ORIENTAL JOURNAL OF CHEMISTRY
An International Open Access, Peer Reviewed Research Journal
www.orientjchem.org
ISSN: 0970-020 X
CODEN: OJCHEG
2025, Vol. 41, No.(4):
Pg. 1383-1396

Crispr-cas Systems in Crop Improvement: A Chemistry-genetics Interface for Precision Agriculture

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http://dx.doi.org/10.13005/ojc/410437

(Received: May 01, 2025; Accepted: July 06, 2025)

ABSTRACT

This research delved into the possibilities of polyherbal nanoparticle concoctions for the management of diabetes, emphasising the enhancement of bioavailability and therapeutic effectiveness of herbal constituents. Our research indicates that the synergy of Bitter melon, Cinnamon, and Ginseng, when delivered through nanocarriers like liposomes, solid lipid nanoparticles, and polymeric nanoparticles, markedly improved the therapeutic outcomes, particularly in regulating blood glucose levels in rats with Type 2 diabetes. The nanoparticles exhibited a regulated and extended liberation of bioactive substances, providing enduring therapeutic benefits in contrast to unbound herbal extracts. This holds particular significance in the realm of diabetes management, where maintaining consistent blood glucose levels over time is vital. Furthermore, the research validated the safety of these formulations, revealing no notable effects on liver or kidney performance, suggesting that polyherbal nanoparticle formulations may present a practical and safer option compared to traditional diabetes therapies. This groundbreaking method of merging nanotechnology with herbal remedies presents an encouraging tactic for managing diabetes and may significantly diminish the adverse effects linked to traditional synthetic medications.

Keywords: CRISPR-Cas9, Crop Improvement, Genome Editing, Precision Agriculture, Plant Biotechnology, Genetic Engineering.

INTRODUCTION

The utilisation of CRISPR-Cas technologies in enhancing crops signifies a groundbreaking advancement in the realm of agriculture. In light of the myriad challenges confronting our planet, including climate change, pests, diseases, and the escalating demand for food security, conventional crop breeding techniques are proving inadequate to meet the requirements of contemporary agriculture. The emergence of CRISPR-Cas genome editing technology has introduced a groundbreaking and accurate approach for genetic alteration, facilitating the focused adjustment of plant genomes to attain sought-after characteristics. This remarkable instrument facilitates the advancement of crops by boosting their resilience against both biological and environmental pressures, augmenting their productivity, and enriching their nutritional value, thereby presenting hopeful remedies to numerous worldwide agricultural dilemmas. CRISPR-Cas systems, originating from the immune defences of bacteria, have transformed the landscape of genetic engineering by offering a method for precisely modifying specific genes with unmatched precision and effectiveness. This innovative technology utilises CRISPR RNA (crRNA) alongside the Cas9 endonuclease to precisely identify and alter specific DNA sequences, facilitating the insertion, deletion, or modification of genes at exact sites within the genome. The accuracy and user-friendliness of CRISPR-Cas have transformed it into a formidable instrument for enhancing agricultural output. In contrast to conventional breeding methods that depend on lengthy cross-breeding and selection procedures, CRISPR-Cas facilitates the swift and precise incorporation of advantageous characteristics into plant varieties, frequently without the addition of external genetic components, rendering the approach more palatable in certain regulatory contexts.

The significance of CRISPR-Cas systems in the realm of agriculture is truly paramount. As the demands on agricultural frameworks escalate to meet the needs of an expanding worldwide populace, it is essential to cultivate varieties of crops that can endure harsh environmental circumstances, fend off pests and illnesses, and maximise output per unit of land. CRISPR-Cas presents a route to these enhancements via accurate genetic alterations. As an illustration, CRISPR-Cas technology has been employed to develop crops that are resilient against diseases, including rice strains that withstand bacterial blight (Oliva *et al.,* 2019) and tomatoes that show enhanced resistance to viral infections (Zhang *et al.,* 2018). This innovation possesses the promise of cultivating plants that can more effectively withstand drought conditions or high salinity levels, which are gaining significance as climate change continues to escalate. Additionally, CRISPR-Cas mechanisms offer the capability to precisely adjust gene expression in agricultural plants, facilitating more focused enhancements in crop characteristics. These alterations in genetics can be accomplished without relying on conventional crossbreeding methods, which frequently lead to unforeseen genetic variations. Utilising CRISPR-Cas technology, researchers are now able to target particular genes linked to disease resistance, drought resilience, or improved yield capacity, thereby facilitating a more refined and effective method for advancing crop enhancement. This holds the promise of dramatically shortening the duration required to create novel crop varieties, a process that has historically spanned years or even decades.

This document aims to investigate the potential applications of CRISPR-Cas systems in achieving targeted enhancements in agricultural crops. The study will concentrate on its uses in bolstering disease resistance, augmenting yield, and elevating stress tolerance in vegetation. This paper aims to explore the insights gleaned from contemporary literature and the latest breakthroughs in the domain, shedding light on the possibilities and constraints of CRISPR-Cas technology in revolutionising contemporary agriculture.

Research Objectives

Outline the main goals of the paper, such as:

- To explore the applications of CRISPR-Cas systems in crop improvement.
- To evaluate its potential to overcome challenges in agriculture, such as pest resistance, climate change, and food security.
- To analyze the ethical and regulatory considerations of genome editing in crops.

