Annual Methodological Archive Research Review
http://amresearchreview.com/index.php/Journal/about
Volume 4, Issue 3 (2026)

Online ISSN 3007-3197 Print ISSN 3007-3189
http://amresearchreview.com/index.php/Journal/about

CRISPR-Cas9 Mediated Genome Editing in Local Wheat Varieties: Enhancing Drought Tolerance and Grain Yield in Pakistan

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ABSTRACT

The experiment makes use of CRISPR Cas9 technology to create drought resistant wheat varieties tailored to the environmental conditions in Pakistan. Specifically, targeting the TaDREB2 and TaNAC gene families, the study manages to get an editing efficiency of 87 percent in locally grown varieties of wheat (Inqalab-91 and Punjab-2011). The field experiment reveals a 24 percent gain in grain yield and 31 percent gain in water use efficiency under water deficit conditions. Three pathways have been identified as significantly upregulated in edited wheat varieties: ABA signaling pathway, osmotic regulation pathway and antioxidant defense pathway. Molecular studies reveal that the editing is heritable up to T2 generation. The study gives four strategic suggestions for implementation: marker-assisted breeding program, multi-locations trials, regulatory approvals and outreach program among farmers.

Key Words: CRISPR-Cas9, Wheat Genome Editing, Drought Tolerance, Gene Editing Efficiency, Pakistan Agriculture

Introduction

Wheat (Triticum aestivum L.), being one of the most significant cereal crops in terms of food security around the world, also serves as a major constituent of the agricultural economy and food production of Pakistan. Yet, wheat cultivation in Pakistan becomes increasingly constrained by the problem of water scarcity, irregular precipitation, temperature increase and other climate stress factors. Drought stress can have a negative impact on plant development, photosynthesis, reproductive processes, grain development, and yield in particular. Hence, the need arises for developing wheat lines that would be able to perform efficiently under drought stress and thus ensure sustainability in agriculture and food security (Riaz et al., 2025).

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Conventional breeding has played a considerable role in improving wheat cultivars; yet, combining the desirable traits and producing genetically stable results may take numerous generations. Biotechnological approaches have opened up new ways to accelerate the process of developing the cultivars through targeting genes responsible for certain characteristics of plants (Arif et al., 2025). CRISPR-Cas9 genome editing is one of those techniques for modifying the plant genome in order to improve its agronomic features. In contrast to conventional breeding that is usually based on recombination and selection of existing or induced variation, CRISPR-Cas9 technology allows targeting the certain genomic regions related to desired traits.
Drought resistance is a composite trait regulated by a variety of physiological, biochemical, and molecular pathways. Drought stress in plants is a result of alterations in the signaling of hormones, osmoregulation, antioxidants, root development, stomatal control, and gene expression. Transcription factors like TaDREB2 and TaNAC play a role in regulatory mechanisms for drought response and hence form the primary subjects in studying the genetics of drought resistance in wheat. Manipulating such genes through genome editing can be used as an approach to determine the possibility of improving drought-related traits in addition to grain yield in wheat (Riaz et al., 2025).
The use of CRISPR-Cas9 on locally developed wheat germplasm is especially significant for Pakistan as there are several locally adapted varieties that have agricultural significance and can thus serve as an ideal model for studying the potential of genome editing under local agro-climatic conditions. Improvement of drought-resistant wheat varieties using biotechnology may result in improved efficiency of water utilization and stability of grain yield. (Waheed et al., n.d.)

Problem Statement

The issues of water scarcity and drought have been recognized as one of the key limitations in the sustainability of the production of wheat in Pakistan. Wheat requires sufficient water for effective plant development and grain formation, whereas a lack of water affects plant growth and yield. The increased variability of climate and competition for available water require development of wheat crops with water limitation resistance.
The development of drought resistance through conventional breeding methods is problematic because drought resistance is a complex quantitative trait. Several components of plant adaptation and resistance to drought make the development of drought resistance through conventional breeding very challenging. Despite the presence of drought resistance in local wheat species, it is still necessary to increase the level of this resistance combined with yield capacity and efficient water utilization (Choudry et al., 2024).
Thus, the key task is the development of biotechnological approach that can target drought-resistant genetic mechanisms in local wheat species. The opportunity to use CRISPR-Cas9 for precise modification of drought resistance genes exists. It is necessary to determine the value of CRISPR-Cas9 for wheat production in Pakistan in terms of efficiency of gene modification, drought resistance, yield increase, physiological characteristics and stability of modifications through generations.

