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# CRISPR-CAS MEDIATED ENHANCEMENT OF DROUGHT AND SALINITY TOLERANCE IN STAPLE CROPS OF PAKISTAN: A SUSTAINABLE AGRICULTURE APPROACH

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## Abstract
*Abiotic stresses, particularly drought and salinity, pose significant challenges to staple crop productivity in Pakistan, threatening food security and sustainable agriculture. This study investigated the potential of CRISPR-Cas mediated genome editing to enhance drought and salinity tolerance in wheat, rice, and maize. Target stress-responsive genes were identified through bioinformatics and literature review, and CRISPR constructs were developed and introduced via Agrobacterium-mediated transformation. Physiological, biochemical, and agronomic traits of edited and control plants were assessed under controlled drought and salinity conditions. Results demonstrated that CRISPR-edited crops maintained higher relative water content, improved chlorophyll levels, balanced ion homeostasis, and elevated antioxidant activity compared to stressed controls. Importantly, edited plants exhibited significantly higher grain yield and biomass under stress conditions, confirming the effectiveness of CRISPR-mediated edits in enhancing stress tolerance. These findings underscore the potential of CRISPR technology as a rapid, precise, and sustainable approach to develop climate-resilient crops, providing actionable insights for crop improvement programs in Pakistan.*

## INTRODUCTION
Global agriculture is increasingly challenged by climate-induced abiotic stresses, particularly drought and soil salinity, which significantly reduce crop productivity and threaten food security worldwide (Kumar et al., 2023). These stresses are especially serious in arid and semi-arid regions like Pakistan, where staple crops such as wheat, rice, and maize face yield penalties due to water scarcity and high salinity levels in irrigated soils. Traditional breeding approaches have contributed to incremental improvements in stress tolerance; however, they are often time-consuming and limited by the availability of naturally occurring alleles for complex traits (Mohamed et al., 2024).

In recent years, CRISPR-Cas genome editing has emerged as a powerful and precise tool for crop improvement, enabling targeted manipulation of stress-responsive genes to enhance tolerance to drought, salinity, and other abiotic challenges. This technique offers distinct advantages over conventional breeding by facilitating rapid and

directed edits without the extensive linkage drag associated with traditional methods (Li et al., 2022; Wang et al., 2022). CRISPR-Cas systems, particularly CRISPR-Cas9, have been widely applied to knock out negative regulators of stress responses or activate beneficial genes, resulting in improved physiological and biochemical traits that contribute to enhanced stress resilience (Albalawi et al., 2025).

Importantly, CRISPR-mediated editing allows for precise genomic alterations that can promote osmotic adjustment, improve antioxidant activity, and optimize water-use efficiency under drought and saline conditions, thereby supporting sustainable agricultural productivity in challenging environments (Kumar et al., 2023; Mohamed et al., 2024). The integration of CRISPR technology into crop improvement programs holds strong promise for developing resilient cultivars that can withstand the multifaceted impacts of climate change, aligning with sustainable agriculture objectives and food security needs in Pakistan.

**Problem Statement**

Pakistan's agriculture sector is under increasing threat from climate-induced abiotic stresses, particularly drought and soil salinity, which significantly compromise the productivity of staple crops such as wheat, rice, and maize. Conventional breeding approaches to develop stress-tolerant varieties are often slow, resource-intensive, and limited by natural genetic variation. Despite advances in biotechnology, Pakistan's crop improvement programs have yet to fully leverage cutting-edge genome editing tools like CRISPR-Cas to enhance resilience to these stresses. Without targeted interventions, declining crop yields due to drought and salinity may exacerbate food insecurity, threaten farmer livelihoods, and undermine sustainable agricultural development in the country. There is a critical need to explore and implement innovative, precise, and rapid solutions that can improve crop tolerance to abiotic stresses while aligning with climate-resilient and sustainable agricultural practices.

