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EXPRESSION ANALYSIS OF *TaWXPL1D* GENE IN WHEAT GENOTYPES UNDER DROUGHT STRESS

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Abstract

Wheat, a globally critical cereal crop, is profoundly affected by drought stress, necessitating advanced strategies to enhance its resilience. This study evaluates the expression level of the *TaWXPL1D* transcription factor (TF) gene, which plays a pivotal role in cuticle biosynthesis, across two bread wheat genotypes, Aran and Gyzyl bughda, under control and drought conditions. Gene expression was analyzed five days post-initiation of water deficit, a timepoint marked by the manifestation of visible stress symptoms. Quantitative real-time PCR (qRT-PCR) was employed to quantify transcript levels, utilizing elongation factor 1 alpha (*Elf1-α*) as a reference gene for normalization. Under optimal conditions, *TaWXPL1D* expression exhibited no significant genotypic variation. However, drought stress induced significant transcriptional alterations, with a pronounced downregulation observed in the drought-sensitive genotype Aran. These results underscore genotype-specific regulatory mechanisms influencing cuticle development under drought stress and provide a foundation for targeted breeding approaches to improve drought tolerance in wheat.

Keywords**:** *Triticum aestivum* L.*; drought; transcription factor; cuticle biosynthesis; gene expression*

1. Introduction

Wheat (*Triticum aestivum* L.) holds a vital position in global agriculture as one of the most widely cultivated crops, supporting billions of people worldwide [5]. Its prominence stems from its exceptional adaptability to a range of climatic and environmental conditions and its ability to serve as a staple food rich in carbohydrates, proteins, and essential nutrients [10]. However, the growing challenge of abiotic stresses, particularly drought, threatens wheat productivity. Drought not only reduces crop yields but also impacts grain quality by altering nutrient composition and kernel development. Addressing these challenges requires innovative approaches to improve the resilience of wheat to water scarcity [6].

According to the World Bank report, Azerbaijan is in 18th place in terms of the risk of severe drought until 2040. Climate models predict that in the period from 2020 to 2040, the temperature will rise by 0.5-2.5 degrees, and precipitation will decrease. According to recent estimates of the Azerkosmos Agency, only 24 percent of the territory of our country has not faced the threat of drought. 15 percent of the territory of Azerbaijan has a very high risk of drought, 8 percent a high risk, 28 percent a medium risk and 25 percent a low risk.

A key aspect of drought tolerance lies in the plant's ability to conserve water and reduce transpiration losses. The cuticle, a lipid-based hydrophobic layer that coats the aerial parts of plants, plays a crucial role in

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minimizing water loss through non-stomatal pathways. Acting as a physical barrier, the cuticle also protects plants from other environmental stressors, including high temperatures, UV radiation, and pathogen attacks. Its functional versatility makes it a significant target for improving drought resistance in crops like wheat [1].

The structure and composition of the cuticle are highly dynamic and influenced by environmental conditions. During drought, plants often exhibit enhanced deposition of epicuticular waxes and a thickened cutin layer, which reduces cuticle permeability. This adaptation is a key defense mechanism that delays dehydration, allowing plants to maintain cellular homeostasis under stress. Understanding these structural changes and the molecular mechanisms that regulate cuticle biosynthesis is essential for developing drought-tolerant cultivars [5].

Recent research has highlighted the role of transcription factors (TFs) in regulating the genes responsible for cuticle formation. By studying how these regulatory networks operate in wheat under drought stress, researchers aim to uncover genetic targets for breeding programs or biotechnological interventions. Enhanced cuticle properties, such as increased wax accumulation and reduced permeability, could significantly improve wheat's water-use efficiency and overall stress resilience. Moreover, integrating knowledge of cuticle dynamics with other physiological and molecular traits, such as root architecture and osmotic adjustment, provides a holistic approach to enhancing drought tolerance. This multidisciplinary strategy could lead to the development of wheat cultivars capable of sustaining high productivity even in regions prone to frequent droughts, thereby ensuring food security for growing populations [2]. This study aims to elucidate the role of wheat WXPL TFs in regulating cuticle biosynthesis under drought conditions. By investigating the expression patterns and transcriptional activities this research seeks to provide insights into the molecular mechanisms that govern cuticle development in wheat. Understanding these regulatory networks will contribute to the development of drought-resilient wheat cultivars, addressing the critical challenge of maintaining crop productivity under climate variability.

2. Materials and methods

Plant materials and growth conditions. The research objects were two local bread wheat genotypes Aran and Gyzyl bughda. Aran – bread wheat hybrids were obtained by individual selection from the populations of Research İnstitute of Crop Husbandry. Potential productivity is 7-8 t/h. The bread quality of the variety is high; Gyzyl bughda was obtained by individual selection of winter wheat genotypes purchased from International Selection Centers. Potential productivity is 5-6 t/h.

