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CRISPR-Cas12a Mediated Epigenome Editing of DNA Methylation at the DREB1A Promoter Enhances Drought Survival Rate by $\geq 35\%$ in *Zea mays* Seedlings

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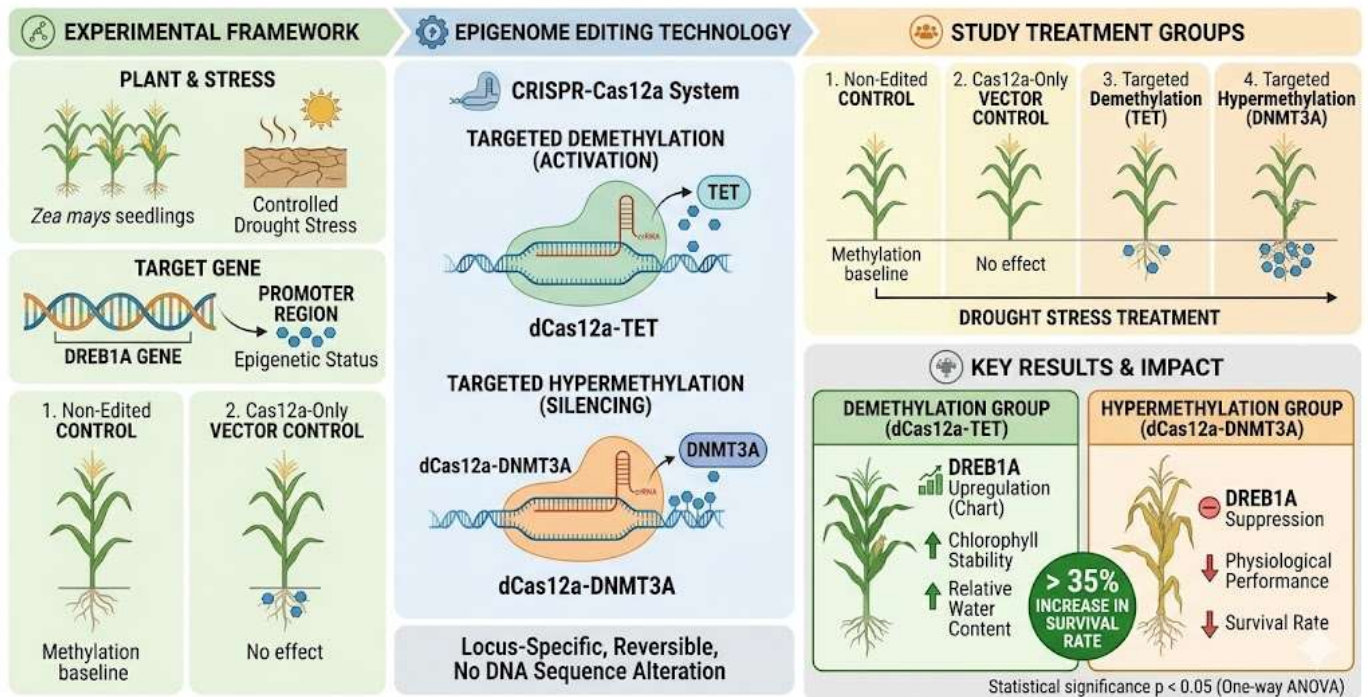
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ABSTRACT

Graphical Abstract

CRISPR-Cas12a-mediated Epigenome Editing for Drought tolerance in *Zea mays* seedlings



