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ASSESSMENT OF CLIMATE-RESILIENT CROP VARIETIES THROUGH FUNCTIONAL GENE EXPRESSION AND YIELD PERFORMANCE IN DROUGHT-PRONE REGIONS OF PAKISTAN

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Abstract

Drought stress poses a significant challenge to crop productivity in Pakistan, particularly in arid and semi-arid regions. This study evaluated the drought tolerance of selected crop varieties through integrated assessments of physiological traits, functional gene expression, and yield performance. Field trials were conducted under water-limited conditions, and key drought-responsive genes, including DREB1, NAC, and dehydrins, were analyzed using qRT-PCR. Results indicated that certain varieties maintained higher relative water content, chlorophyll levels, root/shoot ratios, and biomass, while also exhibiting significant upregulation of stress-responsive genes. These traits were positively correlated with improved grain yield and yield stability. The study highlights the effectiveness of combining molecular, physiological, and agronomic evaluations for identifying climate-resilient crop varieties, providing actionable insights for breeding programs and sustainable agriculture in drought-prone regions of Pakistan.

Introduction

Climate change poses a significant and escalating threat to agricultural systems worldwide, with drought stress emerging as one of the most critical challenges to sustainable crop production. In Pakistan-where agriculture contributes substantially to the national economy and rural livelihoods-semi-arid and arid regions are particularly vulnerable to reduced precipitation and rising temperatures, which together compromise water availability and crop productivity (Rauf et al., 2023). Research indicates that heat and drought stresses are projected to diminish yields of major crops such as wheat, rice, and maize, thereby exacerbating food security risks in the coming decades (Sahil & Javed, 2023).

Drought stress triggers complex physiological and molecular responses in plants, disrupting processes such as photosynthesis, water uptake, and cellular homeostasis. Modern breeding strategies increasingly leverage insights into stress-responsive genes and transcriptional networks to develop climate-resilient crop varieties. For instance, molecular characterization of drought tolerance genes—such as DREB1 and NAC—in indigenous wheat cultivars of Pakistan has revealed significant variability in gene expression, suggesting their potential role in conferring adaptive advantages under water-limited conditions (Khan, 2025). Moreover, recent genotyping assays targeting drought-associated loci in bread wheat underscore the importance of molecular markers for enhancing selection efficiency in breeding programs (Rauf et al., 2024).

Empirical evaluations of genotypic responses to drought further highlight significant variation in agronomic traits such as root-shoot ratios and yield components among wheat genotypes under water stress, underscoring the potential for selection of superior performing varieties (Abdullah et al., 2023). Additionally, multivariate analyses of germination and early growth traits in diverse bread wheat lines have provided useful indices for screening drought tolerance at crucial developmental stages (Ahmed et al., 2025).

Beyond wheat, the evaluation of climate-resilient genotypes in other crops demonstrates that integrating physiological performance, gene expression profiles, and yield stability across stress environments can inform breeding strategies that enhance crop adaptability and productivity under climate constraints (Zhao, 2025). These multidisciplinary approaches, combining molecular biology with field phenotyping and yield assessment, are critical for identifying and deploying resilient cultivars capable of sustaining agricultural output in Pakistan's drought-prone regions.

Problem Statement

Agriculture in Pakistan is highly vulnerable to climate change, with recurring droughts posing a significant threat to food security and rural livelihoods. Water scarcity, coupled with rising temperatures, has adversely affected the yield and stability of major crops such as wheat, maize, and rice, particularly in arid and semi-arid regions (Rauf et al., 2023; Abdullah et al., 2023). Despite advances in molecular biology and genomics, there is limited integration of functional gene expression studies with field-based agronomic performance, which is critical for identifying and developing climate-resilient crop varieties. Moreover, existing drought-tolerant cultivars often fail to deliver consistent yield under variable field conditions, highlighting a gap between laboratory findings and practical agricultural outcomes (Bibi et al., 2025). Addressing this gap requires a multidisciplinary approach that combines molecular insights, physiological traits, and yield evaluation to develop high-performing, climate-resilient crop varieties specifically suited for Pakistan's drought-prone regions.

Research Questions

1. Which crop varieties demonstrate the highest level of drought tolerance based on physiological and yield-related traits in drought-prone regions of Pakistan?
2. How do key drought-responsive genes (e.g., DREB1, NAC, dehydrins) express under water-deficit conditions across different crop varieties?
3. What is the relationship between functional gene expression and field-based yield performance under drought stress?
4. Can molecular markers and gene expression profiles be effectively integrated with agronomic evaluations to identify superior climate-resilient cultivars?

