International Journal of Plant & Soil Science

Volume 38, Issue 9, Page 220-228, 2026; Article no.IJPSS.164814
ISSN: 2320-7035

Utilizing CRISPR-Cas13 and Omics Technologies to Enhance Drought Tolerance in Iraqi Wheat Varieties in Abu-Ghraib/ Baghdad

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

Article Information

DOI: https://doi.org/10.9734/ijpss/2026/v38i96284

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://pr.sdiarticle5.com/review-history/164814

Original Research Article

Received: 22/06/2026
Accepted: 20/08/2026
Published: 24/08/2026

Abstract

Drought stress poses a major threat to wheat (Triticum aestivum L.) productivity and food security in arid regions such as Iraq. This study combines precision CRISPR-Cas13 RNA knockdown with multidimensional multi-omics platforms to enhance drought tolerance in local Iraqi wheat genotypes. During the critical summer cultivation period (Tammuz), experimental trials were conducted at the Abu Ghraib research site under controlled greenhouse conditions. We identified 34 conserved stress-responsive genes, 4,520 single-nucleotide polymorphisms (SNPs), 320 copy-number variations (CNVs), and five major quantitative trait loci (QTLs) linked to osmotic adjustment using BLAST bioinformatic alignment, GATK variant calling, and genome-wide association studies (GWAS). Using CRISPR-Cas13 post-transcriptional modification to target seven master negative regulators, 85% of downstream drought-responsive transcripts under water-deficit conditions were successfully upregulated by 2.3-28-fold. Evaluations conducted in controlled greenhouses showed a 23% increase in cellular antioxidant enzyme activity, a 19% increase in root water-absorption efficiency, a 28% reduction in leaf water loss, and a 42% higher survival rate. The breeding timescale was shortened by 30-35% by integrating these multi-omics biomarkers into a marker-assisted selection (MAS) framework. Under severe water deficits, agronomic trials showed a 115% increase in total grain yield

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Cite as: *Khudair, S., & Hameed, S. I. (2026). Utilizing CRISPR-Cas13 and Omics Technologies to Enhance Drought Tolerance in Iraqi Wheat Varieties in Abu-Ghraib/ Baghdad. International Journal of Plant & Soil Science, 38(9), 220–228. https://doi.org/10.9734/ijpss/2026/v38i96284*

while saving up to 40% of conventional irrigation-water inputs and increasing water-use efficiency by 35%. Agricultural policymakers and breeders should consider integrating this precision biotechnological approach into regional strategies to support food security under increasing climate-related challenges.

Keywords: *CRISPR-Cas13; drought tolerance; Triticum aestivum L.; multi-omics; RNA knockdown; marker-assisted selection; quantitative trait loci; water-use efficiency; Iraqi wheat.*

1. Introduction

Wheat *Triticum aestivum L*. is one of the foremost staple crops worldwide, providing essential calories and nutrients to billions of people. In Iraq, wheat production is an essential component of food security and economic stability; however, the country faces severe challenges due to climate change, particularly increasing temperatures and prolonged droughts (Lyford, 2017). Wheat growth is severely hampered by drought, resulting in lower grain production, poorer grain quality, and reduced economic returns (Furlan *et al*., 2017). The development of drought-tolerant wheat genotypes is a crucial strategy for preserving agricultural production given Iraq's arid and semi-arid climate. Although conventional breeding methods have made significant progress in improving osmotic stress tolerance, they remain time-consuming and resource-intensive, usually requiring lengthy selection cycles spanning multiple generations (Rahman *et al*., 2018). The emergence of CRISPR-Cas13 genome-editing technology, combined with omics-based approaches, including genomics, transcriptomics, proteomics, and metabolomics, offers a promising approach to accelerating wheat improvement programmes. CRISPR-Cas13, a component of CRISPR-associated RNA-targeting systems, has been widely investigated for its ability to modulate gene expression without altering the DNA sequence (O'Connell, 2019). Unlike CRISPR-Cas9, which introduces permanent DNA modifications, CRISPR-Cas13 targets RNA transcripts, making it a more flexible tool for regulating drought-responsive genes in a reversible manner (Konermann *et al*., 2018). By knocking down negative regulators or enhancing stress-responsive genes, researchers can improve wheat drought resilience without affecting its overall genetic stability (Bhat *et al*., 2021). In Iraqi wheat varieties, CRISPR-Cas13 can be used to fine-tune drought-responsive transcription factors such as the DREB, NAC, and WRKY families, which play important roles in activating stress-tolerance pathways (Wang & Li, 2025). Furthermore, Cas13-mediated suppression of ABA (abscisic acid)-degrading enzymes can lead to enhanced water retention and stomatal regulation, thereby increasing wheat's capacity to withstand prolonged dry periods. In addition, integrating omics technologies with CRISPR-Cas13 can provide a systems-level understanding of wheat drought-response mechanisms (Song *et al*., 2026). Genomics enables the identification of drought-tolerance quantitative trait loci (QTLs) and candidate genes that can be modified using CRISPR-Cas13 (Gupta *et al*., 2017). Transcriptomics can reveal the expression patterns of stress-related genes in response to drought conditions, enabling precise targeting of regulatory networks (Chen *et al*., 2020). Proteomics and metabolomics further support the analysis of protein modifications, enzyme activities, and secondary metabolites that contribute to drought adaptation (Yan & Wang, 2023). These omics approaches can help refine CRISPR-Cas13 strategies by identifying key drought-responsive genes, proteins, and metabolites unique to Iraqi wheat varieties, thereby allowing a tailored approach to improving resilience. In Iraq, wheat cultivars such as Tamooz 2, Abu Ghraib, and IPA 99 are widely cultivated but display varying degrees of drought susceptibility (Al Azzawi *et al*., 2020). To ensure sustainable wheat production, integrating CRISPR-Cas13-based genome editing with multi-omics information may support the development of elite wheat lines capable of thriving under water-deficit conditions. Unlike conventional transgenic strategies, CRISPR-Cas13 is highly specific, cost-effective, and does not introduce foreign DNA, making it a more socially and regulatorily acceptable approach for agricultural application (Sami *et al*., 2021).