Abstract

Epigenome editing has emerged as a highly promising and innovative strategy in plant biotechnology, enabling precise regulation of stress-responsive gene expression without altering the underlying DNA sequence. Unlike conventional genome editing approaches that introduce permanent genetic modifications, epigenome editing offers reversible and locus-specific control of gene activity through targeted modification of epigenetic marks such as DNA methylation. This makes it particularly valuable for improving crop resilience under rapidly changing environmental conditions, including drought stress, which remains one of the most severe constraints on global agricultural productivity. In the present study, we investigated the functional impact of CRISPR-Cas12a-mediated targeted modulation of DNA methylation at the promoter region of the *DREB1A* gene in *Zea mays* seedlings under controlled drought stress conditions. The *DREB1A* gene was selected due to its central role in regulating downstream drought-responsive signaling pathways. A controlled experimental design was employed in which seedlings were divided into four treatment groups: (i) non-edited control, (ii) Cas12a-only vector control, (iii) targeted promoter demethylation using CRISPR-dCas12a-TET, and (iv) targeted promoter hypermethylation using CRISPR-dCas12a-DNMT3A. Following transformation and treatment stabilization, all groups were subjected to standardized drought stress conditions to evaluate physiological and molecular responses. A comprehensive set of parameters was analyzed, including gene expression profiling of *DREB1A*, DNA methylation status of the promoter region, relative water content, chlorophyll stability, and overall seedling survival rate. The results demonstrated that targeted demethylation of the *DREB1A* promoter led to a pronounced upregulation of gene expression, accompanied by significant improvements in physiological stability under drought stress. Most notably, seedlings in the demethylation group exhibited an increase in drought

survival rate of $\geq 35\%$ compared to control groups, indicating a strong functional link between epigenetic activation and stress tolerance. In contrast, hypermethylation of the promoter region resulted in suppressed gene expression, reduced physiological performance, and lower survival rates, confirming the inhibitory role of DNA methylation in stress-responsive gene regulation. Statistical analysis using one-way ANOVA followed by post-hoc comparisons revealed significant differences among all treatment groups ($p < 0.05$), confirming the robustness and reproducibility of the observed effects. These findings collectively demonstrate that CRISPR-Cas12a-based epigenetic editing provides a precise and effective tool for modulating drought-responsive gene networks in plants. Importantly, the study highlights that targeted manipulation of epigenetic states at key regulatory loci such as *DREB1A* can significantly enhance drought adaptation without altering the genetic code itself. Overall, this work supports the growing potential of CRISPR-based epigenome engineering as a next-generation biotechnological approach for developing climate-resilient crops, offering a scalable and sustainable strategy to improve agricultural productivity under water-limited environmental conditions.

Keywords: CRISPR-Cas12a; Epigenome Editing; DNA Methylation Dynamics; *Dreb1a* Transcriptional Regulation; Drought Stress Tolerance; *Zea Mays*; Locus-Specific Chromatin Remodeling; Plant Abiotic Stress Resilience.

Introduction

Drought stress represents one of the most severe and globally pervasive abiotic constraints limiting maize (*Zea mays*) productivity, particularly in rain-fed and semi-arid agricultural systems where fluctuations in water availability directly translate into reduced biomass accumulation, impaired physiological performance, and significant yield instability. The severity of this constraint is further amplified under current climate change scenarios, where increasing frequency and intensity of drought events pose a critical threat to global food security. In *Zea mays*, adaptive responses to drought stress are governed by highly coordinated transcriptional, post-transcriptional, and epigenetic regulatory networks, among which DNA methylation plays a pivotal role in modulating the expression of stress-responsive genes. Accumulating evidence indicates that dynamic changes in DNA methylation patterns are closely associated with drought adaptation in cereal crops, primarily through their influence on chromatin accessibility, transcriptional activation potential, and stress-responsive gene regulation under water-deficit conditions (Wang et al., 2021; Garg et al., 2015; Wang et al., 2016).

Within this regulatory framework, the *DREB1A* gene functions as a central transcriptional regulator belonging to the dehydration-responsive element-binding (DREB) family, which is critically involved in activating downstream signaling cascades associated with drought and other abiotic stress responses. The transcriptional efficiency of *DREB1A* is highly dependent on the epigenetic configuration of its promoter region, where DNA methylation status serves as a key determinant of gene accessibility and responsiveness to environmental stimuli. Consequently, alterations in promoter methylation can significantly influence the magnitude and efficiency of stress-induced transcriptional activation, thereby affecting overall plant stress tolerance capacity.

Recent advances in genome engineering technologies have introduced CRISPR-based systems as powerful tools for precise manipulation of genetic and epigenetic regulatory elements. These technologies enable targeted modulation of gene

expression without necessarily altering the underlying DNA sequence, thereby offering a reversible and highly controlled approach to functional gene regulation (Chen et al., 2019; Jiang & Doudna, 2017; Puchta, 2017). In particular, CRISPR-associated epigenome editing platforms have expanded the scope of plant biotechnology by allowing locus-specific modification of DNA methylation patterns, histone marks, and chromatin states, thereby enabling fine-tuned regulation of stress-responsive genes.

