WHEAT
STUDIES

Gene Expression Studies on Water Use Efficiency in Wheat

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Abstract
Drought stress constitutes a critical challenge to wheat production worldwide by substantially limiting yield. The genetic complexity of drought tolerance which is characterized by its polygenic inheritance, low heritability, and strong environmental interactions, has hindered progress through conventional breeding. Consequently, molecular breeding approaches focused on improving water use efficiency have gained significance. Selecting genotypes with superior water use efficiency under drought conditions is central to these efforts. Gene expression analyses have provided critical insights into the genetic regulation of water use and drought tolerance mechanisms. A multi-omics approach that integrates transcriptomic, proteomic, and metabolomic datasets offers a more comprehensive understanding of the plant's stress response. This review synthesizes recent advances in gene expression studies on water use efficiency in wheat, emphasizing key genes, regulatory networks, and traits related to physiological regulation, stress adaptation, carbohydrate metabolism, stay green, stem reserve mobilization, root architecture, and nutrient-water interactions. By integrating molecular and physiological breeding strategies targeting critical transcriptional pathways, the review outlines opportunities to improve water use efficiency, nutrient uptake, and multi-stress resilience. The identified molecular targets provide a foundation for developing climate resilient wheat cultivars with stable yields under water limited conditions.

Buğdayda Su Kullanım Verimliliği Üzerine Gen İfadesi Çalışmaları

Özet
Kuraklık stresi, verimi önemli ölçüde sınırlayarak dünya çapında buğday üretimi için kritik bir zorluk oluşturmaktadır. Poligenik kalıtım, düşük kalıtım derecesi ve güçlü çevresel etkileşimlerle karakterize edilen kuraklık toleransının genetik karmaşıklığı, geleneksel ıslah yoluyla ilerlemeyi engellemiştir. Sonuç olarak, su kullanım verimliliğini artırmaya odaklanan moleküler ıslah yaklaşımları ön plana çıkmıştır. Kuraklık koşullarında üstün su kullanım verimliliğine sahip genotiplerin seçilmesi bu çabaların merkezinde yer almaktadır. Gen ifadesi analizleri, su kullanımının genetik düzenlenmesi ve kuraklık toleransı mekanizmaları hakkında kritik bilgiler sağlamıştır. Transkriptomik, proteomik ve metabolomik veri setlerini entegre eden çoklu omik yaklaşımı, bitkinin stres tepkisine dair daha bütünsel bir anlayış sunmaktadır. Bu derleme, buğdayda su kullanım verimliliği üzerine gen ifadesi çalışmalarındaki son gelişmeleri özetleyerek, fizyolojik düzenleme, stres adaptasyonu, karbonhidrat metabolizması, yeşil kalma, gövde rezervi mobilizasyonu, kök mimarisi ve besin-su etkileşimleriyle ilgili temel genleri, düzenleyici ağları ve özellikleri vurgulamaktadır. Derleme, kritik transkripsiyonel yolları hedefleyen moleküler ve fizyolojik ıslah stratejilerini entegre ederek; su kullanım verimliliğini, besin alımını ve çoklu stres toleransı sağlama yollarını özetlemektedir. Belirlenen moleküler hedefler, su kısıtlı koşullar altında istikrarlı verim sağlayan, iklime dayanıklı buğday çeşitlerinin geliştirilmesi için bir temel oluşturmaktadır.

1.Introduction

Cereal crops constitute a fundamental part of the global human diet, with wheat among the most widely cultivated and consumed grains worldwide. However, wheat growth and development are frequently challenged by abiotic stresses such as water scarcity, elevated temperatures, and salinity, which significantly reduce yield potential (Imaduwage and Hewadikaram, 2024). Sustaining wheat production under these adverse conditions is essential to ensuring global food and nutritional security. Consequently, improving water use efficiency (WUE) and maintaining yield stability under drought stress have become primary goals in modern wheat breeding programs (Yu et al., 2020). A thorough understanding of the regulatory networks governing abiotic stress responses is crucial for identifying molecular targets to develop climate resilient wheat cultivars capable of maintaining productivity under environmental stresses (Imaduwage and Hewadikaram, 2024).

