SARPS: Journal of Advanced Research in Plant Science
July-December 2025 1(1)
https://journalofplantscience.com

Original Article

CRISPR GENE EDITING FOR DEVELOPING STRESS-RESILIENT CROPS

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ABSTRACT

Crop productivity is increasingly threatened by abiotic stresses such as drought, salinity, and temperature extremes, as well as biotic challenges including pathogens and pests. Traditional breeding methods have achieved partial success in enhancing stress tolerance but remain time-consuming and limited by species compatibility. The advent of CRISPR/Cas9 gene-editing technology offers a precise, cost-effective, and rapid approach to developing stress-resilient crops. This paper reviews recent advances in CRISPR-mediated genome editing for improving stress tolerance in major crops, focusing on targeted genes related to abiotic and biotic stress pathways. Methodological approaches, including single-gene knockouts, base editing, and multiplex gene editing, are discussed. Results from published studies demonstrate significant improvements in drought and salt tolerance, disease resistance, and yield stability. The discussion highlights both the promise and limitations of CRISPR, including regulatory concerns and off-target effects. The review concludes that CRISPR technology provides a transformative platform for sustainable agriculture under climate change conditions.

Keywords: CRISPR/Cas9, Gene Editing, Stress-Resilient Crops, Drought Tolerance, Salinity Stress, Genome Engineering, Plant Biotechnology

INTRODUCTION

Global agriculture is under unprecedented pressure due to the dual challenges of climate change and population growth. According to the Food and Agriculture Organization FAO. (2022), the world population is projected to reach 9.7 billion by 2050, requiring a 60–70% increase in food production. At the same time, agricultural productivity is severely constrained by abiotic stresses, such as drought, salinity, flooding, and extreme temperatures, which collectively account for up to 50–70% yield losses in major crops. Biotic stresses, including fungal pathogens, bacterial infections, and insect pests, further threaten global food security.

Traditional breeding techniques have long been employed to enhance crop stress tolerance. However, the polygenic nature of stress responses, coupled with the lengthy breeding cycles and species incompatibility barriers, often limit the effectiveness of conventional methods. Similarly, transgenic approaches have contributed to trait improvement but face regulatory challenges, ethical concerns, and public skepticism over genetically modified organisms (GMOs). This has created an urgent need for innovative, precise, and socially acceptable strategies for crop improvement.

The advent of CRISPR/Cas9 (Clustered Regularly Interspaced Short Palindromic Repeats associated with Cas9 nuclease) has revolutionized the field of plant biotechnology. This technology allows for targeted genome editing by introducing site-specific double-strand breaks in DNA, which are subsequently repaired by cellular mechanisms. The precision, cost-effectiveness, and ease

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Received: 06 September 2025; **Accepted:** 20 October 2025; **Published** 10 November 2025
DOI: 10.29121/SARPS.v1.i1.2025.1
Page Number: 1-4
Journal Title: SARPS: Journal of Advanced Research in Plant Science
Journal Abbreviation: SARPS: J. Adv. Res. Plant Sci.
Online ISSN: 3108-2521
Publisher: Granthaalayah Publications and Printers, India
Conflict of Interests: The authors declare that they have no competing interests.
Funding: This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.
Authors' Contributions: Each author made an equal contribution to the conception and design of the study. All authors have reviewed and approved the final version of the manuscript for publication.
Transparency: The authors affirm that this manuscript presents an honest, accurate, and transparent account of the study. All essential aspects have been included, and any deviations from the original study plan have been clearly explained. The writing process strictly adhered to established ethical standards.

of use of CRISPR systems have made them superior to earlier gene-editing platforms such as zinc finger nucleases (ZFNs) and transcription activator-like effector nucleases (TALENs) Zhang et al. (2019).

In plants, CRISPR technology has been successfully applied to enhance tolerance against abiotic stresses such as drought (by targeting dehydration-responsive genes), salinity (by editing ion transporter genes), and temperature extremes (through regulation of heat-shock factors). Likewise, it has been used to increase resistance to biotic stresses, including fungal and bacterial pathogens, by editing susceptibility genes or activating host immune responses Jaganathan et al. (2018). Notably, the flexibility of CRISPR allows for multiplex editing, enabling simultaneous targeting of multiple genes involved in complex stress pathways, which is essential for building holistic resilience in crops.