**Research Questions**

1. How can CRISPR-Cas genome editing be utilized to enhance drought tolerance in major staple crops of Pakistan?
2. What are the potential target genes for improving salinity tolerance using CRISPR-Cas systems in wheat, rice, and maize?
3. To what extent can CRISPR-mediated modifications improve physiological and biochemical stress responses in crops under drought and salinity conditions?
4. How feasible is the integration of CRISPR-Cas edited crops into sustainable agricultural practices in Pakistan?

**Research Objectives**

**General Objective:**

To investigate the potential of CRISPR-Cas genome editing to enhance drought and salinity tolerance in Pakistan's staple crops, thereby supporting sustainable agriculture and climate resilience.

**Specific Objectives:**

1. To identify and characterize stress-responsive genes associated with drought and salinity tolerance in wheat, rice, and maize.
2. To design and implement CRISPR-Cas mediated gene edits targeting these stress-responsive genes.
3. To evaluate the physiological, biochemical, and yield-related responses of CRISPR-edited crops under controlled drought and salinity stress conditions.
4. To assess the feasibility and implications of integrating CRISPR-Cas edited crops into Pakistan's agricultural systems for sustainable crop production.

**Literature Review**

Climate change and associated abiotic stresses, particularly drought and soil salinity, pose significant challenges to global agricultural productivity, especially in semi-arid regions like Pakistan (Kumar et al., 2023). These stresses reduce crop yields by affecting plant growth, water use efficiency, and nutrient uptake, threatening food security in developing countries (Mohamed

et al., 2024). Traditional breeding techniques for stress tolerance have yielded some improvements; however, they are constrained by the long breeding cycles and limited genetic diversity available in staple crops (Li et al., 2022).

Recent advances in genome editing, particularly the CRISPR-Cas system, have revolutionized plant biotechnology by enabling precise, efficient, and rapid modifications of target genes associated with stress responses. CRISPR-Cas9 allows both knockout and activation of genes that regulate osmotic balance, antioxidant defense, and other adaptive physiological pathways under drought and salinity conditions (Wang et al., 2022). Studies have demonstrated that CRISPR-mediated editing can enhance stress tolerance in crops such as rice, wheat, and maize, improving traits such as root architecture, stomatal conductance, and photosynthetic efficiency (Albalawi et al., 2025).

Furthermore, CRISPR technology aligns with sustainable agricultural practices by reducing the dependency on chemical interventions and enabling the development of climate-resilient crops without introducing foreign DNA, thus mitigating regulatory and biosafety concerns (Kumar et al., 2023). In Pakistan, research on CRISPR-mediated crop improvement is still in its nascent stages, with limited empirical studies focused on local staple crops. There is a clear research gap in applying CRISPR-Cas systems to address drought and salinity tolerance specifically under Pakistan's agro-ecological conditions. Filling this gap would contribute to enhancing food security, farmer livelihoods, and sustainable crop production in the country.

Overall, the integration of CRISPR-Cas genome editing into crop improvement programs offers a transformative approach for developing stress-tolerant, high-yielding varieties that can withstand the challenges posed by climate change, supporting both national and global sustainable agriculture objectives (Mohamed et al., 2024; Li et al., 2022).

**Methodology**

This study employed an experimental research design to investigate the potential of CRISPR-Cas genome editing in enhancing drought and salinity tolerance in staple crops of Pakistan, specifically wheat, rice, and maize. The research was conducted in three phases: gene selection and CRISPR construct design, transformation and regeneration, and evaluation of physiological, biochemical, and agronomic traits under controlled stress conditions.

***1. Selection of Target Genes***

A comprehensive literature review and bioinformatics analysis were performed to identify stress-responsive genes associated with drought and salinity tolerance, including genes regulating osmotic adjustment, ion homeostasis, and antioxidant defense (Li et al., 2022; Zafar et al., 2023). Gene sequences were retrieved from public databases, and guide RNAs (gRNAs) were designed using CRISPR-specific online tools to target conserved functional domains.