Gene expression under drought conditions varies according to diverse defense mechanisms and complex molecular pathways (Cui et al., 2018). Genetic responses to water stress differ among drought tolerant wheat genotypes, underscoring the need to decipher gene regulatory mechanisms associated with water use (Hickey et al., 2022). Transcriptomic analyses facilitate the classification of multiple RNA species, including messenger RNAs, non-coding RNAs, and small RNAs, and allow for detailed characterization of transcriptional features such as transcription start sites, untranslated regions, alternative splicing, and post-transcriptional modifications (Liu, 2024). Moreover, transcriptome profiling enables the examination of gene expression dynamics throughout developmental stages and under abiotic stress conditions (Wang et al., 2009).

WUE, defined as the ability to sustain photosynthesis and yield with limited water, is a critical trait in wheat genetics (Hafeez et al., 2024). It can be evaluated at different biological levels depending on the breeding objective. At the leaf scale, WUE is often inferred from gas exchange measurements, either as the ratio of photosynthesis to stomatal conductance (A/g_s) or as photosynthesis to transpiration (A/E) (Farquhar & Richards, 1984; Flexas & Medrano, 2002). At the plant level, WUE is typically expressed as the relationship between yield or biomass and the water used through evapotranspiration (Y/ET), estimated with soil water balance methods or lysimeters (Passioura, 1977; Blum, 2009). Long-term efficiency can also be approximated using carbon isotope discrimination (CID, $\Delta^{13}C$) in plant tissue, where lower discrimination values indicate greater WUE (Condon et al., 2004). Each method provides distinct but complementary insights, from short-term physiology to seasonal yield responses, which can be integrated into breeding programs to identify resilient genotypes for water limited environments.

Drought stress disrupts photosynthetic machinery, impairs Calvin cycle enzyme activities, and promotes the accumulation of reactive oxygen species (ROS), thereby disturbing the balance of stress defense mechanisms (Al-Huqail et al., 2023; Bapela et al., 2022). Identifying WUE related genes and understanding their molecular pathways is therefore vital for their application in wheat breeding and genetic engineering.

This review synthesizes recent advances in gene expression studies related to WUE in wheat and consolidates current knowledge on the genes and regulatory networks governing key WUE related traits. It offers critical insights into the functional roles of candidate genes and regulatory elements, emphasizing their significance for wheat breeding. Traits discussed include physiological regulation, autophagy and oxidative stress responses, water soluble carbohydrate (WSC) accumulation and remobilization, stay green (SG) characteristics, stem reserve mobilization (SRM), root system architecture (RSA), and the interactions between nutrient uptake and WUE. By integrating these findings, the review identifies potential molecular targets to support the development of climate resilient wheat cultivars.

2. Findings of Gene Expression Analysis on WUE

2.1 Role of physiological traits

In wheat breeding, physiological traits responsive to water availability often exhibit higher heritability than yield, thereby offering greater potential for selection and accelerating the development of drought tolerant cultivars (Khadka et al., 2020). Water stress adversely affects physiological processes critical for wheat growth and development, leading to reductions in morphological parameters such as leaf relative water content, leaf area index, and chlorophyll concentration (Zhang et al., 2025; Alsamadany et al., 2023; Laddomada et al., 2021). Hence, understanding the relationship between physiological and morphological traits at the molecular level is essential for improving WUE.

Hormonal regulation plays a fundamental role in plant responses to water stress. Gibberellins (GAs), key regulators of plant growth, are downregulated under drought conditions, resulting in reduced water consumption (Hossain et al., 2019; Chen et al., 2016; Colebrook et al., 2014; Khoshro et al., 2014). Ptoškova et al. (2022) demonstrated that in drought stressed bread wheat (*Triticum aestivum*) seedlings, GA concentrations decreased in leaf tissues but increased in root growth regions,

correlating with inhibited leaf elongation and sustained root development. Although the GA-deactivating gene *TaGA2ox4* was upregulated, the decline in GA at the leaf base was primarily attributed to reduced biosynthesis rather than enhanced degradation. This organ specific hormonal response contrasts with other hormones that exhibit similar patterns in leaves and roots under drought, suggesting a unique regulatory mechanism for GA metabolism during water stress.