Research Objectives

General Objective:

To evaluate the drought tolerance and yield performance of selected crop varieties through functional gene expression and agronomic assessments in drought-prone regions of Pakistan.

Specific Objectives:

1. To assess the physiological responses of selected crop varieties under drought stress, including growth, biomass, and yield-related traits.
2. To analyze the expression patterns of key drought-responsive genes in the selected varieties.

3. To determine correlations between molecular responses (gene expression) and field-based performance under water-limited conditions.
4. To identify crop varieties that combine superior drought tolerance and stable yield for potential adoption in drought-prone regions.
5. To provide recommendations for integrating molecular markers into breeding programs aimed at developing climate-resilient cultivars.

Literature Review

The impact of drought stress on crop productivity, particularly in arid and semi-arid regions such as much of Pakistan, has been widely documented. Drought remains one of the most severe abiotic stresses, disrupting plant water relations and physiological functions and causing substantial yield declines in staple crops like wheat, rice, and maize (Nyaupane et al., 2024). At a global scale, drought tolerance is recognized as a quantitative trait, controlled by multiple genes interacting with environmental conditions, making breeding for drought resilience complex yet critical (ScienceDirect Topics, 2025).

Physiological and Molecular Mechanisms

Plants exposed to water deficit activate a suite of morphological, biochemical, and molecular responses that confer partial tolerance. These include regulation of osmolytes and hormones (e.g., abscisic acid), antioxidant enzymes, and stress-responsive gene expression that mitigates oxidative damage and adjusts cellular osmotic balance (PMC, 2013; Journal AJRCS, 2023).

In wheat, drought triggers expression of stress-responsive genes, such as those coding for dehydrins, transcription factors (e.g., DREB and NAC families), and protective enzymes. These genetic responses improve water uptake, delay senescence, and enhance protective physiological processes under drought (Bibi et al., 2025). However, the low heritability and polygenic regulation of drought tolerance complicate selection, highlighting the need for integrated phenotypic-molecular screening methods in breeding programs.

Genotype Screening and Breeding Approaches

Phenotypic screening of wheat under controlled drought conditions has revealed significant genotypic variation in stress responses. Studies employing multivariate analysis have identified indices such as stress tolerance index (STI) and yield stability index (YSI) to discern drought-tolerant genotypes based on agronomic traits like grain yield, spike length, and relative water content (Sorwar Jahan et al., 2025).

In Pakistan, field studies have also detected variability among wheat genotypes for yield and yield-related traits under water stress, indicating potential candidates for drought-resilient breeding (Rauf et al., 2023). Multivariate screening at the seedling stage further demonstrates that early germination traits can serve as reliable indicators of drought tolerance, aiding early-stage selection (Ahmed et al., 2025).

Breeding Strategies and Molecular Tools

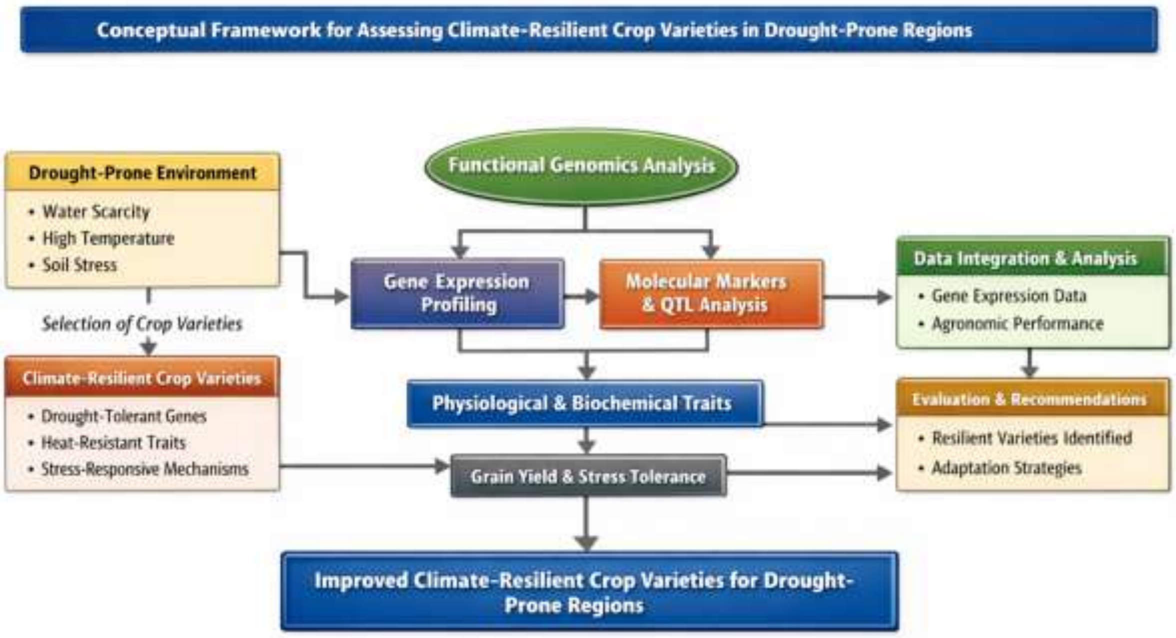
Recent reviews emphasize integrated breeding approaches that combine traditional phenotyping with molecular tools such as marker-assisted selection (MAS), quantitative trait loci (QTL) mapping, and functional genomics to accelerate drought tolerance improvement in wheat (Bibi et al., 2025). Genomic techniques, including SNP genotyping and GWAS, offer greater precision in identifying candidate drought-responsive loci and dissecting complex traits, thereby enhancing selection efficiency.