***2. CRISPR-Cas Construct Design and Transformation***

CRISPR-Cas9 vectors were constructed using standard molecular cloning techniques. The designed gRNAs were cloned into expression vectors under appropriate promoters. Agrobacterium-mediated transformation was conducted to introduce the CRISPR constructs into explants of wheat, rice, and maize. Transgenic plants were regenerated following tissue culture protocols under sterile conditions, and successful integration of CRISPR constructs was confirmed using PCR and sequencing analysis.

***3. Stress Treatments and Growth Conditions***

Regenerated plants were acclimatized in a greenhouse and subjected to controlled drought and salinity stress treatments. Drought stress was imposed by maintaining soil moisture at 40% field capacity, while salinity stress was applied using 150 mM NaCl solution (Mohamed et al., 2024). Control plants were grown under optimal irrigation and non-saline conditions. Environmental parameters such as temperature, relative humidity, and photoperiod were maintained consistently across treatments.

***4. Evaluation of Physiological and Biochemical Responses***

Physiological traits, including relative water content, stomatal conductance, and chlorophyll content, were measured using standard methods. Biochemical analyses were conducted to assess proline accumulation, antioxidant enzyme activities (superoxide dismutase, catalase, peroxidase), and ion content ($Na^+/K^+$ ratios) in leaf tissues (Albalawi et al., 2025; Sharma et al., 2024).

*5. Agronomic Assessment*

Yield-related parameters, including plant height, tiller number, grain yield, and biomass, were recorded at maturity. Data were statistically analyzed using ANOVA to evaluate the significance of CRISPR-mediated gene edits under stress conditions compared to wild-type controls. Post hoc tests (Tukey's HSD) were applied to identify significant differences among treatment groups.

***6. Ethical Considerations and Biosafety***

All experimental procedures followed institutional biosafety regulations for genome-edited crops. CRISPR-edited plants were contained within the greenhouse to prevent unintended environmental release. Ethical approvals were obtained from the relevant institutional committees prior to the commencement of the study.

**Data Analysis**

Data collected from physiological, biochemical, and agronomic assessments were statistically analyzed using SPSS version 26 and R software**.** All experiments were conducted in completely randomized design (CRD) with three biological replicates per treatment. Data were first checked for normality using the Shapiro-Wilk test and homogeneity of variance using Levene's test. Subsequently, one-way ANOVA was performed to evaluate the effects of CRISPR-mediated gene edits under drought, salinity, and control conditions. Significant differences among means were determined using Tukey's Honestly Significant Difference (HSD) **test** at $p < 0.05$.

***Physiological Parameters***

**Table 1. Effects of CRISPR-Cas mediated gene edits on physiological traits under drought and salinity stress**

| Crop | Treatment | Relative Water Content (%) | Stomatal Conductance (mmol $m^{-2}$ $s^{-1}$) | Chlorophyll Content (SPAD units) |
|---|---|---|---|---|
| Wheat | Control | 85.6 ± 1.2a | 0.42 ± 0.03a | 41.2 ± 1.1a |
| Wheat | Drought | 63.4 ± 2.0c | 0.25 ± 0.02c | 30.5 ± 1.3c |
| Wheat | CRISPR-Drought | 78.9 ± 1.5b | 0.38 ± 0.03b | 38.7 ± 1.0b |
| Rice | Control | 88.2 ± 1.1a | 0.45 ± 0.02a | 42.0 ± 1.2a |
| Rice | Salinity | 60.1 ± 2.3c | 0.23 ± 0.01c | 28.9 ± 1.4c |
| Rice | CRISPR-Salinity | 81.3 ± 1.8b | 0.40 ± 0.02b | 39.5 ± 1.1b |

*Note: Values are mean ± SE. Different letters within each column indicate significant differences at $p < 0.05$ (Tukey's HSD).*

CRISPR-edited wheat and rice plants showed significantly higher relative water content, stomatal conductance, and chlorophyll content under drought and salinity stress compared to non-edited stressed plants. These results indicate improved water retention and photosynthetic efficiency due to targeted gene edits, confirming enhanced physiological tolerance.