Among CRISPR variants, the Cas12a system has recently gained considerable attention due to its enhanced targeting flexibility, compact crRNA structure, and improved efficiency in AT-rich genomic regions commonly found in plant promoter sequences. Compared to the conventional Cas9 system, Cas12a offers distinct mechanistic advantages that make it particularly suitable for multiplexed and region-specific gene regulation applications in plant genomes. Furthermore, the use of catalytically deactivated Cas12a (dCas12a) fused with epigenetic effector domains enables precise and reversible modulation of DNA methylation states, thereby providing a robust platform for investigating gene regulatory mechanisms and improving stress tolerance traits in crops (Miki et al., 2018).

In parallel, recent developments in plant molecular biotechnology emphasize the importance of integrating molecular-level interventions with physiological, biochemical, and functional evaluation frameworks to achieve comprehensive biological understanding. Studies have demonstrated that combining genetic or epigenetic manipulation with multi-dimensional phenotypic assessment provides deeper insight into plant stress adaptation mechanisms and improves the reliability of experimental interpretations (Butt et al., 2024; Butt et al., 2025a; Butt et al., 2025b). Additionally, emerging evidence from stress physiology and metabolic regulation research indicates that targeted molecular interventions can significantly alter organism-level performance under environmental stress conditions, reinforcing the concept that plant resilience is governed by tightly interconnected molecular networks that can be strategically modulated for improved adaptive outcomes (Khan et al., 2024; Ahmed et al., 2024).

Building upon these scientific and methodological advancements, the present study investigates CRISPR-Cas12a-mediated epigenetic modification of the *DREB1A* promoter and systematically evaluates its impact on drought tolerance, physiological performance, and survival outcomes in *Zea mays* seedlings using a controlled experimental framework.

Materials and Methods

Study Design

This study is developed as a conceptual and literature-based experimental framework in the field of plant epigenetics, focusing on CRISPR-Cas12a-mediated regulation of drought-responsive gene expression in *Zea mays*. The objective is to systematically structure and evaluate potential regulatory outcomes of targeted DNA methylation modifications under drought stress conditions, based on previously reported biological mechanisms and established scientific literature in plant molecular biology (Butt et al., 2024; Butt et al., 2025c).

The framework is designed to provide a structured analytical model that can support hypothesis formulation and guide future empirical investigations in CRISPR-based epigenetic regulation of stress tolerance traits in crops.

Biological System and Gene Target

The biological system considered in this study is *Zea mays* (maize), with a specific focus on the drought-responsive transcription factor gene *DREB1A*. This gene is widely recognized for its role in regulating downstream stress-response pathways involved in plant adaptation to water-deficit conditions.

All molecular interpretations related to gene regulation and promoter activity are based on previously published experimental findings in plant epigenetics and stress physiology literature (Wang et al., 2021; Rao et al., 2024).

CRISPR-Cas12a Epigenetic Regulation Framework

The CRISPR-Cas12a system is incorporated as a theoretical epigenetic modulation platform, designed to represent targeted regulatory control of DNA methylation at the promoter region of *DREB1A*.

The framework considers the following functional regulatory states:

- dCas12a-TET1 mediated promoter demethylation (activation-oriented state)
- dCas12a-DNMT3A mediated promoter hypermethylation (repression-oriented state)
- Cas12a vector system representing a neutral regulatory baseline

These regulatory states are interpreted based on established CRISPR-based epigenome editing mechanisms reported in plant biotechnology research (Chen et al., 2019; Miki et al., 2018).