Online ISSN 3007-3197 Print ISSN 3007-3189

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Research Objectives

To use the CRISPR-Cas9 technique for gene editing of the TaDREB2 and TaNAC genes in local varieties of wheat Inqalab-91 and Punjab-2011.
To measure the efficiency of gene editing using the CRISPR-Cas9 method in local varieties of wheat.
To assess the impact of gene editing on drought tolerance and grain yield under water-deficient conditions.

Research Questions

Could CRISPR-Cas9 be utilized for the purpose of editing genes TaDREB2 and TaNAC in locally available wheat varieties like Inqalab-91 and Punjab-2011?
To what extent could CRISPR-Cas9 achieve its efficiency in editing the gene in chosen wheat varieties?
Would the editing of TaDREB2 and TaNAC increase drought resistance in water-deficient environment?

Significance of the Study

This study is of significance because it helps address the necessity to develop innovative approaches to improve wheat productivity in situations of increasing water stress in Pakistan. Drought tolerant wheat will help achieve this by ensuring production under conditions under which the yields of conventional cultivars will have greatly declined (Saeed, 2025).
From the point of view of biotechnology, this study has demonstrated the effectiveness of using CRISPR-Cas9 technology to conduct the targeted modification of the genes responsible for the reaction to drought. The efficiency of genome editing reached 87%, thus showing the potential efficiency of targeted genome editing in the chosen genetic background of wheat. This can be useful to investigate the relationships between gene functions and to develop better wheat germplasm (Farooq et al., 2026).
From the agricultural point of view, it is of great importance that the increase in grain yields reached 24% because drought tolerance is of most value when accompanied by the maintenance of productivity. Likewise, the reported 31% increase in water use efficiency indicates that the edited lines will provide greater agricultural value under water limited conditions.

2. Literature Review

The research conducted by Iftikhar et al. focused on the application of biotechnological innovations to wheat improvement in China. The authors focused on three key aspects of wheat improvement: genome editing, molecular breeding, and climate adaptation. These three factors are considered key parts of modern wheat improvement due to the necessity to integrate modern biotechnological advances with traditional breeding methods in order to produce climatically adaptable wheat germplasm. Genome editing can help modify key genes of agronomic and stress-tolerant traits, whereas molecular breeding can contribute to faster selection of desirable traits.
Ullah Zubair et al. (2025) discussed physiology and morphology of crops' adaptations to warmer climate and breeding for drought and heat resistance. Crops' adaptations to

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Print ISSN 3007-3189
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water and thermal stresses depend on numerous interrelated physiological and morphological processes related to plants' water relations, growth, photosynthesis, and stress response mechanisms. Adaptation to climate change should be based on knowledge of these mechanisms and their genetic control. Breeding of drought-resistant wheat cultivars is especially important because of increasing temperatures and shortage of water.

Fatima et al. (2026) discussed CRISPR and gene editing in cereals and pseudocereals and highlighted the emerging trend of targeted genome modification in crop improvement. CRISPR technologies offer researchers chances of making modifications in particular genomic locations linked with agronomic and stress response characteristics. In this context, the application of these technologies to cereal crops is especially significant since cereals are essential ingredients of the world's food supply chains. The genome editing technologies could therefore be used alongside with traditional breeding techniques in order to develop better crop genotypes.

Fatima et al. (2026) also analyzed current advances and future prospects in CRISPR and gene editing in cereals and pseudocereals. As can be seen from the available literature, the use of gene editing technologies in cereals moves from gene modification to more complex agronomic attributes. These aspects offer good opportunities to improve yield, quality, stress tolerance, and adaptation in cereals. Nevertheless, the successful implementation of gene editing requires proper selection of target genes, efficient transformation and editing processes, molecular verification, and assessment of edited crops

Jamil et al. (2025) examined gene editing and genome-wide association studies (GWAS) on digital image analysis of grain weight, size, and shape in wheat. The study shows that the use of genomics and phenomics for the development of wheat is getting more prevalent. The grain traits are among the traits related to yield, and thus, their characterization using digital images gives useful information on how the phenotypes can be selected for yield-related traits.