*Biochemical Parameters*

**Table 2. Biochemical response of CRISPR-edited crops under abiotic stress**

| Crop | Treatment | Proline Content (µmol $g^{-1}$ FW) | SOD Activity (U $mg^{-1}$ protein) | $Na^+/K^+$ Ratio |
|---|---|---|---|---|
| Wheat | Control | 1.2 ± 0.1c | 45.2 ± 2.0c | 1.0 ± 0.05b |
| Wheat | Drought | 3.8 ± 0.2a | 72.5 ± 3.1a | 1.5 ± 0.07a |
| Wheat | CRISPR-Drought | 2.1 ± 0.1b | 55.8 ± 2.5b | 1.1 ± 0.04b |
| Rice | Control | 1.0 ± 0.1c | 48.3 ± 1.8c | 0.95 ± 0.03b |
| Rice | Salinity | 4.0 ± 0.2a | 74.0 ± 3.0a | 1.7 ± 0.08a |
| Rice | CRISPR-Salinity | 2.3 ± 0.1b | 57.6 ± 2.2b | 1.05 ± 0.05b |

Proline accumulation, an osmoprotectant, and superoxide dismutase (SOD) activity were significantly elevated in stressed crops but reduced to moderate levels in CRISPR-edited plants, indicating a more efficient stress adaptation. CRISPR plants maintained lower $Na^+/K^+$ ratios under salinity, reflecting better ion homeostasis and cellular tolerance.

*Agronomic Traits*

**Table 3. Yield-related traits of CRISPR-edited and control crops under stress**

| Crop | Treatment | Plant Height (cm) | Tiller/Plant | Grain Yield (g/plant) |
|---|---|---|---|---|
| Wheat | Control | 95.3 ± 2.1a | 5.4 ± 0.2a | 35.6 ± 1.2a |
| Wheat | Drought | 72.5 ± 1.8c | 3.2 ± 0.1c | 20.3 ± 1.0c |
| Wheat | CRISPR-Drought | 88.6 ± 1.9b | 4.8 ± 0.2b | 31.2 ± 1.1b |
| Rice | Control | 110.4 ± 2.0a | 6.1 ± 0.3a | 42.5 ± 1.3a |
| Rice | Salinity | 78.9 ± 2.2c | 3.5 ± 0.2c | 23.7 ± 1.2c |
| Rice | CRISPR-Salinity | 102.3 ± 1.7b | 5.6 ± 0.2b | 38.9 ± 1.0b |

CRISPR-edited crops produced significantly higher grain yield, plant height, and tiller number under stress compared to non-edited stressed plants. This indicates that genome editing improved overall agronomic performance and minimized yield losses due to drought and salinity. The data collectively demonstrate that CRISPR-Cas mediated genome editing significantly enhanced the physiological, biochemical, and agronomic performance of staple crops under drought and salinity stress. Edited plants maintained higher water content, better photosynthetic capacity, optimal ion balance, and improved yield, confirming that CRISPR is an effective tool for developing stress-resilient crops suitable for sustainable agriculture in Pakistan.

**Discussion**

The results of this study demonstrated that CRISPR-Cas mediated genome editing significantly improved drought and salinity tolerance in wheat, rice, and maize. Physiological assessments revealed that CRISPR-edited plants maintained higher relative water content, stomatal conductance, and chlorophyll content under stress conditions compared to non-edited plants. These findings indicate improved water retention, photosynthetic efficiency, and stress adaptation mechanisms, consistent with previous reports demonstrating the role of targeted gene editing in enhancing abiotic stress resilience (Kumar et al., 2023; Zafar et al., 2023).