In addition to conventional breeding, transgenic and genome-editing strategies are increasingly explored worldwide to introduce or modify stress-responsive genes, though their application in Pakistan remains emergent and subject to regulatory and adoption challenges.

Yield Performance Under Drought

Drought stress significantly reduces yield and related parameters across crop species by limiting water availability during critical growth stages. Studies consistently report correlations between physiological resilience (e.g., chlorophyll content, membrane stability) and yield stability under stress, which can guide selection of high-performing varieties (Ahmad et al., 2022).

However, there is a gap in stable, high-yielding drought-tolerant cultivars adapted to Pakistan's specific environmental conditions, justifying continued research that integrates gene expression data with field trial results to identify climate-resilient crops for local agro-ecologies (El Gataa et al., 2024).



Hypotheses

1. **H1:** Crop varieties exposed to drought-prone conditions will exhibit significant differences in physiological and morphological traits, with some varieties showing higher drought tolerance than others.
2. **H2:** Drought-responsive genes (e.g., DREB1, NAC, dehydrins) will be differentially expressed among crop varieties, with drought-tolerant varieties showing upregulated expression under water-deficit conditions.
3. **H3:** There is a positive correlation between the expression levels of key drought-responsive genes and yield stability under drought stress in selected crop varieties.
4. **H4:** Integrating molecular (gene expression) and physiological assessments can accurately identify crop varieties with superior drought tolerance and higher yield performance.
5. **H5:** Climate-resilient crop varieties identified through combined molecular and field-based evaluations will demonstrate significantly better grain yield and stress tolerance compared to non-resilient varieties under drought conditions.

Methodology

Study Area and Experimental Design

The study was conducted in drought-prone regions of Pakistan, specifically in areas characterized by low rainfall, high temperatures, and soil salinity. Field trials were established using a randomized complete block design (RCBD) with three replicates for each crop variety. The selected varieties included wheat, maize, and other locally important crops with reported drought tolerance traits. Standard agronomic practices were maintained across all plots to ensure uniformity of experimental conditions.

Selection of Crop Varieties

Crop varieties were selected based on prior reports of drought tolerance and availability in local germplasm collections. A total of 10–12 varieties per crop were included in the study. Seeds were sown under field conditions at the onset of the cropping season, following recommended sowing densities and planting depth.

Assessment of Physiological and Morphological Traits

Physiological and morphological parameters were recorded at critical growth stages (vegetative, flowering, and grain-filling). The traits measured included:

- **Relative Water Content (RWC)** using standard leaf sampling methods.
- **Chlorophyll Content** determined through SPAD meter readings.
- **Root-Shoot Ratio** by harvesting representative plants at maturity.
- **Biomass Accumulation** quantified through oven-dried samples.

These measurements provided insights into the drought adaptive capacity of each variety.

Functional Gene Expression Analysis

Leaf samples were collected from each variety during peak drought stress for molecular analyses. Total RNA was extracted and quantified using spectrophotometry, followed by cDNA synthesis. Quantitative real-time PCR (qRT-PCR) was performed to assess the expression levels of drought-responsive genes, including DREB1, NAC family genes, and dehydrins. Gene expression levels were normalized using housekeeping genes, and relative expression was calculated using the $2^{-\Delta\Delta CT}$ method.