Despite its promise, challenges remain, including potential off-target mutations, delivery methods for plant cells, and regulatory ambiguities in different countries. Nevertheless, CRISPR offers a transformative opportunity for developing stress-resilient crops that can withstand adverse environments while ensuring yield stability. This review aims to provide a comprehensive overview of the methodological approaches, experimental findings, and future directions of CRISPR-mediated stress tolerance in plants, with particular emphasis on its potential role in addressing global food security.

METHODOLOGY

The methodology adopted in this review involves a systematic approach combining literature survey, data extraction, and critical analysis of published studies related to CRISPR/Cas9 applications in crop improvement. In addition, general workflows for CRISPR-based genome editing in plants are outlined to provide a comprehensive overview of how this tool is used for stress-resilience breeding.

1) Literature Review and Data Collection

Relevant articles were collected from peer-reviewed journals, including Nature Biotechnology, Plant Physiology, Frontiers in Plant Science, Trends in Biotechnology, and The Plant Cell. Databases such as PubMed, Scopus, and Web of Science were searched using keywords including “CRISPR/Cas9 in plants,” “stress tolerance,” “abiotic stress,” “biotic resistance,” and “crop improvement.” Only studies published between 2015–2025 were considered to ensure updated insights.

2) Gene Target Identification

Genes associated with abiotic stress tolerance (e.g., DREB, HKT1, NHX1, HSPs) and biotic stress resistance (e.g., MLO, SWEET, R genes) were identified. These genes were chosen based on their documented role in drought resistance, salinity regulation, heat response, and pathogen defense.

3) CRISPR/Cas9 Construct Design

Guide RNAs (gRNAs) specific to the selected genes were designed using online tools such as CRISPR-P and CHOPCHOP. The gRNAs were integrated into CRISPR/Cas9 vectors under plant-compatible promoters for efficient delivery.

4) Delivery Methods

Two widely used delivery strategies were reviewed:

Agrobacterium-mediated transformation for stable gene editing in crops like rice, tomato, and maize.

Particle bombardment and protoplast transfection for transient and stable expression in recalcitrant species.

5) Screening and Validation

Edited plants were screened using PCR-based genotyping, Sanger sequencing, and high-throughput next-generation sequencing (NGS) to confirm targeted mutations. Phenotypic screening under stress conditions (e.g., drought simulation, salt exposure, pathogen inoculation) was also evaluated.

6) Data Analysis

Results from selected studies were compiled to compare the efficiency of CRISPR-mediated gene editing in conferring stress tolerance. The focus was on measurable parameters such as growth performance, stress survival rates, yield stability, and biochemical indicators.

RESULTS

The application of CRISPR/Cas9 technology in crop plants has demonstrated remarkable progress over the past decade. Multiple studies have reported successful editing of genes linked with abiotic and biotic stress pathways, leading to improved tolerance against drought, salinity, and pathogens. Table 1 summarizes selected examples of CRISPR-mediated gene editing in major crops and their outcomes.

Table 1

Table 1 Selected Applications of CRISPR/Cas9 in Stress-Resilient Crops

Crop	Target Gene	Stress Type	Outcome
Rice	OsSWEET14	Bacterial Blight Resistance	Enhanced resistance
Wheat	TaMLO	Powdery Mildew Resistance	Loss of Susceptibility
Maize	ZmHKT1	Salt Tolerance	Improved ion homeostasis
Tomato	SiML01	Fungal Resistance	Improved pathogen defense
Soybean	GmFAD2	Drought Tolerance	Improved water-use efficiency

To assess the research trend, published data between 2016 and 2024 were analyzed. The results indicate a steady increase in the number of CRISPR-based plant studies, with a sharp rise after 2018, coinciding with the growing acceptance of CRISPR as a reliable genome-editing tool. Figure 1 shows the growth trend in CRISPR applications in plants.