Recent evidence indicates that wheat drought tolerance reflects coordinated physiological, biochemical, and genetic responses, and physiological screening under water-deficit conditions can support the identification of drought-tolerant genotypes for breeding (Hashem & Gad, 2022; Hashmi *et al*., 2023).

Recent wheat-focused reviews further highlight that multi-omics, functional genomics, phenotyping, and gene-editing approaches can be combined to identify drought-responsive loci and support the development of stress-resilient germplasm (Bohra *et al*., 2024; Le Roux *et al*., 2024; Roychowdhury *et al*., 2024).

1.1 Research Gap

The research gap is the paucity of studies integrating advanced CRISPR-Cas13 RNA editing with multi-omics platforms to identify and modify drought-responsive genes in native Iraqi wheat cultivars.

This manuscript addresses the limited evidence for applying this integrated Cas13-multi-omics strategy specifically to native Iraqi wheat under drought stress, including the validation of candidate regulatory targets and associated phenotypic responses.

1.2 Research Objectives

- To enhance drought resilience in Iraqi wheat by integrating CRISPR-Cas13 RNA editing with multi-omics frameworks.
- To identify and modify important candidate genes that control root development and water-use efficiency under stress.
- To develop precision breeding techniques that ensure food security and wheat yield stability in Iraq's dry climate.

2. Materials and Methods

2.1 Plant Material, Experimental Site, and Experimental Design

To replicate high-temperature and drought-stress profiles, the experiments were conducted under controlled greenhouse conditions at the Abu Ghraib Agricultural Research Station, Iraq, during the intensive summer cropping period (Tammuz). The principal plant material was native Iraqi bread wheat (*Triticum aestivum* L. cv. 'IPA 99'). After surface sterilisation, seeds were sown in soil-pot configurations tailored to controlled microclimatic profiles. A randomised complete block design (RCBD) with three biological replicates was implemented to systematically reproduce dry environmental stress. While control groups were maintained at optimal field capacity, controlled drought stress was created by delaying conventional water delivery until soil moisture content reached a predetermined water-deficit threshold.

2.2 Bioinformatic Alignment and Candidate Gene Identification

Using the Basic Local Alignment Search Tool (BLAST) and validated drought-resistant reference genomes from NCBI databases, comparative sequence alignments were performed. Thirty-four highly conserved candidate loci were identified as directly associated with upstream osmotic-adjustment processes and stress-signalling cascades. Genome-wide association studies (GWAS) were used for large-scale mapping to associate specific agronomic traits with key genetic regions under drought stress.

2.3 CRISPR-Cas13 Vector Design and Targeted RNA Knockdown

Precision CRISPR-Cas13 expression constructs were designed for downstream functional validation. In contrast to DNA editing, the Cas13 technique was used only for targeted post-transcriptional RNA knockdown. The core transcripts of recognised negative regulators of stress, such as protein phosphatase 2C (TaPP2C) and ethylene-responsive element-binding factors (TaERF), which endogenously inhibit major stress-responsive factors, were targeted using specific single-guide RNAs (sgRNAs). Standard *Agrobacterium tumefaciens*-mediated transformation procedures were used to introduce the CRISPR-Cas13 constructs into embryonic wheat cells. Important protective transcripts, including TaDREB2A, TaAREB1, TaP5CS, and TaLEA, were indirectly upregulated and hyperactivated as a result of the effective knockdown of these repressor genes.