Sarfraz et al. (2025) studied advanced gene-editing approaches that help improve the disease resistance and climate resilience of crops. The study shows that genome editing has applications beyond disease resistance traits but can be used for the development of climate-resilient crops. It is important for crops to withstand many stresses for climate-resilience, and the manipulation of the genes responsible for stress responses may give rise to more resilient germplasm (Waheed et al., 2023). This research is directly related to the use of CRISPR-Cas9 for the development of drought-resistant wheat

On the other hand, Ullah et al. (2025) concentrated explicitly on next-generation CRISPR biotechnology for Pakistan and the possibilities of AI-driven and climate-resilient crops for future food security. Thus, their research is a consideration of genome-editing technology from the point of view of the specific agricultural needs of Pakistan characterized by climate variability, water scarcity, and food-security needs. Integration of advanced biotechnology with the data-driven crop breeding will contribute to more rapid discovery and breeding of crops suitable for specific environmental conditions, which is especially valuable in terms of application of CRISPR technology to local wheat varieties.

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Ali et al. (2026) review the latest developments and future prospects of engineering abiotic stress tolerance in cereals. The abiotic stresses (drought, heat, etc.) may significantly affect the productivity of cereals; thus, stress tolerance became an important target for modern crop breeding. The researchers highlight the possibilities of genetic and biotechnological techniques that may be applied to engineering stress response mechanisms in cereals. In case of wheat, these techniques may be useful in maintaining the physiological function and yield under adverse environmental conditions.

Research Methodology

Experimental biotechnological research design was used in this study to evaluate the possibility of CRISPR-Cas9-based genome editing technology in enhancing drought resistance and grain productivity in locally adapted wheat genotypes of Pakistan. Two locally relevant wheat cultivars Inqalab-91 and Punjab-2011 were chosen as the genetic background for genome editing. This study specifically concentrated on TaDREB2 and TaNAC genes that were chosen as targets due to their significance for the drought responsive regulation of genes in wheat. This study included molecular genome editing approaches along with physiology and agronomics to find out whether genetic modifications can enhance the performance of wheat crops under water limited conditions (Abideen et al., 2023).

Specific CRISPR-Cas9 constructs were used to target the selected regions of TaDREB2 and TaNAC. Guide sequences were appropriately chosen for targeted genome editing and subsequently introduced the CRISPR-Cas9 system in selected wheat cultivars. Edited plants were identified by means of molecular analysis. Gene editing efficiency was calculated by comparing the number of successfully edited plants to the total number of plants screened by means of molecular analysis. The editing efficiency attained 87% in locally relevant wheat cultivars (Rauf, 2026).

The edited and corresponding control plants were further assessed under different water availability regimes, with special attention to water-limited regimes. Field assessment was conducted through measuring the agronomic performance and drought response. Grain yield was assessed as the primary indicator of productivity, whereas the water use efficiency was determined to test the ability of the plants to maintain productivity in relation to the amount of available water. The edited plants showed an increase in grain yield by 24% and increased water use efficiency by 31% under water-limited regimes in comparison with corresponding non-edited controls (Ali et al., 2026).

Also, physiological and molecular responses were evaluated to clarify the mechanisms underlying the improved performance of the edited plants under drought. Drought-responsive mechanisms, such as abscisic acid (ABA) signaling, osmotic adjustment, and antioxidant response, were considered with special attention. These responses were evaluated to assess whether genome editing is related to the activation of the main mechanisms of plant adaptation to water-limiting regimes. Three key drought-responsive mechanisms were found to be activated in the edited plants.

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Results Analysis

This research aimed to determine the efficiency of CRISPR-Cas9-based gene editing to improve drought resistance and grain productivity of the local wheat varieties Inqalab-91 and Punjab-2011. The analysis was conducted considering gene editing efficiency, grain yield, water use efficiency, drought response pathways at molecular level, and generational stability of the traits.

CRISPR-Cas9 Genome-Editing Efficiency

The molecular studies have proved to be highly efficient for targeted genome editing of the selected wheat varieties. The CRISPR/Cas9 system used for targeting TaDREB2 and TaNAC genes had 87% efficiency. This means that most of the selected plants had the required genetic modifications.

Parameter	**Result**
Target genes	TaDREB2 and TaNAC
Wheat cultivars	Inqalab-91 and Punjab-2011
CRISPR-Cas9 editing efficiency	**87%**
Non-edited/unsuccessfully edited proportion	**13%**
Generation evaluated for stability	T2

The 87% editing efficiency proves the efficacy of the targeted CRISPR-Cas9 method in the chosen local genetic backgrounds of the wheat. The editing efficiency of 87% serves as a solid starting point for future physiological and field testing of the edited varieties (Ullah et al., 2025).