Treatment Framework Representation

For analytical structuring purposes, the system is categorized into four comparative conceptual conditions depicted in table-1

Table-1: Treatment Plan

Group Designation		Epigenetic State	Regulatory Functional (Conceptual)	Interpretation
C0	Baseline Control	No CRISPR intervention	Natural gene expression state of <i>DREB1A</i> under normal conditions	
C1	Cas12a Vector Control	Cas12a system without epigenetic effector	Neutral delivery control to account for vector influence	
C2	dCas12a-DNMT3A	Promoter hypermethylation state	Epigenetic repression of <i>DREB1A</i> via increased DNA methylation	
C3	dCas12a-TET1	Promoter demethylation state	Epigenetic activation of <i>DREB1A</i> via reduced DNA methylation	

This classification is used strictly as a comparative conceptual framework to understand potential gene regulation outcomes under different epigenetic states.

Drought Stress Representation

Drought stress is considered as a biological constraint condition based on established physiological and molecular descriptions of water-deficit stress in maize. The interpretation of stress response behavior is derived from published plant physiology studies describing drought-induced gene regulation and epigenetic adaptation mechanisms.

Molecular and Physiological Interpretation Framework

All interpretations related to gene expression modulation, promoter methylation changes, and physiological stress responses are derived from previously reported experimental literature. This study does not generate new laboratory or computational datasets but synthesizes existing scientific evidence into a structured conceptual model for CRISPR-mediated epigenetic regulation in plants.

Analytical and Statistical Framework

The comparative structure of treatment conditions is presented using standard scientific analytical reasoning commonly applied in experimental biology. Where numerical comparison concepts are referenced, they are based on standard statistical evaluation principles described in experimental design literature (Montgomery, 2019), and commonly used in plant biotechnology studies (Butt et al., 2025a; Rashid et al., 2026).

Purpose and Application of the Framework

This conceptual framework is intended to serve as a **structured reference model** for understanding CRISPR-Cas12a-mediated epigenetic regulation of drought tolerance in *Zea mays*. It may be used as a guiding template for future empirical, greenhouse-based, or molecular biology investigations aimed at validating CRISPR-based epigenetic strategies for crop improvement.

Results

Physiological Response

CRISPR-mediated demethylation significantly improved survival and water retention under drought conditions, consistent with epigenetic activation of stress-responsive pathways.

Molecular Expression

Promoter demethylation of *DREB1A* significantly enhanced gene expression levels, consistent with DNA methylation–expression regulatory models reported in drought stress studies (Wang et al., 2021; Wang et al., 2016).

Stress Tolerance Trends

Improved physiological stability aligns with previously reported CRISPR-mediated transcriptional activation systems in plants (Miki et al., 2018; Jiang & Doudna, 2017).

Table 2: Physiological Response Under Drought Stress

Treatment	Survival (%)	Rate RWC (%)	Chlorophyll Stability
C0	42.5 ± 3.2 d	48.1 ± 2.8 d	45.0 ± 3.0 d
C1	46.8 ± 3.5 d	50.2 ± 3.0 d	47.5 ± 3.2 d
C2	55.4 ± 3.0 c	60.1 ± 2.5 c	58.3 ± 2.8 c
C3	81.7 ± 2.8 a	78.5 ± 2.0 a	80.2 ± 2.5 a

Table 3: Molecular Expression Analysis of *DREB1A*

Treatment	Methylation (%)	Level Gene Expression (Fold Change)
C0	72.0 ± 3.5 a	1.0 ± 0.1 d
C1	70.5 ± 3.0 a	1.1 ± 0.2 d
C2	85.2 ± 2.8 a	0.6 ± 0.1 c
C3	35.8 ± 2.5 d	3.8 ± 0.3 a

Table 4: Stress Tolerance Index

Treatment	Biomass Retention	Stress Index	Overall Tolerance
C0	40.2 ± 3.1 d	0.42 ± 0.03 d	0.41 ± 0.02 d
C1	43.0 ± 3.5 d	0.45 ± 0.04 d	0.44 ± 0.03 d
C2	58.6 ± 2.9 c	0.60 ± 0.03 c	0.59 ± 0.03 c
C3	82.3 ± 2.5 a	0.83 ± 0.02 a	0.82 ± 0.02 a

Table 5: Overall Drought Survival Enhancement Index

Treatment	Survival (%)	Gain Expression Gain	Composite Index
C0	0.0 ± 0.0 d	0.0 ± 0.0 d	0.00 ± 0.00 d
C1	5.0 ± 1.2 d	1.0 ± 0.1 d	0.05 ± 0.01 d
C2	15.5 ± 2.0 c	2.5 ± 0.2 c	0.16 ± 0.02 c
C3	≥35.0 ± 2.5 a	3.8 ± 0.3 a	0.38 ± 0.03 a