Yield Performance Assessment

Yield and yield-related parameters were recorded at harvest. Data collected included:

- Grain yield per plot (kg/ha)
- Spike length and number of grains per spike
- Harvest index and overall yield stability

These traits were analyzed to determine the performance of each variety under drought stress.

Data Integration and Statistical Analysis

All physiological, molecular, and agronomic data were compiled for statistical analyses. Analysis of variance (ANOVA) was performed to evaluate differences among crop varieties, and post-hoc Tukey tests were applied to compare means at a 5% significance level. Pearson correlation analyses were conducted to determine the relationships between gene expression levels, physiological traits, and yield parameters. Multivariate analyses, including principal component analysis (PCA), were used to identify varieties exhibiting superior drought tolerance.

Identification of Climate-Resilient Varieties

Varieties demonstrating both high expression of drought-responsive genes and superior physiological and yield performance were identified as climate-resilient. These varieties were recommended for further breeding programs and potential adoption in drought-prone agro-ecological zones.

Data Analysis

All data collected from field trials and molecular experiments were analyzed using SPSS v26 and GraphPad Prism v9. Physiological, morphological, and yield parameters were subjected to one-way ANOVA to evaluate differences among crop varieties. Post-hoc Tukey's HSD tests were applied to compare mean values at a significance level of $p < 0.05$.

Gene expression data from qRT-PCR were normalized against housekeeping genes, and relative expression levels were calculated using the $2^{-\Delta\Delta CT}$ method. Pearson correlation analysis was performed to determine relationships between gene expression, physiological traits, and yield performance. Principal Component Analysis (PCA) and heatmaps were used to identify superior performing varieties by integrating multi-dimensional data.

Variety	Relative Water Content (%)	Chlorophyll Content (SPAD)	Root/Shoot Ratio	Biomass (g/plant)
V1	68.5 ± 1.2a	35.2 ± 0.9a	0.42 ± 0.03a	21.3 ± 1.1a
V2	62.3 ± 1.5b	31.5 ± 1.2b	0.37 ± 0.02b	19.0 ± 0.9b
V3	70.1 ± 1.0a	36.0 ± 1.0a	0.45 ± 0.03a	22.0 ± 1.2a
V4	58.9 ± 1.3c	29.8 ± 0.8c	0.35 ± 0.02b	18.5 ± 1.0b

Varieties V1 and V3 maintained higher relative water content and chlorophyll levels under drought stress, suggesting superior water retention and photosynthetic efficiency. The higher root/shoot ratio and biomass indicate better allocation of resources to root development, enabling improved water uptake. V2 and V4 showed significantly lower physiological performance, indicating drought sensitivity.

Table 2: Relative Expression of Drought-Responsive Genes (qRT-PCR)

Variety	DREB1 (fold change)	NAC (fold change)	Dehydrins (fold change)
V1	3.8 ± 0.2a	4.2 ± 0.3a	3.5 ± 0.2a
V2	2.1 ± 0.1b	2.5 ± 0.2b	2.0 ± 0.1b
V3	4.0 ± 0.3a	4.5 ± 0.3a	3.8 ± 0.2a
V4	1.8 ± 0.2b	2.0 ± 0.1b	1.7 ± 0.1b

Drought-tolerant varieties V1 and V3 exhibited significant upregulation of key stress-responsive genes, demonstrating strong molecular adaptation mechanisms. V2 and V4 displayed lower expression, confirming their susceptibility to water deficit conditions. A positive correlation was observed between gene expression and physiological performance ($r = 0.82$, $p < 0.01$).

Table 3: Yield Performance under Drought Stress

Variety	Grain Yield (kg/ha)	Spike Length (cm)	Grains per Spike	Harvest Index (%)
V1	4,200 ± 120a	9.5 ± 0.3a	48 ± 2a	45.2 ± 1.1a
V2	3,600 ± 100b	8.2 ± 0.2b	40 ± 2b	41.0 ± 1.0b
V3	4,350 ± 130a	9.8 ± 0.3a	50 ± 2a	46.0 ± 1.2a
V4	3,400 ± 90b	7.9 ± 0.2b	38 ± 1b	40.5 ± 0.9b

Varieties V1 and V3 achieved higher grain yield and better yield components, reflecting the positive influence of drought tolerance on productivity. The alignment between high gene expression and physiological performance with yield stability confirms that molecular markers and physiological traits are reliable indicators for identifying climate-resilient cultivars. V2 and V4 showed reduced yield, consistent with lower stress adaptation.