The plants were grown in controlled environment chambers under control and drought conditions using a completely randomized design. Twenty seeds of each genotype were pregerminated in filter paper. Seeds were stratified at 4˚C for 24 h and kept at room temperature for 5 days until the coleoptile emerged. Germinated seedlings were transplanted to two (for control and drought treatments) multi-cell seed trays (96 cell pots, with 5 cm^3 volume for a single cell) containing soil-sand mixture (3:1 ratio) and transferred in a growth chamber at 16:8 h light/dark period 24˚C and 19˚C respectively, relative humidity 50%. Plants were watered twice a day with 5 ml of tap water. The 3-leaf stage (12-day-old) plants, at which plants are more sensitive to abiotic stresses, were subjected at to water deficiency by withholding irrigation. Samples were immediately frozen in liquid nitrogen and kept at -80˚C until required. *RNA extraction and cDNA synthesis.* Total RNA was extracted from leaf material using the Monarch Total RNA Miniprep Kit (New England Biolabs, Inc.) following the manufacturer's instructions. Genomic DNA contamination was removed using RNase-free DNase I. The quality and quantity of the extracted RNA were assessed by agarose gel electrophoresis. RNA concentration was measured spectrophotometrically using a NanoDrop Thermo Scientific-2000C (USA). Single-stranded cDNA synthesis was performed from the total RNA using the LunaScript RT SuperMix Kit (New England Biolabs, Inc.) according to the manufacturer's instructions, in a final volume of 20 µl.

Quantitative Real-Time PCR. PCR was conducted using a Mic Real-Time PCR system in a total reaction volume of 20 µl. Each reaction mixture contained 10 µl of Luna Universal qPCR Mix (New England Biolabs, Inc.), 1 µl of 1:5 diluted cDNA, 0.5 µl each of forward and reverse primers (10 µM), and 7 µl of nuclease- free water. The PCR protocol included an initial denaturation step at 94°C for 60 seconds, followed by 45 cycles of 95°C for 15 seconds and 60°C for 30 seconds. No-template controls (NTCs) were included for each primer pair. Each reaction was performed in triplicate (technical replicates) for each of the three biological replicates. Primer sequences used for expression analysis are listed in Table 1. Primer efficiency for each pair was determined by the standard curve method using serial dilutions of cDNA, calculated using the formula: Efficiency (%) = (10^(-1/slope) - 1) × 100. Dissociation curves for each

amplicon were analyzed to confirm the specificity of the amplification reactions. Fold change in gene expression (stressed versus control) was calculated using the 2^−ΔΔCt method (Livak and Schmittgen, 2001).

Table 1. Sequences of primers used for qRT-PCR

Gene	Direction	Sequences
TaWXPL1D	F	CCTGTTCGTCTCCTTGTTCACG
	R	CGCCTGGCCGATTACTACAG

3. Results and discussion

This study investigated the transcript levels of the *TaWXPL1D* gene, with elongation factor 1 alpha (*Elf1-α*) selected as the housekeeping gene due to its stable and consistent expression across experimental conditions. The research focused on two wheat genotypes, Gyzyl bughda and Aran. For the *Elf1-α* gene, the average Cq values for Gyzyl bughda were 19.30 (control) and 19.00 (stress), and for "Aran," 19.39 (control) and 19.21 (stress). These minor deviations (less than one Cq cycle) indicate the stability of *Elf1-α* across conditions, affirming its suitability as a reference gene. In contrast, for the target *TaWXPL1D* gene, Gyzyl bughda exhibited average Cq values of 19.17 (control) and 19.11 (stress), while Aran showed 19.07 (control) and 19.89 (stress). The significant increase in Cq under stress for Aran suggests a suppression of *TaWXPL1D* expression in this genotype. Analysis of relative gene expression, normalized to the reference gene, revealed a decrease in *TaWXPL1D* transcript levels under stress in both genotypes. These findings highlight genotype-specific differences in the regulation of this transcription factor, potentially linked to cuticle biosynthesis and drought response (Fig. 1).