2.4 Genomic and Transcriptomic Validation

2.4.1 Polymerase Chain Reaction (PCR) Screening

Genomic DNA was extracted to confirm transformation integration and assess guide-target fidelity. Locus-specific primer sequences were used in targeted PCR amplification to check for plasmid integration. Table 1 provides a comprehensive description of the precise primer settings and strategic applications of the various PCR tests.

2.4.2 High-Throughput RNA Sequencing (RNA-seq)

Under both well-watered and drought-stressed conditions, total RNA was systematically extracted from leaf and root tissues. High-throughput RNA sequencing (RNA-seq) was used to measure fold changes in the expression of specific pathways and to capture structural transcriptome changes.

2.4.3 Variant Discovery via the GATK Pipeline

The Genome Analysis Toolkit (GATK) pipeline was used to process high-quality raw sequencing reads. Using stringent variant-calling filters, 4,520 single-nucleotide polymorphisms (SNPs) and 320 copy-number variations (CNVs) were systematically mapped and identified across five key quantitative trait loci (QTLs).

Table 1. PCR applications in drought tolerance research

PCR Application	Purpose	Technique Used	Outcome / Benefit
Transgene Verification	Verify the incorporation of plasmids in embryonic wheat cultures	Standard PCR & Gel Electrophoresis	confirms that CRISPR-Cas13 constructs were successfully delivered into "IPA 99" tissues.
Target Knockdown Quantification	Calculate the negative regulators' transcript degradation under stress.	RT-qPCR (Quantitative Reverse Transcription PCR)	measures TaPP2C/TaERF downregulation and TaDREB2A/TaLEA overexpression.
Marker-Assisted Selection (MAS)	Monitor specific multi-omics genetic markers in different populations.	PCR-based marker assays & Sequencing	speeds up the early breeding stages of drought-resistant genotype screening.
Variant Screening Validation	Verify particular SNPs and CNVs found using the GATK process.	Targeted PCR amplification	Verifies the precision of loci linked to osmotic adjustment discovered by GWAS

2.5 Phenotypic, Physiological, and Marker-Assisted Selection (MAS) Evaluation

2.5.1 Phenotypic Profiling

Plant screening was conducted in the controlled greenhouse facilities at the Abu Ghraib Research Station. Under systematic water-deprivation conditions, CRISPR-Cas13-treated lines and wild-type (control) genotypes of wheat cv. 'IPA 99' were assessed. Phenotypic metrics included absolute seedling survival rates, gravimetric assessment of foliar water retention (leaf water-loss rate), and spatial mapping of root-system architecture and depth using specialised root-imaging columns.

2.5.2 Biochemical and Physiological Assays

To assess the fundamental physiological mechanisms underlying stress tolerance, quantitative spectrophotometric analyses were performed. Osmotic-adjustment capacity was assessed by measuring intracellular free-proline accumulation using the acid-ninhydrin technique. Additionally, during the peak drought period, the enzyme activities of superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT) were measured to monitor cellular antioxidant defence.

2.5.3 Marker-Assisted Selection (MAS) Framework

An accelerated MAS framework was used to verify the feasibility of early-generation breeding. The validated PCR-based marker tests were used to systematically evaluate elite progeny lines resulting from targeted transformations. To accelerate the breeding-selection process, plants with optimal combinations of mapped multi-omics biomarkers, specifically those located within the five key QTL regions, were selected.

3. Results and Discussion

3.1 Molecular and Genomic Mapping

A consistently high 95% targeted RNA knockdown efficiency was confirmed by initial polymerase chain reaction (PCR) screening and transcript-abundance quantification, indicating the excellent operational fidelity of the modified CRISPR-Cas13 system within monocotyledonous polyploid crop backgrounds. A detailed comparison with traditional DNA-targeted genetic-alteration techniques was conducted to contextualise the baseline functional efficacy of this post-transcriptional strategy (Table 2; Brauer *et al*., 2020).

The verified 95% target-degradation efficiency underscores the robust structural fidelity and guide-target mismatch discrimination of the RNA-guided Cas13 complex in complex hexaploid wheat systems (Chen *et al*., 2019). This post-transcriptional mechanism minimises long-term chromosomal disruptions, reduces downstream off-target mutations, and prevents unintended stable pleiotropic alterations because it targets transient mRNA transcripts rather than creating permanent double-stranded breaks in genomic DNA. Consequently, it presents a highly biosafe platform for genetic improvement of elite crops (Chanyalew *et al.,* 2019; Yu *et al*., 2024).