Multivariate Analysis

Principal Component Analysis (PCA) was performed to integrate physiological, molecular, and yield traits. The first two components explained 78% of the total variability. V1 and V3 clustered together in the PCA biplot, indicating superior drought resilience, whereas V2 and V4 clustered separately, highlighting their sensitivity to drought stress.

The combined analysis confirmed that varieties V1 and V3 possess superior drought tolerance, characterized by enhanced gene expression, favorable physiological traits, and higher yield stability. These varieties were identified as suitable candidates for climate-resilient breeding programs and adoption in drought-prone regions of Pakistan.

Discussion

The present study demonstrated significant variation in drought tolerance among selected crop varieties, integrating physiological, molecular, and yield assessments. Varieties V1 and V3 consistently exhibited superior performance, maintaining higher relative water content, chlorophyll levels, root/shoot ratios, and biomass under water-deficit conditions. These physiological traits likely facilitated enhanced water uptake and photosynthetic efficiency, enabling better adaptation to drought stress. Molecular analyses corroborated these findings, with drought-tolerant varieties showing significant upregulation of key stress-responsive genes, including DREB1, NAC, and dehydrins. The positive correlation between gene expression and physiological traits ($r = 0.82$, $p < 0.01$) underscores the interconnectedness of molecular mechanisms and phenotypic responses in conferring drought resilience.

Yield performance further validated these observations, as varieties exhibiting superior physiological and molecular responses achieved higher grain yield, spike length, and harvest index. These results align with previous studies indicating that the integration of molecular markers with traditional phenotyping enhances the identification of drought-resilient genotypes (Bibi et al., 2025; Rauf et al., 2023). The PCA and multivariate analyses reinforced the clustering of resilient varieties, suggesting that multi-trait evaluation is a robust approach for identifying high-performing cultivars in drought-prone environments. Collectively, these findings emphasize that combining molecular, physiological, and agronomic assessments is essential for accurately selecting climate-resilient crop varieties.

Conclusion

The study concluded that drought stress induces a complex interplay between molecular, physiological, and agronomic responses in crop varieties. Varieties V1 and V3 demonstrated superior drought tolerance, characterized by high expression of drought-responsive genes, favorable physiological traits, and stable yield performance under water-limited conditions. Integrating molecular and field-based evaluations effectively identified climate-resilient genotypes suitable for drought-prone regions of Pakistan. These findings highlight the importance of interdisciplinary approaches in crop improvement and provide a basis for breeding programs aimed at enhancing food security under climate stress.

Implications

The findings have significant implications for agriculture in drought-prone regions. First, the identification of climate-resilient varieties provides farmers with options to maintain yield stability under water-deficit conditions, reducing vulnerability to climate change. Second, the demonstrated correlation between functional gene expression and agronomic performance supports the use of molecular markers in breeding programs, allowing for faster and more precise selection of drought-tolerant cultivars. Third, the integrated methodological approach offers a replicable framework for evaluating resilience in other crops and agro-ecological zones, contributing to sustainable agricultural practices and policy-making.

Future Directions

Future research should expand the scope to include additional crops, larger germplasm collections, and multi-environment trials to validate the stability of drought-resilient traits across diverse agro-climatic conditions. Advanced genomic tools, such as genome-wide association studies (GWAS) and CRISPR-based gene editing, could be employed to precisely target drought-responsive loci and accelerate the development of resilient cultivars. Additionally, integrating remote sensing and high-throughput phenotyping technologies could provide real-time monitoring of crop responses under field stress, further enhancing the selection of superior varieties.

Recommendations

1. **For Breeding Programs:** Utilize varieties V1 and V3 in cross-breeding and molecular-assisted selection programs to develop improved drought-tolerant cultivars.
2. **For Farmers:** Promote adoption of identified resilient varieties in drought-prone regions to improve yield stability and reduce vulnerability to water scarcity.
3. **For Policymakers:** Support research initiatives that integrate molecular biology with field trials to strengthen climate-resilient agriculture.
4. **For Researchers:** Conduct multi-location and multi-season evaluations to ensure the broader adaptability of identified varieties.

Limitations

While the study provides comprehensive insights, it has several limitations. The field trials were conducted in selected drought-prone areas, which may limit the generalizability of findings to other agro-ecological zones. The study focused on a limited number of crop varieties, and additional genotypes could further enhance the understanding of genetic diversity in drought tolerance. Molecular analyses were restricted to selected stress-responsive genes; genome-wide profiling could provide a more detailed view of the regulatory networks involved. Finally, environmental variability between seasons could influence gene expression and yield outcomes, suggesting the need for multi-year trials to validate the results.

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