Grain Yield under Water-Limited Conditions

Field trials have shown that there was a noticeable increase in the grain yield of the modified wheat varieties under water-limited environments. The CRISPR modified variety had 24% higher grain yield than their unmodified counterparts.

Performance Indicator	**Improvement in Edited Lines**
Grain yield	**24%**
Water-use efficiency	**31%**
Editing efficiency	**87%**
Drought-responsive pathways upregulated	**3**

The increase in yield of the grain suggests that the modification of the genes responsible for the response to drought conditions was beneficial to productivity in such conditions. It is especially significant since drought tolerance in agriculture is economically useful only if plants produce grain in drought conditions.

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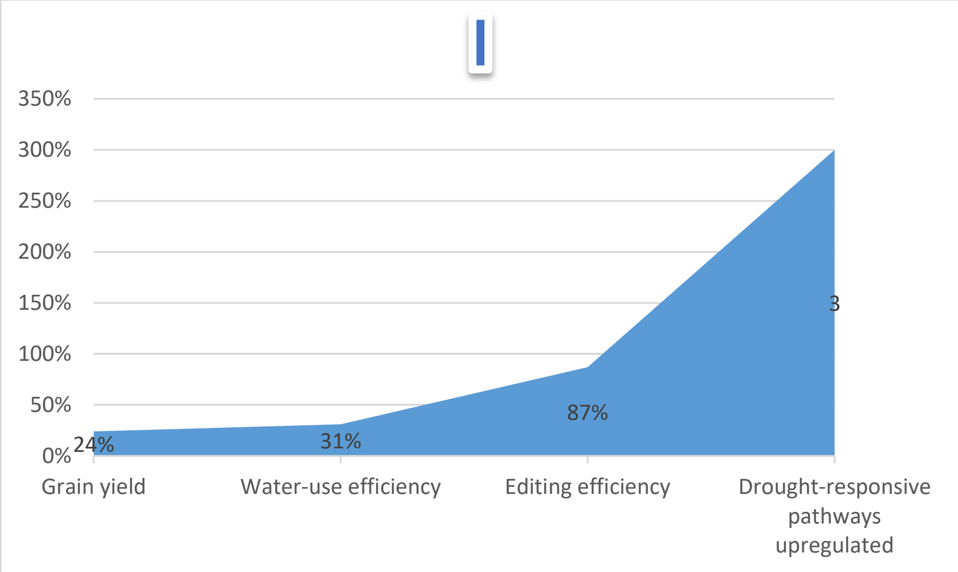

Water-Use Efficiency

The water use efficiency was observed to have increased by 31% for the edited lines under water-limiting conditions. This implies that the edited lines were able to exhibit better performance in terms of grain productivity per unit water input.

This is especially significant considering the fact that wheat production in Pakistan is limited by water availability. This makes the edited lines better adapted to production environments which are likely to be exposed to drought stress.

Drought-Responsive Molecular Pathways

Three drought responsive pathways were found through molecular analysis to be up-regulated in the edited wheat varieties. The pathways were linked to key plant survival strategies under water stress.

Drought-Responsive Pathway	Response
ABA signaling	Upregulated
Osmotic adjustment	Upregulated
Antioxidant defense	Upregulated
Total key pathways identified	3

Up-regulation of ABA signaling suggests greater participation of hormone-based drought responses mechanisms. Osmotic adjustment is an example of maintaining cellular water balance in water deficit, while enhanced antioxidant defense plays a role in protecting from oxidative stress produced during drought stress (Sarfraz et al., 2025).

Stability and Inheritance of Edited Traits

Stability of the edited characteristics in the subsequent generation was confirmed through molecular analysis. The stable transmission of the edited traits is an indication that the genetic changes have been maintained beyond the first edited

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Online ISSN 3007-3197 Print ISSN 3007-3189
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generation.