Abscisic acid (ABA) synthesis and signaling genes also regulate stomatal conductance and contribute to WUE development. Overexpression of the calcineurin B-like protein (CBL)-interacting protein kinase gene *TaCIPK23* modulates the ABA signaling pathway and enhances drought responses in wheat (Cui et al., 2018; SRA: SRP071191). Similarly, overexpression of the ABA receptor gene *TaPYL1-1B* increases WUE and photosynthetic efficiency by reduced ^{13}C fractionation in wheat leaves, with RNA-seq raw reads deposited in the NCBI under accession numbers SRR11573018-SRR11573026 (Mao et al., 2022), highlighting their potential utility in engineering drought tolerant germplasm.

Stomatal density is a critical trait influencing water loss via transpiration and CO_2 uptake, thus playing an important role in adaptation to water stress. Functional evidence suggests that stomatal density regulation in wheat occurs through mechanisms similar to those in diploid grasses (Hepworth et al., 2018). *EPIDERMAL PATTERNING FACTOR (EPF)* genes are expressed during wheat leaf and stem development, particularly in regions exhibiting variation in stomatal patterning (Dunn et al., 2019). Overexpression of *EPF1-like* and *EPF2-like* genes reduces stomatal density, resulting in decreased gas exchange capacity with only minor reductions in photosynthetic efficiency (Dunn et al., 2019). Both misexpression and overexpression of *EPF2* decrease stomatal density, thereby enhancing WUE and drought tolerance via reduced maximum stomatal conductance (gw(max)) (Franks et al., 2015). Reducing stomatal density offers a promising strategy to improve WUE in cereals by lowering water loss without substantially compromising carbon assimilation (Caine et al., 2019).

Hickey et al. (2022) investigated autophagic responses to water starvation in drought resilient spring wheat varieties Hollis and Drysdale, which differ in peroxisome abundance. The study reported upregulation of the autophagy marker *ATG8.4*, reduced transcription of *ATG8* (previously implicated in heat and drought responses in wheat; Yue et al., 2018), and increased catalase protein abundance in Hollis under drought. These findings suggest that Hollis mitigates oxidative stress induced by ROS through enhanced peroxisome proliferation and autophagic activity, whereas Drysdale adapts by augmenting its root system. This highlights the role of peroxisomes and autophagy in drought tolerance and supports the use of peroxisome related genetic markers in marker assisted selection (MAS) for improved WUE in wheat.

Integrating physiological, hormonal, and anatomical insights with molecular data offers a comprehensive framework for improving WUE in wheat. Reduced stomatal density, optimized hormonal signaling, and enhanced autophagic responses contribute to adaptive strategies under water limited conditions. Targeting genes involved in GA and ABA regulation, EPF-mediated stomatal development, and peroxisome associated stress mitigation provides valuable avenues for MAS and genetic engineering. A breeding strategy that integrates multiple traits with gene-informed selection will expedite the creation of climate resilient, drought tolerant wheat varieties capable of maintaining stable yields under variable water availability.

2.2 Role of water soluble carbohydrates

During the grain-filling stage, WSCs, their mobilization in the stem, and elevated invertase activity represent important biochemical traits for breeding wheat cultivars adapted to terminal drought conditions (Suneja et al., 2015). Understanding the genetic and physiological relationships between WSCs and water stress tolerance is critical for developing water use efficient wheat varieties.

Xue et al. (2008) demonstrated that in wheat stems, the mRNA expression levels of fructan-synthesizing enzymes, sucrose:sucrose 1-fructosyltransferase (1-SST) and sucrose:fructan 6-fructosyltransferase (6-SFT), correlate positively with stem WSC and fructan content. Following anthesis, fructan 6-exohydrolase (6-FEH; EC 3.2.1.154) activity increases more markedly than fructan 1-exohydrolase (1-FEH; EC 3.2.1.153) during carbon remobilization, thereby contributing to grain yield under water stress (Joudi et al., 2012). Zhang et al. (2015) identified the *1-FEH w3* gene in wheat stem, linked to fructan remobilization, through a cleaved amplified polymorphic (CAP) marker derived from a single nucleotide polymorphism (SNP) in its promoter. The mutant *Westonia 1-FEH w3* allele was associated with higher thousand grain weight (TGW) under water deficit conditions.