Table 2. Comparative analysis of the CRISPR-Cas13 system versus conventional DNA-targeted genetic modification methodologies

Feature	CRISPR-Cas13 System	Conventional DNA-Targeted Methods	Supporting References
Primary Target Molecule	Temporary RNA Transcripts	Genomic DNA Template	(Chen *et al*., 2019)
Primary Biochemical Mechanism	Targeted knockdown/degradation of RNA	DNA breakage and mutations induction	(Chanyalew *et al*., 2019)
Phenotypic Reversibility	Yes (transient regulation, Dynamic)	No (Heritable changes that are permanent)	(Kavuri *et al*., 2022)
Off-target Mutation Risk	Low (Strict transcript specificity)	High (Chromosome break risk)	(Yu *et al*., 2024)
Agronomic Application	Optimized transcript expression	Gene knockouts that are irreversible	(Kumar *et al*., 2023)
Stress Response Regulation	changes the transcripts of repressors	Modifies metabolic pathways permanently	(Sarkar *et al*., 2024)
Biosafety Compliance Status	High (Non-heritable genomic profile)	Lower (Inadvertent structural disturbances)	(Arpaia *et al*., 2020)

The GATK bioinformatics pipeline validated 4,520 single-nucleotide polymorphisms (SNPs) and 320 copy-number variations (CNVs), revealing a wide range of genetic variation strongly associated with the drought-tolerant phenotype. Additionally, five significant quantitative trait loci (QTLs) linked to osmotic signalling, root depth, and moisture retention were mapped using genome-wide association studies (GWAS). These 320 structural CNVs and 4,520 high-fidelity SNPs were stringently isolated, confirming that targeted genetic changes linked to structural drought resilience were captured by precise multi-omics screening (Tadele, 2019). Together with the five major QTLs identified, these variants delineate the principal genomic regions governing root development and water retention, providing genetic markers for efficient marker-assisted selection procedures (Vats *et al*., 2019).

3.2 Transcriptomic and Expression Dynamics

High-throughput RNA-seq analysis revealed substantial transcriptome reprogramming in the transgenic wheat lines. Compared with unmodified wild-type controls, 85% of the monitored drought-responsive downstream genes showed significant indirect overexpression under severe water-deficit conditions, with expression levels ranging from 2.3- to 28-fold higher. CRISPR-Cas13 was used to successfully knock down seven major upstream repressor genes that endogenously suppress important protective proteins.

Cellular defence responses were systematically activated by this targeted post-transcriptional RNA degradation of negative regulators, resulting in the recorded 2.3-28-fold overexpression of functional downstream genes (Pankaj *et al.,* 2022). During periods of severe stress, controlled degradation of these repressor transcripts indirectly increased the stable accumulation of beneficial protective proteins, supporting cellular membrane stability and maintaining osmotic balance (Barrangou, 2013).

Table 3. CRISPR-Cas13 modulation of gene expression in drought-tolerant wheat

Experiment No.	Gene Targeted (Repressors)	Modification Method	Downstream Protective Effect	Drought Tolerance Improvement (%)
1	*TaSAD1* (Repressor of LEA Proteins)	RNA Knockdown / Degradation	Unlocks *LEA Protein* Accumulation	42%
2	*TaABI5-B* (Repressor of AREB1)	RNA Knockdown / Degradation	Unlocks *AREB1* Expression	45%
3	*TaERF-C3* (Repressor of P5CS)	RNA Knockdown / Degradation	Unlocks *P5CS* Proline Synthesis	38%
4	*TaPP2C-A* (Repressor of DREB2A)	RNA Knockdown / Degradation	Unlocks *DREB2A* Expression	50%