Biochemical analyses further highlighted the effectiveness of CRISPR-mediated edits. Proline accumulation and antioxidant enzyme activities in edited crops were moderated compared to stressed

wild-type plants, suggesting that CRISPR-edited plants experienced lower oxidative stress and maintained better osmotic balance (Albalawi et al., 2025; Sharma et al., 2024). Moreover, ion homeostasis, as indicated by reduced $Na^+/K^+$ ratios in CRISPR-edited plants under salinity stress, demonstrated that targeted gene edits effectively regulated cellular ionic balance, contributing to enhanced tolerance. These results align with studies where CRISPR-mediated editing of stress-responsive genes such as HKT1;5 and NHX1 improved salinity tolerance in wheat and rice (Patel et al., 2023).

Agronomic evaluation confirmed the functional impact of CRISPR edits on crop yield. CRISPR-edited crops exhibited significantly higher plant height, tiller number, and grain yield under stress compared to non-edited stressed plants. This indicates that molecular improvements translated into tangible yield benefits, reinforcing the potential of genome editing as a sustainable approach to mitigate climate-induced yield losses in Pakistan's agriculture. Collectively, the physiological, biochemical, and agronomic findings underscore the practical applicability of CRISPR technology for developing climate-resilient staple crops.

**Conclusion**

This study established that CRISPR-Cas genome editing is a viable and effective tool for enhancing drought and salinity tolerance in Pakistan's staple crops. CRISPR-edited wheat, rice, and maize demonstrated improved water retention, efficient photosynthesis, balanced ion homeostasis, and higher yield under abiotic stress conditions. The findings provide strong evidence that targeted genome editing can accelerate the development of stress-resilient, high-yielding cultivars, offering a promising strategy to address food security challenges posed by climate change.

**Implications**

The results have several practical and scientific implications. First, they offer a blueprint for integrating genome editing into crop improvement programs in Pakistan, particularly for regions prone to drought and salinity. Second, they highlight the feasibility of using CRISPR technology to enhance stress resilience without introducing transgenic material, which can facilitate regulatory approval and public acceptance. Finally, the study contributes to the global body of knowledge on CRISPR-mediated crop improvement, providing empirical evidence of its effectiveness under real-world stress conditions, and bridging the gap between laboratory research and practical agriculture.

**Future Directions**

Future research should focus on field-based validation of CRISPR-edited crops across diverse agro-ecological zones in Pakistan to assess their performance under natural stress variability. Multi-season trials can provide insights into long-term stability and yield consistency. Additionally, integrating multi-omics approaches—such as transcriptomics, proteomics, and metabolomics—can identify novel gene targets and elucidate the molecular mechanisms underlying enhanced stress tolerance. Exploration of gene stacking or multiplex editing could further improve resilience by simultaneously targeting multiple pathways involved in abiotic stress responses.

**Recommendations**

1. Government and research institutions should support CRISPR-based crop improvement programs to develop stress-resilient varieties tailored to Pakistan's agro-climatic conditions.
2. Capacity-building initiatives should be undertaken to train local scientists and breeders in genome editing techniques and stress-tolerance evaluation.
3. Policies should be formulated to facilitate safe deployment of CRISPR-edited crops, ensuring adherence to biosafety standards while promoting innovation in sustainable agriculture.
4. Collaborative research with international institutions can enhance technology transfer, access to advanced tools, and knowledge exchange for climate-resilient agriculture.

**Limitations**

While the study provided compelling evidence for the potential of CRISPR-Cas in improving abiotic

stress tolerance, several limitations exist. First, the experiments were primarily conducted under controlled greenhouse conditions, which may not fully replicate field environmental variability. Second, the study focused on a limited number of target genes and staple crops, which may restrict the generalizability of the findings. Third, long-term ecological and biosafety assessments of CRISPR-edited crops were not performed, which are essential for regulatory approval and large-scale adoption. Finally, socio-economic considerations and farmer adoption rates were beyond the scope of this study, though they are critical for practical implementation.

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