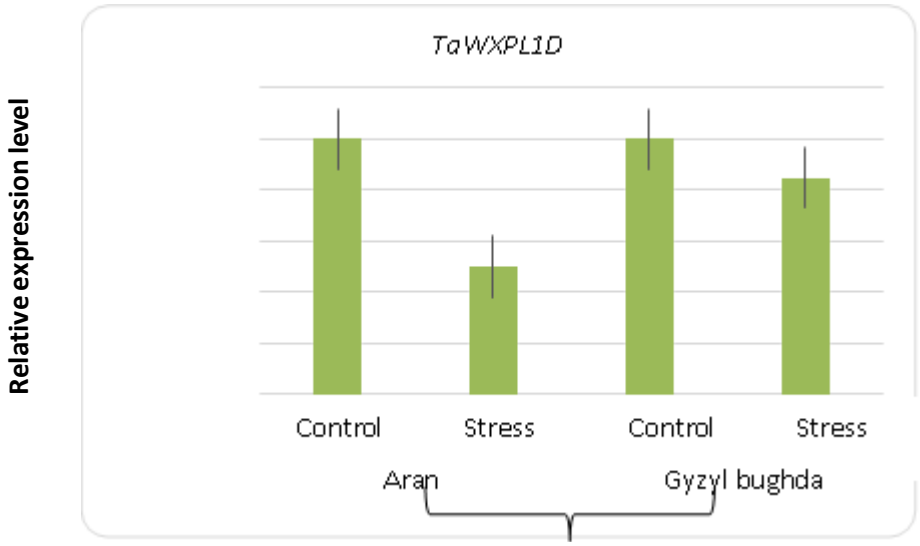

Fig.1. Transcript levels of *TaWXPL1D* gene in leaves of wheat plants under drought stress. Level of mRNA determined by qRT-PCR using elongation factor (*Elf1-α*) gene as an internal control. The fold change in expression was determined according to the 2−ΔΔCt method. Each value is mean ± standard deviation of three biological replicates.

The cuticle, a hydrophobic layer covering aerial plant organs, plays a crucial role in protecting plants against environmental stresses, including drought. It serves as a barrier to non-stomatal water loss, contributing significantly to plant water conservation under drought conditions [7]. The composition and structure of the cuticle, including epicuticular waxes and cutin, are critical determinants of its efficiency in reducing water loss. The dynamic nature of the cuticle's biosynthesis and regulation allows plants to adapt to varying environmental challenges, particularly under water-limiting conditions [11].

Transcription factors (TFs) are key regulators of gene expression networks involved in cuticle biosynthesis. Among them, the AP2/ERF and MYB families have been extensively studied in various plant

species for their roles in cuticle development and stress tolerance [4, 12]. These TFs activate genes responsible for the synthesis of cuticular components, such as very long chain fatty acids, wax esters, and β-diketones, which are essential for enhancing the drought tolerance of plants. However, the molecular mechanisms underlying the regulation of cuticle biosynthesis in wheat (*Triticum aestivum* L.), especially under drought conditions, remain largely unexplored [2, 9].

Recent studies have identified wheat homologues of WAX PRODUCTION (WXP) transcription factors, designated as WXP-like (WXPL) TFs, which are orthologous to the well-characterized WXP1 and WXP2 TFs from Medicago *truncatula*. These WXPL TFs have been implicated in regulating cuticle-related genes, including those encoding 3-ketoacyl-CoA synthase and cytochrome P450 monooxygenase [3, 9]. Their expression is influenced by drought stress, with distinct patterns observed between drought-tolerant and drought-sensitive wheat genotypes. Notably, the drought-tolerant genotype RAC875 exhibits a thicker cuticle and higher wax deposition compared to the drought-sensitive genotype Kukri, highlighting the potential role of WXPL TFs in mediating these phenotypic differences [2]. In *Medicago truncatula*, WXP1 and WXP2 TFs, are involved in cuticle biosynthesis and show drought and ABA-dependent expression. Overexpression of WXP1 in *Medicago sativa* and both WXP genes in *Arabidopsis* increased wax deposition and drought tolerance. However, WXP2 showed sensitivity to low temperatures and growth disturbances in transgenic plants, unlike WXP1, which holds promise for enhancing drought and frost tolerance. Several WXP orthologues, such as RAP2.4 in *Arabidopsis*, ZmDBF1 in maize, and GmDREB2 in soybean, also regulate stress responses. In wheat, TaWXP-like (TaWXPL) TFs, homologues of WXP1 and WXP2, were identified and shown to activate cuticle biosynthesis genes under drought conditions [2, 3].

4. Conclusions

This study provides insights into the transcriptional regulation of the *TaWXPL1D* gene, a key player in cuticle biosynthesis, under drought conditions in wheat genotypes. Significant genotypic differences were observed, with a pronounced downregulation in the drought-sensitive genotype Aran. These findings underscore the role of *TaWXPL1D* in the adaptive response to drought and highlight genotype-specific mechanisms influencing cuticle development.

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