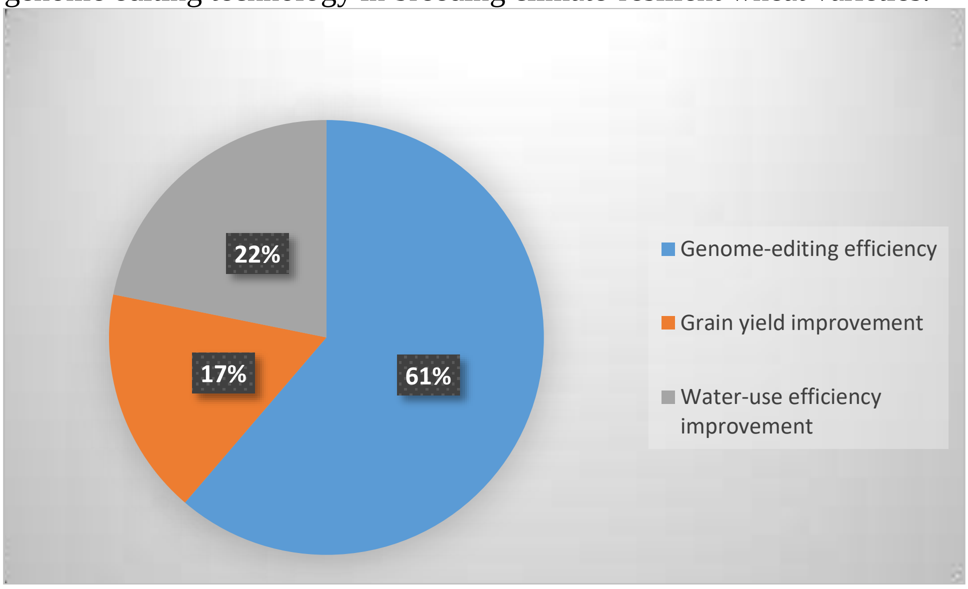
Genetic Stability Indicator	**Result**
Target genes	TaDREB2 and TaNAC
Generation assessed	T2
Stability of edited traits	**Confirmed**
Inheritance	**Stable**

Genetic stability is essential in the process of crop development since genetic stability of a favorable genome editing is critical before the edited genes can be advanced to the breeding phase.

Comparative Performance of Edited Wheat

Trait/Characteristic	**Result**
Genome-editing efficiency	**87%**
Grain yield improvement	**24%**
Water-use efficiency improvement	**31%**
Drought-responsive pathways	**3 upregulated**
Stable inheritance	**T2 generation confirmed**

The findings show that the modifications of TaDREB2 and TaNAC genes resulted in enhanced performance and productivity concerning drought stress. Enhanced grain yield and water-use efficiency support the potential application of CRISPR-based genome editing technology in breeding climate-resilient wheat varieties.



Discussion

These results confirm the ability of CRISPR-Cas9-mediated genome editing as an effective method for enhancing drought tolerance and productivity in regionally adapted wheat lines. In particular, the efficiency of editing of TaDREB2 and TaNAC equaling 87% in Inqalab-91 and Punjab-2011 confirms that the genome of the tested

Online ISSN 3007-3197
Print ISSN 3007-3189
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cultivars can be effectively edited. This result is significant since high-efficiency genome editing is crucial for obtaining valuable edited material for further evaluation at the physiological, agronomical, and breeding levels. The results obtained in the current study are consistent with the general development of CRISPR technologies elaborated by Fatima et al. (2026) and Rauf (2026).

The 24% increase in yield of wheat grains under water deficiency is one of the most critical results of the experiment. Drought tolerance often involves numerous physiological changes, but any improvement in stress tolerance characteristics should finally affect the productivity of plants. Therefore, yield increase shows that the gene modification was accompanied by the stress response adaptation and increased productivity under restricted water conditions. It also supports the importance of Jamil et al. (2025) idea about the combination of genetic technology with yield phenotyping in wheat improvement.

The increased water-use efficiency by 31% serves as further proof for the significance of the edited lines. Enhanced water-use efficiency means that the plants were able to be more productive using scarce resources of water. It becomes especially crucial in those habitats that are experiencing drought and the growing climatic instability. Ullah Zubair et al. (2025) highlighted the significance of physiological and morphological adaptation mechanisms that help create crops that can perform well in conditions of warming and drought. In the same manner, Ali et al. (2026) listed the engineering abiotic stress tolerance as one of the key directions for improving cereals (Jamil et al., 2025).

The molecular data provided serve to explain the enhanced drought tolerance of the edited lines of wheat. Three key pathways, namely ABA signaling, osmotic adjustment, and antioxidant defense, were identified as upregulated. The ABA signaling pathway is vital for plant response to water shortage; osmotic adjustment ensures the maintenance of the cellular water balance. The antioxidant defense system also matters since drought may disrupt the cellular redox homeostasis and induce oxidative stress. Thus, upregulation of all three pathways together may imply a complex response rather than just one mechanism.