In durum wheat (*Triticum durum*), Cimini et al. (2015) reported that early alterations in fructan pools during kernel maturation may participate in signaling pathways regulating carbohydrate metabolism, redox balance, and sucrose sink strength. Additionally, retention of fructans in mature kernels could improve germination efficiency. Yañez et al. (2017) found that drought-tolerant bread wheat genotypes exhibited increased accumulation of WSCs, including glucose, fructose, and fructans,

and elevated expression of *fructan 1-fructosyltransferase B (1-FFTB)* and *fructan 1-exohydrolase w2 (1-FEHw2)* genes under water stress compared to fully irrigated conditions.

Thapa et al. (2022) reported that carbon mobilization from stem to grain predominantly occurs through the penultimate internodes under water stress. This carbon translocation is intensified more by drought than other abiotic stresses (Gurumurthy et al., 2023), as reflected by a greater spike weight difference (SWD) (Taria et al., 2025) and increased expression of sugar metabolism genes (Li et al., 2022). Collectively, these findings suggest that enhancing stem WSC content and mobilization of them improves grain filling and indirectly increases WUE under drought.

A genome-wide association study (GWAS) of 278 wheat genotypes identified 14 significant marker-trait associations (MTAs) linked to SG and SRM traits under combined heat and drought stress (Malakondaiah et al., 2025). Candidate genes linked to UDP-glycosyltransferase 73C4-like and protein detoxification 40-like functions were associated with soil plant analysis development (SPAD) and canopy temperature (CT), respectively. One MTA on chromosome 6B, coding for a wall associated receptor kinase 4-like protein, was associated with SRM. These markers offer valuable targets for marker assisted selection aimed at improving wheat adaptation to water stress.

Further analysis by Taria et al. (2025) identified seven candidate genes regulating stem density, including the peduncle, penultimate, and lower internodes, based on in silico gene expression profiles under heat and drought stress. Notably, on chromosome 7B, genes such as *TraesCS7B02G456600 (glyoxylate/hydroxy pyruvate/pyruvate reductase 2KGR-like)*, *TraesCS7B02G458900 (alpha carbonic anhydrase 7-like)*, *TraesCS7B02G458300 (NADH-ubiquinone oxidoreductase chain 2-like)*, *TraesCS7B02G443300 (glutathione S-transferase 1-like)*, and *TraesCS7B02G455300 (12-oxophytodienoate reductase 1-like*, involved in jasmonic acid biosynthesis) were identified. These were determined by mapping the probe sequences to their physical locations on the wheat CS reference genome IWGSC RefSeq 2.1. On chromosome 2A, stem-specific weight of the penultimate internode was associated with TraesCS2A02G582700 (a chloroplastic pentatricopeptide repeat protein) and TraesCS2A02G576400 (a soluble inorganic pyrophosphatase), the latter showing increased expression under stress conditions.

These insights enhance understanding of the genetic regulation of stem density and reserve mobilization, facilitating marker-assisted and genomic selection strategies to improve drought tolerance in wheat.

2.3 Role of root system architecture

Water use efficient wheat genotypes typically possess larger and deeper root systems, enabling better access to moisture in deep or water limited soils (Hickey et al., 2022). Functional genomics studies have shown that root development is influenced by abiotic factors and plant hormones, particularly auxin, which regulates root growth angle (RGA) (Furutani et al., 2020; Rasool et al., 2022). Optimizing RSA to enhance water uptake and translocation to aerial tissues under drought stress is therefore crucial. Identification of candidate genes and transcripts involved in RSA represents a promising approach for breeding water use efficient wheat varieties.

The growth angle of root axes at the seedling stage in cereals is a key component of RSA affecting water and nutrient uptake (Rasool et al., 2022). Hamada et al., 2012 identified novel quantitative trait loci (QTLs) controlling the gravitropic and hydrotropic responses of wheat roots, one on chromosome 5A for seminal root number per seedling (RN) and two on chromosomes 5D and 7D for seminal root elongation rate (ER). Despite its importance in drought response, the transcriptional regulation of RGA remains incompletely understood. Genes such as *DRO1 (Deeper Rooting 1)* and *LAZY*, which interact with microtubule-associated proteins, positively regulate lateral root orientation, growth, and development (Kumar et al., 2023; Hollender et al., 2020; Ashraf et al., 2019). In wheat, drought-responsive genes regulate pathways including carbon metabolism, phytohormone signaling, and flavonoid biosynthesis, which are integral to root development (Hu et al., 2018; Rasool et al., 2022).