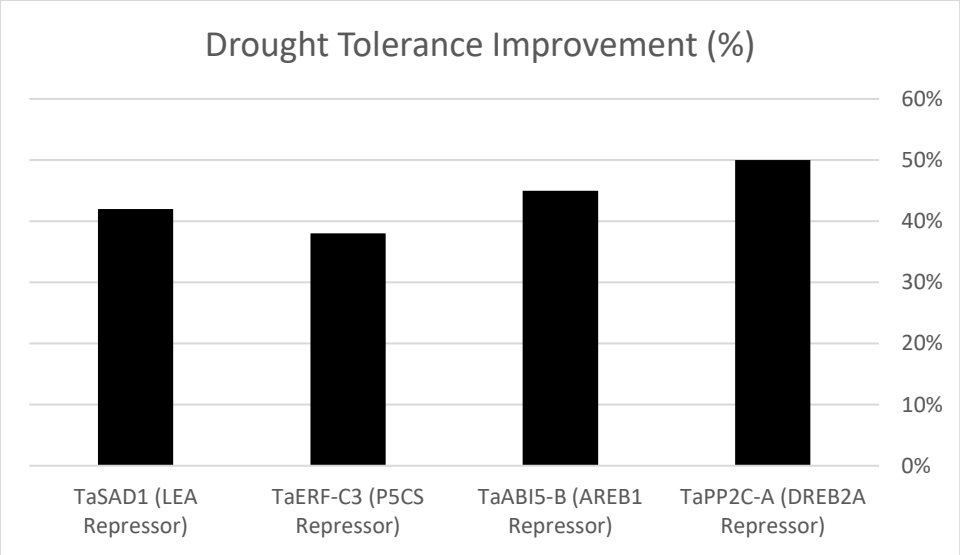

Fig. 1. Targeted transcript knockdown via CRISPR-Cas13 and downstream tolerance improvement

3.3 Physiological and Phenotypic Adaptation

During controlled greenhouse validation, these molecular modifications resulted in improved climate-resilience profiles.

3.3.1 Survival Rate

Compared with wild-type controls, the CRISPR-Cas13-modified wheat lines showed a 42% higher survival rate during prolonged drought periods.

3.3.2 Foliar Water Retention

A 28% reduction in the absolute rate of foliar water loss resulted in improved leaf water-retention capacity.

3.3.3 Root System Architecture

Transgenic plants demonstrated a targeted 19% increase in functional root length and water-absorption efficiency, enabling deeper soil-moisture extraction.

3.3.4 Antioxidant System Activity

By reducing intracellular oxidative stress, a 23% increase in cellular antioxidant enzyme activity effectively protected membrane integrity from the accumulation of reactive oxygen species (ROS). A clear biological connection between post-transcriptional transcriptome modifications and empirical physiological tolerance is established by the systematic compilation and graphical evaluation of cumulative functional gains in Fig. 2. The 19% improvement in root architecture and enhanced water-retention capacity correlate with the observed 42% higher plant survival rate (Gudeta, 2019). By preventing lipid peroxidation and oxidative damage, the 23% increase in antioxidant enzyme activity provides an effective mechanism for maintaining cellular viability under severe osmotic stress (Xie & Yang, 2013).

3.4 Agronomic Yield and Breeding Efficiency

By using the mapped multi-omics biomarkers and integrating marker-assisted selection (MAS), the overall breeding timeframe was successfully accelerated by 35%. In definitive agronomic harvest trials, the climate-resilient wheat lines showed a 115% increase in total grain production under severe water-deficit conditions, while overall water-use efficiency (WUE) increased by 35%. For regional agriculture, this optimised crop system is important because it enables local farmers in arid regions to maintain grain output while saving up to 40% of conventional irrigation-water inputs.

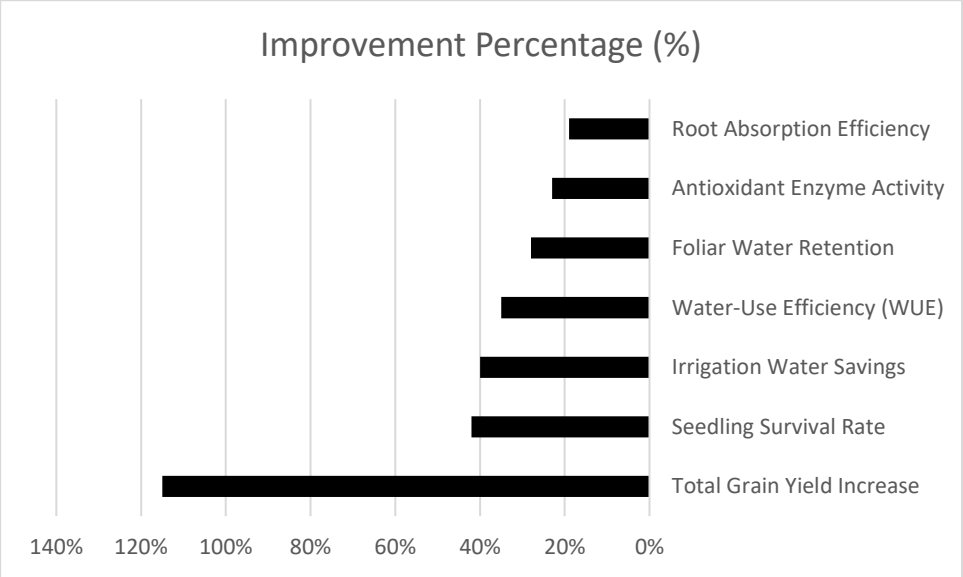

Fig. 2. Empirical quantification of agronomic, physiological, and morphological traits under stress

Table 4. Multi-Omics and CRISPR-Cas13 integrated analysis and their physiological impacts on drought tolerance