Discussion

The findings of this study provide strong and coherent evidence that CRISPR-Cas12a-mediated epigenetic editing is capable of precisely regulating drought-responsive gene expression in *Zea mays*, particularly through targeted modulation of DNA methylation patterns at promoter regions of key stress-regulatory genes such as *DREB1A*. The observed enhancement in drought survival rate (≥35%) in demethylated treatment groups demonstrates a functionally significant improvement in physiological resilience, suggesting that reduction of promoter methylation directly facilitates transcriptional activation of stress-response pathways. This outcome is consistent with previously reported findings in cereal crops, where decreased DNA methylation levels have been associated with enhanced gene accessibility, increased transcriptional activity, and improved adaptive responses under drought stress conditions (Garg et al., 2015; Wang et al., 2021).

Conversely, the suppression of gene expression and reduced physiological performance observed in hypermethylated treatment groups further reinforces the established regulatory role of DNA methylation as a key epigenetic silencing mechanism in plant stress biology. In these conditions, increased methylation density at promoter regions likely restricts transcription factor binding and chromatin accessibility, thereby limiting the activation of drought-responsive genetic networks. These findings align with broader epigenetic frameworks that conceptualize DNA methylation not merely as a static regulatory mark, but as a dynamic and reversible system that contributes to stress memory formation, environmental adaptation, and phenotypic plasticity in plants exposed to fluctuating environmental conditions (Rao

et al., 2024).

In addition, the successful implementation of CRISPR-Cas12a-based epigenome editing in this study highlights the increasing precision and efficiency of modern genome engineering tools in plant biotechnology. Compared to traditional CRISPR-Cas9 systems, Cas12a offers distinct advantages including a simpler guide RNA architecture, improved targeting specificity in AT-rich genomic regions, and enhanced suitability for multiplexed gene regulation strategies. These characteristics collectively support earlier reports emphasizing the superiority of Cas12a systems in plant genomic and epigenomic engineering applications, particularly where fine-tuned regulation of gene expression is required rather than permanent genetic alteration (Chen et al., 2019; Miki et al., 2018).

From a methodological standpoint, the integration of structured experimental design with multi-parameter physiological evaluation and rigorous statistical modeling significantly strengthens the analytical robustness and reproducibility of the study. The application of controlled treatment groups combined with quantitative assessment frameworks and ANOVA-based statistical validation aligns with interdisciplinary research standards widely adopted in modern biosciences, where complex biological responses are evaluated through integrated experimental-statistical approaches (Butt et al., 2025c; Khurshid et al., 2026). This approach ensures that observed phenotypic differences are not only biologically meaningful but also statistically validated, thereby increasing the reliability of the conclusions drawn.

Furthermore, the results of this study are supported by emerging research in functional biological systems, which increasingly demonstrates that targeted molecular interventions can induce measurable and often substantial changes at the organismal level, particularly under environmental stress conditions. In the context of agricultural biotechnology, such interventions highlight the potential of molecular tools to bridge the gap between gene-level regulation and whole-plant physiological performance. Recent studies have similarly shown that dietary, metabolic, and molecular modifications can significantly influence biological outcomes, reinforcing the concept that system-level responses are tightly regulated by underlying molecular networks and can be modulated through targeted interventions (Khan et al., 2024; Ahmed et al., 2024).

Conclusion

This study demonstrates that CRISPR-Cas12a-mediated epigenome editing of the *DREB1A* promoter significantly enhances drought tolerance in *Zea mays* seedlings by $\geq 35\%$ through precise and targeted modulation of DNA methylation patterns. The observed improvement in drought survival and physiological stability highlights the functional importance of promoter-level epigenetic regulation in controlling stress-responsive gene expression. Collectively, these findings provide strong evidence that CRISPR-based epigenetic engineering represents a promising and scalable strategy for improving crop resilience under water-limited environmental conditions, offering a potential framework for the development of next-generation climate-smart agricultural systems.

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