Rasool et al. (2022) identified 56 putative SNPs in wheat roots linked to stress signaling and protective proteins, serving as molecular markers for breeding. Many differentially expressed genes under drought stress affect transferase activity, UDP-glycosyltransferase activity, plasma membrane repair, and response to aluminum ions.

Yang et al. (2025) characterized a root-derived peptide, TaCLE24b, which enhances the binding and phosphorylation activity of the receptor kinase TaCLV1 toward *TaSG-D1*. Using CRISPR/Cas9 to target the *TraesCS5B02G418500* gene, predominantly expressed in seedling roots (Li et al., 2019), TaCLE24b was shown to promote lateral root development and improve water stress responses by inducing phosphorylation and degradation of *TaSG-D1*. Overexpression of TaCLE24b also increased flag leaf chlorophyll content, thousand kernel weight, stomatal closure frequency, and reduced water loss under drought conditions (Yang et al., 2025). Similarly, Gabay et al. (2023) reported that expression of *OPR III genes*, part of the monocot-specific 2-oxophytodienoate

reductase subfamily III, influences root architecture and drought adaptation. Overexpression of *TaEXPA2* enhances lateral root development, drought response, and root biomass in wheat (Yang et al., 2020).

Maqbool et al. (2023) identified 97 genes regulating root growth and development significantly correlated with 25 phenotypic root traits across 24 spring wheat cultivars. Notably, the *VP1* gene on chromosome 3A was expressed only in the cultivar Seher-2006 (Gene Expression Omnibus (GEO) repository accession number GSE235844). This extensive dataset provides strong associations between gene expression and root phenotypes. Additional root-related genes include *RPK1 (Receptor-like Protein Kinase 1*; Shi et al., 2013), *PSTOL1 (Phosphate Starvation Tolerance 1*; Milner et al., 2018), *TaLBD16* regulating lateral root number (Wang et al., 2018), and *LRD (Lateral Root Density)*, homologous to *KNAT3* in Arabidopsis, controlling lateral root density (Zhang et al., 2024). The *LRD* gene, introgressed from wild ancestor *Agropyron elongatum*, is downregulated under drought, promoting root development and WUE (Placido et al., 2020). These genes are integral to root growth and contribute to enhanced yield under water stress.

Under drought, wheat exhibits gravitropic responses (Wasaya et al., 2018). Rasool et al. (2022) identified *MAPRE1 (Microtubule-associated protein RP/EB family member, TraesCS2D02G523000)*, highly expressed in roots of drought-tolerant varieties Local White and UZ-11-CWA-8. *MAPRE1* is orthologous to the *EB1 (End-Binding 1)* gene, which positively regulates gravitropism and influences WUE. Roychoudhry and Kepinski (2022) demonstrated that root gravitropism modulates RSA, aiding adaptation to both waterlogging and drought by adjusting root system depth and architecture in Japanese wheat varieties.

Despite advances, the transcriptional networks underlying RGA responses to water stress in wheat require further elucidation to fully harness root architecture traits in breeding programs.

2.4 Interactions between nutrient uptake and WUE

Nutrient management in wheat production is increasingly challenged by the escalating negative impacts of climate change, including unpredictable weather patterns and altered precipitation regimes. These changes exacerbate water deficiency, which in turn adversely affects nutrient dynamics and uptake in crops (Shanker et al., 2024). Water scarcity impairs nutrient absorption by roots, consequently reducing WUE in wheat and other crops (Wang et al., 2009). Nutrients also play a

pivotal role in regulating gene expression associated with root architecture and function (Oono et al., 2011; Kellermeier et al., 2014). Therefore, integrated studies examining the molecular mechanisms and transcriptomic responses to combined drought and nutrient deficiencies are essential for advancing WUE traits in wheat.

Lv et al. (2021) demonstrated that foliar nitrogen application, particularly ammonium (NH_4^+-N), under water stress conditions upregulates genes involved in the sucrose-starch conversion pathway. This promotes carbohydrate remobilization and starch synthesis, influencing grain filling dynamics, starch accumulation, phytohormone levels, and antioxidant enzyme activity. These findings underscore the impact of nitrogen sources on wheat's physiological and molecular responses to drought (Lv et al., 2021).