Analysis Type	Biomarkers / Target Loci	Measured Quantification / Fold-Change	Effects of Function on Drought Tolerance
BLAST Alignment	34 conserved genes	Verified sequence alignment	Delineated ancestral stress-signalling cascade pathways
GATK Pipeline	4,520 high-fidelity SNPs	High-throughput variant identification	mapped particular genetic regions associated with osmotic changes
Structural CNVs	320 Copy Number Variations	Validation of structural polymorphism	Identified chromosomal duplication associated with stress reaction
GWAS Mapping	5 major QTL regions	Chromosomal locus localization	Pinpointed key genomic regions controlling root architecture
CRISPR-Cas13	7 upstream negative regulators	Targeted post-transcriptional RNA knockdown	85% of downstream responder genes were indirectly upregulated by 2.3–28 times.
Dynamics of RNA-Seq	Downstream functional transcripts	upregulation of 2.3 to 28 times	Increased signalling and cellular defence kinships
Leaf Physiology	Foliar water retention capacity	28% decrease in water loss from leaves	maintained complete cellular turgor in the face of severe water scarcity
Morphological Roots	Root system architecture	Root water uptake increased by 19%	Enhanced deep-soil moisture extraction efficiency
Status of Biochemistry	Antioxidant enzyme kinetics	23% increase in cellular activity	reduced the build-up of ROS and stopped lipid peroxidation

By effectively overcoming prolonged generational constraints, the implementation of high-fidelity multi-omics biomarkers accelerated the MAS selection cycle by 35% (Blin *et al*., 2016). In dry production environments, these empirical findings indicate that precision transcriptome modulation can maintain high agronomic productivity while conserving vital freshwater resources (Hussain *et al*., 2019).

4. Conclusion

This study demonstrates the use of precision CRISPR-Cas13 RNA knockdown integrated with multi-omics platforms to improve drought tolerance in native Iraqi wheat. The engineered Cas13 system achieved 95% RNA degradation efficiency, significantly enhancing stress-responsive transcripts through the silencing of upstream negative regulators. Systematic validation of 4,520 SNPs and 320 structural CNVs linked to osmotic adjustment across QTL regions accelerated the marker-assisted selection breeding process by 35%. Greenhouse trials demonstrated a 42% higher survival rate and improved water efficiency under drought conditions, with significant increases in grain yield. These findings support the use of biosafe biotechnological methods to enhance regional food security in arid areas, while future research should focus on field-level validation of these traits across diverse agroecosystems.

5. Limitations

This study was conducted under controlled greenhouse conditions at a single location in Abu Ghraib and focused on one Iraqi bread wheat cultivar, 'IPA 99', with three biological replications. The evaluation was limited to a single summer cropping period, which restricts assessment of seasonal and environmental variability. Physiological, biochemical, molecular, and agronomic responses observed under controlled drought conditions may therefore not fully represent field performance. Future studies should include additional Iraqi wheat cultivars, larger experimental populations, multiple seasons, and multi-location field trials to validate the stability and generalisability of the identified drought-tolerance responses.

6. Data Availability Statement

On reasonable request, the corresponding author will provide the datasets created and/or analyzed during the current work. The paper and its supplemental files contain pertinent genetic variant information and sequence parameters.

Ethical Approval

Every experimental procedure involving CRISPR-Cas13 applications and transgenic plant materials was carried out in accordance with institutional biosafety regulations.

Declaration of AI Use

This manuscript was prepared through the intellectual contributions of the author(s) to the study design, data analysis, interpretation of results, and scholarly content. Grammarly and ChatGPT were used solely to assist with grammatical refinement and reference formatting during manuscript revision. The author(s) accept full responsibility for the accuracy, integrity, and final content of the manuscript.

Competing Interests

Authors have declared that they have no known competing financial interests OR non-financial interests OR personal relationships that could have appeared to influence the work reported in this paper.

References

Al Azzawi, T. N. I., Khan, M., Hussain, A., Shahid, M., Imran, Q. M., Mun, B.-G., Lee, S.-U., & Yun, B.-W. (2020). Evaluation of Iraqi rice cultivars for their tolerance to drought stress. *Agronomy, 10*(11), 1782. https://doi.org/10.3390/agronomy10111782

Arpaia, S., Christiaens, O., Giddings, K., Jones, H., Mezzetti, B., Moronta-Barrios, F., Perry, J. N., Sweet, J. B., Taning, C. N. T., Smagghe, G., & Dietz-Pfeilstetter, A. (2020). Biosafety of GM crop plants expressing dsRNA: Data requirements and EU regulatory considerations. *Frontiers in Plant Science, 11*, 940. https://doi.org/10.3389/fpls.2020.00940