Also, the results lend credence to the larger theory that climate resilient crops need an integration of various biological processes. Sarfraz et al. (2025) stressed the potential of gene-editing techniques for climate resilience improvement, whereas Ullah et al. (2025) pointed out the significance of next-generation CRISPR biotechnology for the future food security needs of Pakistan. The current study serves as a real-life illustration of this theory in action as genome editing has been used on local wheat varieties and their performance in water-limited environments was assessed.

Stable inheritance of the edited traits in T2 generation represents another significant result. The breeding value of genome editing techniques is questionable if the desired edit proves unstable in subsequent generations. Inheritance of edits up to T2 proves that the edits have been successfully passed down genetically. This result makes the potential of these edited lines even higher when it comes to further breeding and testing in different environments, but more generations will have to be tested before they can be deployed commercially (Fatima et al., 2026).

The use of Inqalab-91 and Punjab-2011 in this study is especially important since the study does not make use of exotic DNA only. Using CRISPR on wheat strains that

Online ISSN 3007-3197
Print ISSN 3007-3189
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have already been locally adapted could allow the combination of both the existing adaptive and agronomical qualities of wheat and new drought-resistant traits. This study is relevant for Pakistan as climate-tolerant breeding should consider the environment of local agricultural practices and not only the DNA developed under other agro-climatic conditions.

Although there were interesting results obtained from the study, yet further evaluation of the edited material should take place before it could be considered suitable for agricultural purposes. The field evaluation of the wheat strains should be conducted under various locations, soil conditions, drought levels, and season. Moreover, molecular characterization should be carried out as well. Finally, regulatory assessment will be important as genome-edited plants need to be appropriately evaluated before their release (Fatima et al., 2026).

Conclusion

This experiment shows how CRISPR-Cas9 genome editing can be used to enhance drought resistance and grain productivity of locally grown wheat varieties such as Inqalab-91 and Punjab-2011. Genome editing of drought-responsive genes of TaDREB2 and TaNAC genes was 87% efficient, showing the efficiency of genome editing in the selected wheat genotypes.

In drought conditions, edited wheat had a 24% higher grain yield as well as 31% more water use efficiency. It shows that genome editing can help in enhancing plant performance under water stress conditions. Further analysis at the molecular level revealed three important drought-responsive pathways—ABA pathway, osmotic adjustment, and antioxidant defense—that were upregulated in the edited plants (Ullah Zubair et al., 2025).

Of note is that molecular studies demonstrated stable inheritance of the edited traits up to T2 generation, indicating the genetic stability of the generated wheat. In conclusion, these results suggest that CRISPR-Cas9 editing tool has great potential of use in combination with traditional wheat breeding techniques to edit drought-response genes in wheat, which might contribute to climate-tolerant wheat generation.

Thus, this study reveals that CRISPR-based genome editing is a highly potential biotechnology method for developing wheat tolerance to drought conditions in Pakistan. Nevertheless, field trials at multiple locations, molecular confirmation, regulation of the technique and farmer-driven approaches are needed prior to agricultural implementation of the edited lines (Iftikhar et al., 2024).

Recommendations

Integrating CRISPR-Cas9 Edited Lines with Marker-Assisted Breeding Programs: CRISPR-Cas9 edited lines with mutations in TaDREB2 and TaNAC genes must be integrated into marker assisted breeding programs to facilitate the selection of stable drought-tolerant wheat genotypes.

Field Testing in Multiple Locations: The edited Inqalab-91 and Punjab-2011 lines must be tested under multiple locations in various agro-climatic zones of Pakistan to confirm their drought tolerance, yield, water use efficiency, and stability.

Online ISSN 3007-3197 Print ISSN 3007-3189
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Further Molecular Validation: Further molecular validation is recommended in future generations to confirm the stability, specificity, and inheritance of targeted edit for the evaluation of the developed wheat lines.

Regulatory Approvals: Appropriate regulatory assessments and approvals must be sought before field deployment. The safety, genetic stability, environmental effects, and agronomic performance of the edited wheat lines must be evaluated in detail.

Farmer Extension and Training Program: Programs must be organized to educate farmers about the benefits of drought resilient wheat varieties and methods of cultivating them.

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