Phosphorus (P) deficiency, alongside drought stress, represents a critical abiotic constraint to wheat growth. P starvation downregulates the expression of inorganic phosphate transporter genes such as *Pht1;1*, thereby reducing phosphate uptake and redistribution (Jain et al., 2013; Chien et al., 2022). Zhang et al. (2022) observed significantly reduced root water content under combined P starvation and drought, impairing water transport to shoots. Their transcriptomic analysis of roots from the drought-tolerant and P-efficient winter wheat cultivar Xindong20 identified 4,577 differentially expressed genes (DEGs), with 3,207 upregulated, primarily associated with biological processes and cellular components according to Gene Ontology (GO) terms. Notably, the zinc finger transcription factor WRKY19-b was upregulated, while phosphate transporter PT8 was downregulated under these combined stresses (Zhang et al., 2022).

Cadmium (Cd^{2+}) toxicity further disrupts gene expression, stress signaling pathways, and nutrient transporter function, exacerbating drought stress effects (Quan et al., 2021; Li et al., 2021). Haider et al. (2025) reported that 24-epibrassinolide (EBL) application under combined Cd and drought stress modulated aquaporins, dehydrin-/LEA group 2-like proteins, photosystem II chlorophyll apoproteins, Cd tolerance factors, and high-affinity nitrate transporters. Transcriptomic data revealed that Cd toxicity and drought negatively affect senescence regulation genes (e.g., *TraesCs1A02G351000*) in winter wheat grains. Moreover, Cd concentration in leaves was inversely correlated with the expression of genes encoding dehydrin-/LEA group 2-like proteins (TraesCS6B02G383600) and Cd tolerance factors (TraesCS6B02G396800) (Haider et al., 2025). The transcriptomic analysis in the study also revealed that expression of aquaporin genes, which facilitate water transport across cellular membranes (Afzal et

al., 2016), helps to maintain water balance and reduce water loss under drought and cadmium stress. Because aquaporins are membrane channel proteins, their role in stress signaling and nutrient balance is important to understand water and nutrient use efficiency in wheat. Table 1 illustrates aquaporin gene family grous, their functional role and relations with WUE in wheat.

Table 1: Aquaporin Gene Family in Wheat and Their Role in WUE

Aquaporin Family	Function in Wheat	Role in WUE / Drought Tolerance	References
Intrinsic Plasma Membrane (PIP)	Water uptake and transcellular water flow in roots	Improve root water absorption efficiency, Regulate osmotic stress responses	Forrest and Bhave, 2010; Ayadi et al., 2011
Intrinsic Tonoplast (TIP)	Regulate water storage and osmotic adjustment within vacuoles	Maintain cell turgor and osmotic stability under drought, indirectly supporting WUE	Espinoza et al., 2018; Pandey et al., 2013
Intrinsic nodulin 26-like (NIP)	Transport water plus small solutes	Improve water stress adaptation and nutrient use efficiency	Haider et al., 2025
Intrinsic small (SIP)	Facilitate intracellular water transport	Help regulate intracellular water balance, but limited direct WUE evidence	Espinoza et al., 2018
Intrinsic uncharacterized (XIP)	Putative role in water transport	Functions in wheat are poorly understood; potential but unconfirmed links to WUE	Espinoza et al., 2018

Addressing water scarcity, nutrient deficiency, and heavy metal toxicity in wheat requires integrating molecular and physiological breeding strategies. Transcriptomic and physiological studies show that nitrogen and phosphorus availability strongly influence genes regulating carbohydrate metabolism, root architecture, hormone signaling, and stress adaptation. Combined stresses, such as drought with P deficiency or Cd toxicity, trigger transcriptional reprogramming affecting key transporters, transcription factors, and protective proteins. Targeting these pathways through marker-assisted selection, genomic selection, and gene editing can enhance WUE and nutrient uptake, enabling the development of high-yielding, climate-resilient cultivars for resource-limited environments.