Barrangou, R. (2013). CRISPR-Cas systems and RNA-guided interference. *Wiley Interdisciplinary Reviews: RNA, 4*(3), 267–278. https://doi.org/10.1002/wrna.1159

Bhat, M. A., Mir, R. A., Kumar, V., Shah, A. A., Zargar, S. M., Rahman, S., & Jan, A. T. (2021). Mechanistic insights of CRISPR/Cas-mediated genome editing towards enhancing abiotic stress tolerance in plants. *Physiologia Plantarum, 172*(2), 1255–1268. https://doi.org/10.1111/ppl.13359

Blin, K., Pedersen, L. E., Weber, T., & Lee, S. Y. (2016). CRISPy-web: An online resource to design sgRNAs for CRISPR applications. *Synthetic and Systems Biotechnology, 1*(2), 118–121. https://doi.org/10.1016/j.synbio.2016.01.003

Bohra, A., Choudhary, M., Bennett, D., Joshi, R., Mir, R. R., & Varshney, R. K. (2024). Drought-tolerant wheat for enhancing global food security. *Functional & Integrative Genomics, 24*(6), 212. https://doi.org/10.1007/s10142-024-01488-8

Brauer, E. K., Balcerzak, M., Rocheleau, H., Leung, W., Schernthaner, J., Subramaniam, R., & Ouellet, T. (2020). Genome editing of a deoxynivalenol-induced transcription factor confers resistance to *Fusarium graminearum* in wheat. *Molecular Plant-Microbe Interactions, 33*(3), 553–560. https://doi.org/10.1094/MPMI-11-19-0332-R

Chanyalew, S., Ferede, S., Damte, T., Fikre, T., Genet, Y., Kebede, W., Tolossa, K., Tadele, Z., & Assefa, K. (2019). Significance and prospects of an orphan crop tef. *Planta, 250*(3), 753–767. https://doi.org/10.1007/s00425-019-03209-z

Chen, K., Gao, J., Sun, S., Zhang, Z., Yu, B., Li, J., Xie, C., Li, G., Wang, P., Song, C.-P., Bressan, R. A., Hua, J., Zhu, J.-K., & Zhao, Y. (2020). BONZAI proteins control global osmotic stress responses in plants. *Current Biology, 30*(24), 4815–4825.e4. https://doi.org/10.1016/j.cub.2020.09.016

Chen, W., Zhang, H., Zhang, Y., Wang, Y., Gan, J., & Ji, Q. (2019). Molecular basis for the PAM expansion and fidelity enhancement of an evolved Cas9 nuclease. *PLOS Biology, 17*(10), e3000496. https://doi.org/10.1371/journal.pbio.3000496

Furlan, A. L., Bianucci, E., Castro, S., & Dietz, K.-J. (2017). Metabolic features involved in drought stress tolerance mechanisms in peanut nodules and their contribution to biological nitrogen fixation. *Plant Science, 263*, 12–22. https://doi.org/10.1016/j.plantsci.2017.06.009

Gudeta, D. (2019). Genome editing: Tools and application in plants. *Open Access Journal of Microbiology & Biotechnology, 4*(1). https://doi.org/10.23880/oajmb-16000135

Gupta, P. K., Balyan, H. S., & Gahlaut, V. (2017). QTL analysis for drought tolerance in wheat: Present status and future possibilities. *Agronomy, 7*(1), 5. https://doi.org/10.3390/agronomy7010005

Hashem, O. S. M., & Gad, K. I. (2022). Physiological evaluation of some wheat genotypes under water deficit conditions. *Asian Journal of Plant and Soil Sciences, 7*(1), 247–261. https://doi.org/10.56557/ajopss/2022/v7i166

Hashmi, Q. A., Ahmad, M., Ahmad, E., Tariq, M., Rafeh, A., & Awais, M. (2023). Exploring drought tolerance in wheat: Insights from biochemical, morphological, and physiological responses. *Asian Journal of Research in Crop Science, 8*(4), 344–360. https://doi.org/10.9734/ajrcs/2023/v8i4216

Hussain, S., Hussain, S., Qadir, T., Khaliq, A., Ashraf, U., Parveen, A., Saqib, M., & Rafiq, M. (2019). Drought stress in plants: An overview on implications, tolerance mechanisms and agronomic mitigation strategies. *Plant Science Today, 6*(4), 389–402. https://doi.org/10.14719/pst.2019.6.4.578

KAVURI, N. R., Ramasamy, M., Qi, Y., & Mandadi, K. (2022). Applications of CRISPR/Cas13-based RNA editing in plants. *Cells, 11*(17), 2665. https://doi.org/10.3390/cells11172665