Figure 1

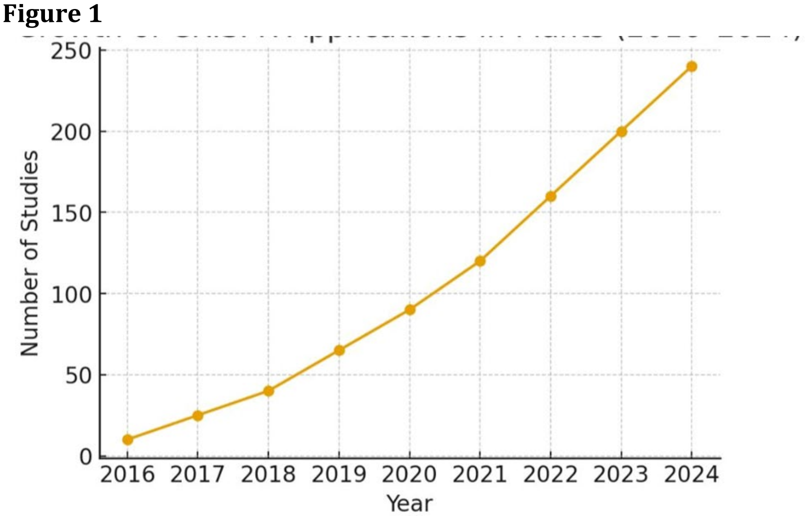

Figure 1 Growth of CRISPR Applications in Plant Science (2016–2024)

In addition to published data, the general workflow for CRISPR-mediated stress tolerance in plants can be summarized as a sequence of key steps:

1) Identification of stress-responsive genes.
2) Design of guide RNAs (gRNAs).
3) Construction of CRISPR/Cas9 vector.
4) Delivery into plant cells (via Agrobacterium or particle bombardment).
5) Screening and validation of edited plants.
6) Evaluation under stress conditions.

DISCUSSION

The findings of this review highlight the transformative potential of CRISPR/Cas9 technology in the development of stress-resilient crops. Compared with traditional breeding and transgenic approaches, CRISPR offers unparalleled precision, rapidity, and flexibility in genome modification. The ability to target specific stress-responsive genes, such as DREB for drought tolerance or SWEET genes for pathogen resistance, has enabled significant improvements in crop resilience under controlled and field conditions.

The increase in CRISPR-based plant studies since 2016 Figure 1 reflects the scientific community's recognition of its utility in addressing global agricultural challenges. The examples summarized in Table 1 demonstrate that gene-edited plants exhibit measurable improvements in stress tolerance, including enhanced water-use efficiency, improved ion homeostasis, and reduced pathogen susceptibility. These outcomes are particularly relevant in the context of climate change, where crops are frequently exposed to multiple stresses simultaneously.

Despite these promising results, several limitations persist. Off-target effects remain a concern, particularly in polyploid crops with complex genomes. Additionally, efficient delivery methods and regeneration systems are required for species that are recalcitrant to transformation. Regulatory frameworks also vary globally; while some countries treat CRISPR-edited crops similarly to conventionally bred plants, others subject them to strict GMO regulations, which could hinder commercialization. Furthermore, public acceptance of gene-edited crops remains an important factor influencing their adoption.

Future research should focus on refining base editing and prime editing technologies to minimize off-target effects, developing universal transformation methods for difficult crops, and expanding the use of multiplex editing to simultaneously regulate complex stress pathways. Integrating CRISPR with other modern tools, such as transcriptomics, proteomics, and nanotechnology-based delivery, may further enhance the efficiency and precision of plant genome engineering.

CONCLUSIONS

CRISPR/Cas9 gene-editing technology has emerged as a powerful tool for developing crops with enhanced resilience to abiotic and biotic stresses. The reviewed studies demonstrate its effectiveness in improving drought tolerance, salinity resistance, and disease defense mechanisms. While technical, regulatory, and societal challenges remain, CRISPR holds significant promise for ensuring global food security in the face of climate change and increasing population demands. With continued advancements in delivery methods, regulatory harmonization, and public awareness, CRISPR has the potential to revolutionize sustainable agriculture and crop improvement strategies worldwide.

ACKNOWLEDGMENTS

None.

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