weakest transcriptional responses to stress, indicating limited reliance on ethylene or ABA pathways for stress adaptation. Despite their subdued responses, these cultivars may still provide useful genetic resources for specific breeding objectives requiring baseline tolerance.

Overall, this clustering analysis highlights the unique transcriptional profile of Beni Suef 7 and the shared stress response mechanisms among the other groups. The diversity in transcriptional strategies underscores the potential for leveraging specific cultivars in breeding programs targeting drought and salinity tolerance.

Abiotic stresses, particularly drought and salinity, significantly hinder global wheat (Triticum aestivum) production, especially in arid and semi-arid regions like Saudi Arabia. The urgent need for identifying stressresilient wheat cultivars and understanding the molecular mechanisms that underpin their adaptability is paramount for sustainable agricultural practices in these challenging environments. This study investigates the expression of two pivotal transcription factors, TaERF3 and TabZIP1, across fourteen Egyptian wheat cultivars subjected to controlled drought and salinity stress conditions. The findings indicate substantial variability in transcriptional responses among the cultivars, with certain varieties exhibiting remarkable resilience, thus positioning them as potential candidates for breeding programs tailored to the climatic conditions of Saudi Arabia.

TaERF3 is integral to ethylene-mediated stress responses, notably in regulating species (ROS) reactive oxygen detoxification, osmotic balance, and stomatal conductance. The significance of ERF transcription factors in enhancing stress tolerance has been well documented. For example, the overexpression of OsERF71 in rice (Oryza sativa) has been shown to improve drought tolerance by modulating root architecture, thereby enhancing water uptake efficiency (Nahas et al., 2019). Additionally, in wheat, TaERF3 has been reported to bolster both salt and drought tolerance by enhancing water retention and minimizing oxidative damage (Ali et al., 2022). In this study, the cultivar Beni Suef 7 exhibited the highest expression levels of *TaERF3*, particularly under conditions of salinity and severe drought stress, indicating its capacity to activate ethylene signaling pathways effectively. This unique transcriptional profile positions Beni Suef 7 as a prime candidate for breeding programs focused on developing stress-resilient wheat varieties.

TabZIP1, a member of the bZIP family, plays a crucial role in abscisic acid (ABA)mediated stress signaling, which is vital for osmotic adjustments and water-use efficiency during drought and salinity stress. Research on OsbZIP23 in rice has demonstrated its involvement in enhancing drought tolerance through the upregulation of ABA-dependent gene expression (Hamdi et al., 2020). In the current study, Giza 186 consistently showed upregulation of *TabZIP1* under both drought and salinity stress, suggesting a strong reliance on ABA pathways for effective stress mitigation. The cultivar's ability to maintain osmotic regulation and water-use efficiency underscores its potential for thriving in the water-scarce environments characteristic of Saudi Arabia.

The clustering analysis of cultivars based on their transcriptional responses provided deeper insights into the diversity of stress adaptation strategies. Beni Suef 7 formed a distinct single-sample cluster, highlighting its unique transcriptional behavior, while Giza 186 clustered with cultivars such as Gimmeza 12 and Sakha 93, which also exhibited balanced expression of both transcription exceptional factors. The performance of Beni Suef 7 in TaERF3 expression, combined with Giza 186's robust TabZIP1 expression across stress conditions, emphasizes their complementary roles in stress tolerance mechanisms. This complementarity renders these cultivars particularly valuable for inclusion in breeding programs aimed at developing dual stress-tolerant varieties. The physiological and biochemical responses of wheat to abiotic stresses are complex and multifaceted. Drought and salinity stress lead to the accumulation of ROS, which can cause oxidative damage, thereby impairing cellular integrity and functionality (Begcy et al., 2019). To counteract this oxidative stress, plants have evolved intricate antioxidant defense mechanisms, which include both enzymatic and non-enzymatic pathways (Dugasa et al., 2018). The role of *TaERF3* and *TabZIP1* in modulating these responses highlights their importance in the overall stress tolerance framework of wheat.

The identification of stress-resilient wheat cultivars is especially pertinent for Saudi Arabia, where agricultural practices must adapt to extreme environmental conditions, including high temperatures, limited water availability, and salinity-prone soils. Integrating Beni Suef 7 and Giza 186 into progressive breeding programs could significantly enhance wheat productivity in the region. By merging molecular insights with traditional breeding techniques, these cultivars can serve as parental lines for developing new varieties with improved drought and salinity tolerance. Furthermore, the expression profiles of TaERF3 and TabZIP1 can act as molecular markers, facilitating the selection of stress-resilient genotypes and expediting the breeding process.

CONCLUSIONS

This study offers a comprehensive evaluation of the transcriptional responses of fourteen wheat cultivars under drought and salinity stress, emphasizing the significance of Beni Suef 7 and Giza 186 as exceptional candidates for breeding programs aimed at developing climate-resilient wheat varieties. Beni Suef 7 demonstrated a unique capacity to upregulate TaERF3, a key regulator of ethylene-mediated stress adaptation, under severe drought and salinity conditions, while Giza 186 consistently expressed TabZIP1, critical for ABA-mediated osmotic regulation and water-use efficiency. These cultivars, with their distinct yet complementary stress response mechanisms, represent valuable genetic resources for improving the resilience of wheat crops to abiotic stresses. Their integration into breeding programs holds promise for enhancing agricultural

productivity in arid and semi-arid regions, particularly Saudi Arabia, where hyper-arid climate, water scarcity and soil salinity are significant challenges. Moreover, the use of transcriptional markers such as TaERF3 and TabZIP1 provides a precise and efficient approach to selecting stress-tolerant facilitating targeted breeding genotypes, strategies. This study not only underscores the adaptability of Egyptian wheat cultivars challenging environments to but also establishes a molecular basis for leveraging these insights in developing wheat varieties tailored to the future demands of global agriculture in the face of climate change.

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