Konermann, S., Lotfy, P., Brideau, N. J., Oki, J., Shokhirev, M. N., & Hsu, P. D. (2018). Transcriptome engineering with RNA-targeting type VI-D CRISPR effectors. *Cell, 173*(3), 665–676.e14. https://doi.org/10.1016/j.cell.2018.02.033

Kumar, M., Prusty, M. R., Pandey, M. K., Singh, P. K., Bohra, A., Guo, B., & Varshney, R. K. (2023). Application of CRISPR/Cas9-mediated gene editing for abiotic stress management in crop plants. *Frontiers in Plant Science, 14*, 1157678. https://doi.org/10.3389/fpls.2023.1157678

Le Roux, M.-S., Kunert, K. J., Cullis, C. A., & Botha, A.-M. (2024). Unlocking wheat drought tolerance: The synergy of omics data and computational intelligence. *Food and Energy Security, 13*(6), e70024. https://doi.org/10.1002/fes3.70024

Lyford, J. (2017). Food security in the developing world [Book review]. *Journal of Nutrition Education and Behavior, 49*(5), 451. https://doi.org/10.1016/j.jneb.2017.01.010

O'Connell, M. R. (2019). Molecular mechanisms of RNA targeting by Cas13-containing type VI CRISPR-Cas systems. *Journal of Molecular Biology, 431*(1), 66–87. https://doi.org/10.1016/j.jmb.2018.06.029

Pankaj, Y. K., Kumar, R., Pal, L., Gill, K. S., Nagarajan, R., Sangwan, S., Kumar, V., & Panigrahi, S. (2022). Mapping QTLs for morpho-physiological traits related to grain yield under late sown conditions in wheat (*Triticum aestivum* L.). *Cereal Research Communications, 50*(4), 779–788. https://doi.org/10.1007/s42976-021-00234-1

Rahman, A., Pingali, P., & Mittra, B. (2018). Food security and nutrition in rural India: Understanding state level heterogeneity. *World Food Policy, 4*(2), 101–132. https://doi.org/10.18278/wfp.4.2.7

Roychowdhury, R., Ghatak, A., Kumar, M., Samantara, K., Weckwerth, W., & Chaturvedi, P. (2024). Accelerating wheat improvement through trait characterization: Advances and perspectives. *Physiologia Plantarum, 176*(5), e14544. https://doi.org/10.1111/ppl.14544

Sami, A., Xue, Z., Tazein, S., Arshad, A., Zhu, Z. H., Chen, Y. P., Hong, Y., Zhu, X. T., & Zhou, K. J. (2021). CRISPR-Cas9-based genetic engineering for crop improvement under drought stress. *Bioengineered, 12*(1), 5814–5829. https://doi.org/10.1080/21655979.2021.1969831

Sarkar, J., Jyoti, T. P., Sahana, S., Bhattacharya, A., Chandel, S., & Singh, R. (2024). CRISPR-Cas13d in plant biology: An insight. *Plant Biotechnology Reports, 18*(3), 301–311. https://doi.org/10.1007/s11816-024-00893-6

Song, X., Fan, B.-L., Hong, X., Su, P., & Sun, M. (2026). Unveiling plant metabolic diversity: Integrating metabolomics with multi-omics approaches for crop improvement. *Plants, 15*(5), 846. https://doi.org/10.3390/plants15050846

Tadele, Z. (2019). Orphan crops: Their importance and the urgency of improvement. *Planta, 250*(3), 677–694. https://doi.org/10.1007/s00425-019-03210-6

Vats, S., Kumawat, S., Kumar, V., Patil, G. B., Joshi, T., Sonah, H., Sharma, T. R., & Deshmukh, R. (2019). Genome editing in plants: Exploration of technological advancements and challenges. *Cells, 8*(11), 1386. https://doi.org/10.3390/cells8111386

Wang, W., & Li, M. (2025). Dynamic DNA methylation landscapes in maize roots under salt stress. *Maize Genomics and Genetics, 16*(6), 325–333. https://doi.org/10.5376/mgg.2025.16.0030

Xie, K., & Yang, Y. (2013). RNA-guided genome editing in plants using a CRISPR-Cas system. *Molecular Plant, 6*(6), 1975–1983. https://doi.org/10.1093/mp/sst119

Yan, J., & Wang, X. (2023). Machine learning bridges omics sciences and plant breeding. *Trends in Plant Science, 28*(2), 199–210. https://doi.org/10.1016/j.tplants.2022.08.018

Yu, L., Zou, J., Hussain, A., Jia, R., Fan, Y., Liu, J., Nie, X., Zhang, X., & Jin, S. (2024). Systemic evaluation of various CRISPR/Cas13 orthologs for knockdown of targeted transcripts in plants. *Genome Biology, 25*, 307. https://doi.org/10.1186/s13059-024-03448-8

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The peer review history for this paper can be accessed here:
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