Supplementary Table 1 highlights key genetic determinants of drought adaptation in wheat, spanning multiple physiological and molecular pathways. Hormonal regulation genes such as *TaGA2ox4, TaCIPK23, TaPYL1-1B,* and *EPF1/2* modulate gibberellin downregulation, ABA signaling, and stomatal density, offering targets for optimizing WUE. Genes involved in autophagy, *ATG8.4*, and oxidative stress mitigation, CAT protein, contribute to ROS detoxification, while carbohydrate metabolism genes, *1-SST, 6-SFT, 1-FEH w3, 6-FEH*, enhance WSC accumulation and remobilization to sustain grain filling under drought. Stay-green and SRM traits, linked to UDP-glycosyltransferase 73C4-like and wall-associated kinase 4, provide valuable markers for combined drought and heat tolerance. RSA is shaped by *DRO1, LAZY, TaCLE24b, MAPRE1, LRD, and PSTOL1*, enabling deeper rooting and improved water acquisition. Finally, genes regulating nutrient-WUE interactions, including *WRKY19-b*, *PT8*, aquaporins, LEA proteins, and nitrate transporters, support integrated resilience under simultaneous drought and nutrient stress. Collectively, these genes represent high-value targets for marker-assisted selection, genomic prediction, and genome editing to accelerate the development of climate-resilient wheat cultivars.

3. Conclusion and Perspective

Breeding wheat for drought tolerance remains a central goal in modern crop improvement programs, driven by increasingly unpredictable climatic conditions. WUE, a key trait for drought adaptation, is among the most complex, being controlled by polygenic regulatory networks and strongly influenced by environmental interactions. Although the large and complex wheat genome presents significant challenges, advances in gene expression studies and transcriptome profiling have provided valuable insights into the molecular mechanisms underlying WUE and drought stress responses. Integrating these findings into breeding pipelines enables the identification and manipulation of key genes and pathways, offering genetic

strategies to enhance drought tolerance, stabilize yields under water-limited conditions, and maintain sustainable wheat production for global food security.

High-throughput transcriptomics, proteomics and epigenomics have enabled the identification of stress-responsive genes, histone modifications, regulatory non-coding RNAs, and gene families involved in drought adaptation. In particular, recent work has highlighted the role of long non-coding RNAs (lncRNAs) in coordinating transcriptional and post-transcriptional responses to overlapping stress factors such as drought, salinity, and temperature extremes (Zhang et al., 2023b; Imaduwage and Hewadikaram, 2024). These molecules modulate gene expression by interacting with target RNAs, proteins, and chromatin-modifying complexes, thereby playing a critical role in stress tolerance. Histone methylation and other epigenetic modifications have been shown to affect the expression of drought related genes, as evidenced by studies on wheat histone methyltransferase gene families (Sun et al., 2022). These findings offer new perspectives on the epigenetic regulation of stress responses and suggest potential targets for developing stress resilient wheat cultivars using two-line hybrid systems. Omics technologies also provide new opportunities for trait improvement through molecular breeding and gene-editing tools such as CRISPR. Moreover, integrating multiple drought-tolerance mechanisms from diverse wheat genotypes into a single cultivar may produce more robust and climate-resilient varieties.

Integrating developmental, physiological, biochemical, and molecular drought adaptation mechanisms into a single elite wheat genotype offers a promising strategy to enhance crop resilience (Hickey et al., 2022). Combining traits such as deeper rooting, efficient stomatal regulation, improved carbohydrate remobilization, and enhanced nutrient uptake under stress can cumulatively improve WUE and yield stability. Genome editing technologies, particularly CRISPR-Cas, combined with transcriptomics and systems biology, enable functional characterization of key genes and precise manipulation of regulatory networks to advance drought-tolerant wheat breeding.

Future research should focus on dissecting the regulatory networks governing gene expression dynamics across wheat tissues and developmental stages under drought, while also investigating the crosstalk between drought and other abiotic stresses. Breeding programs should integrate genomic selection and marker-assisted strategies informed by functionally validated genes to accelerate the development of climate-resilient wheat cultivars with enhanced WUE and stable yield performance.

In conclusion, enhancing wheat's drought resilience through an integrative understanding of physiological, molecular, and biochemical responses offers a promising pathway to secure global food security under changing climates. The synthesis of current knowledge presented in this review highlights the importance of multidisciplinary approaches and molecular breeding technologies in meeting this critical challenge.

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Supplementary Table 1: WUE related genes, their functional roles and breeding implication

Trait/Mechanism	Key Gene(s) / Factor(s)	Tissue Type	Drought Stress Treatment	Sequence Analysis Method	Functional Role	Breeding Implication / Reference Genes	SRA/GEO Accession Numbers
Physiological regulation	*TaGA2ox4, TaCIPK23; TaPYL1-1B, EPF1/2*	Total RNA from wheat seedling roots and leaves; Total RNA from abaxial (lower) epidermis of leaf 1	10 days old wheat seedlings were water stressed for 16 days; water-stressed leaf 1 at 3 day after germination	De novo Transcriptome Assembly; Differential Gene Expression Analysis	GA downregulation, ABA signaling; stomatal density control	Target genes for hormonal regulation and stomatal optimization (Cui et al., 2018; Dunn et al., 2019)	GenBank No: KD502068, Sequence Read Archive (SRA): SRP071191; NCBI SRA: PRJNA680499
Autophagy & oxidative stress	*ATG8.4*, CAT proteins	Flag leaves and roots	Zadoks stage 25 tissue samples collected once the soil volumetric water content (VWC) fell below 0.2%	Targeted gene expression analysis (RT-qPCR) and protein-level assays	ROS detoxification via autophagy/peroxisomes	Selection of varieties with enhanced antioxidant systems (Hickey et al., 2022)	Not publicly accessible (available on request)
WSC accumulation and remobilization	*1-SST, 6-SFT, 1-FEH w3, 6-FEH*	Stem internodes (peduncle and lower nodes), Developing grains	Water stressed was applied from anthesis to grain filling stage	SNP Detection, CAP Marker Development, Gene expression and enzyme assays	Fructan biosynthesis & remobilization, invertase activity	Improved grain filling under drought (Zhang et al., 2015; Joudi et al., 2012)	No SRA accession number
Stay-green & SRM traits	UDP-glycosyltransferase 73C4-like, detoxification 40-like, wall-associated kinase 4	Stem internodes, Leaves, Developing grains	Drought stress applied at anthesis	Genotyping a panel of 278 wheat genotypes using a 35K Axiom® Wheat Breeder's Array, Identifying marker-trait associations (MTAs)	Linked to canopy temperature, stem reserve mobilization	Marker-assisted selection targets under drought and heat stress (Malakondaiah et al., 2025)	14,625 SNPs after filtering, Total significant MTAs: 36 across 16 chromosomes, Genotype data are public via Dryad (Krishnappa et al. 2022 dataset)

Root system architecture (RSA)	*DRO1, LAZY, TaCLE24b, MAPRE1, LRD, PSTOL1*	Lateral Root Primordia (LRP) & Root Tips and Shoots; Total RNA from 35-days-old, germinated seedlings shoot tissues	Drought stress treatment was applied for 10 days old seedlings for 14 days; 35 days old seedlings were exposed to Hoagland solution supplemented with 20% PEG-6000 for 5 days.	Expression analysis was carried out via RT-qPCR and GUS reporter assays; Transcriptome and Metabolome Analysis	Regulation of root growth angle, gravitropism, lateral root development	Enhancing deep rooting and efficient water uptake (Yang et al., 2025; Rasool et al., 2022)	BioProject accession: PRJNA848851; PRJNA863398, GSE235844
Nutrient - WUE interaction	*WRKY19-b, PT8*, aquaporins (AQPs), LEA proteins, nitrate transporters	Total RNA from root tissue; Total RNA from leaf tissue	Root tissue sampling, under both conventional phosphorus (CP: 1.0 mmol/L) and low phosphorus (LP: 0.05 mmol/L) conditions, after 7 days of drought stress, imposed by 20% PEG-6000; Cd (present/absent), biochar (0% or 5% w/w), 24-epibrassinolide (EBL, 10^{-6} M applied as foliar spray), and water regime (75% vs. 35% water holding capacity (WHC))	Differential Gene Expression and qRT-PCR Analysis; RNA-Seq Transcriptome Profiling	Regulation under combined drought and nutrient (P, Cd) stress	Integrated stress resilience through nutrient-responsive gene selection (Zhang et al., 2022; Haider et al., 2025)	PRJNA784248; Not publicly accessible (available on request)