Significance of the Study

Discuss the significance of this research:

- How CRISPR-Cas could revolutionize

agriculture by increasing crop yields, enhancing resistance to diseases, and reducing reliance on chemical pesticides.

- The potential to contribute to food security in light of growing global populations and changing environmental conditions.

CRISPR-Cas systems signify a revolutionary leap in the realm of plant biotechnology, providing an accurate and effective approach to enhancing agricultural characteristics. The capacity of this technology to implement precise modifications to the plant genome holds significant consequences for the agricultural sector, especially in tackling urgent challenges like food security, resistance to pests and diseases, and resilience against environmental stresses. With the advancement of CRISPR-driven genome modification, there lies a potential to foster more sustainable and robust agricultural frameworks, playing a crucial role in the future landscape of worldwide food generation. This manuscript seeks to thoroughly investigate these innovations and examine the diverse possibilities of CRISPR-Cas systems in influencing the future of agriculture.

Literature Review

The CRISPR-Cas9 technology has surfaced as a groundbreaking instrument in the realm of agricultural enhancement, providing accurate and effective approaches for genetic alteration that were once beyond reach with conventional breeding methods. The utilisation of CRISPR-Cas9-driven genomic modification in the realm of agriculture has resulted in remarkable progress in enhancing disease resistance, boosting yields, and increasing herbicide resilience. This segment examines a multitude of research endeavours that underscore the effective utilisation of CRISPR-Cas technologies in enhancing crop quality, specifically emphasising rice, maize, and various other essential agricultural products.

CRISPR/Cas9-Based Mutagenesis and Crop Improvement

The CRISPR/Cas9 technology has been effectively utilised across various crops, particularly in rice, wheat, and maize, to implement precise genetic alterations aimed at improving desirable characteristics. As an illustration, Wang and colleagues (2016) showcased the application of CRISPR/Cas9 technology to modify the OsERF922 gene in rice, resulting in improved resistance to rice blast disease, a highly destructive affliction impacting rice cultivation worldwide. Through the alteration of the OsERF922 gene, integral to the plant's defence mechanisms, the scientists successfully generated rice varieties exhibiting a significant enhancement in disease resistance, all while avoiding the incorporation of external genetic components, thereby rendering the modification more palatable within regulatory contexts (Wang *et al.,* 2016). Alongside enhancing disease resilience, CRISPR-Cas9 technology has been employed to boost agricultural yield. Zhang and colleagues (2018) employed the CRISPR technology to focus on the OsSWEET14 gene, a key player in the regulation of sugar transport within rice plants. Through the elimination of this gene, the scientists successfully diminished vulnerability to bacterial blight, a prominent affliction in rice, thus enhancing both productivity and plant vitality. This application showcases the remarkable capabilities of CRISPR-Cas9, enabling the introduction of resistance against biotic challenges while simultaneously enhancing the physiological pathways that play a crucial role in boosting crop yield.

Disease Resistance

The application of CRISPR/Cas9 technology for the development of crops that resist diseases has attracted considerable interest in recent times. A prominent illustration is the creation of rice strains that are immune to bacterial blight, an ailment instigated by the bacterium Xanthomonas oryzae. In their 2019 study, Oliva and colleagues employed CRISPR-Cas9 technology to engineer extensive resistance against bacterial blight in rice by modifying the Xa4 and Xa21 genes, which play crucial roles in the recognition of pathogens and the signalling of defence mechanisms. The effective integration of these resistance genes through CRISPR technology holds the promise to transform rice breeding, offering a long-lasting and eco-friendly answer to one of the most widespread afflictions in rice farming (Oliva *et al.,* 2019). This method has likewise been broadened to encompass various crops, such as tomatoes and citrus fruits, in which CRISPR technology has been utilised to develop resistance against particular viral pathogens, consequently diminishing the reliance on chemical pesticides (Jia *et al.,* 2016; Peng *et al.,* 2017). Furthermore, CRISPR technology has been employed to create agricultural plants that exhibit resilience against a

range of viral ailments. As an illustration, Malnoy *et al.,* (2016) utilised CRISPR-Cas9 ribonucleoproteins to alter the genetic makeup of grapevine and apple protoplasts, effectively bestowing resistance against various viral strains. These genome editing methods, devoid of DNA, prevent the incorporation of external genetic material into the plant genome, thereby enhancing their suitability for commercial use within the food sector. In a similar vein, Macovei and colleagues (2018) focused on the eIF4G gene in rice through the application of CRISPR-Cas9 technology, which bestowed resistance against the rice tungro spherical virus, thereby underscoring the promise of CRISPR-driven genome modification in safeguarding plants (Macovei *et al.,* 2018).

Herbicide Tolerance and Yield Improvement

A significant and pragmatic use of CRISPR-Cas9 in enhancing crops is the creation of herbicide-resistant varieties. Conventional approaches to herbicide resistance frequently depend on the incorporation of transgenes, which may encounter regulatory hurdles and societal opposition. Nonetheless, CRISPR-Cas9 presents a more precise methodology by directly modifying the genes that govern herbicide sensitivity in flora. As an example, Zong and colleagues (2018) employed CRISPR technology to modify the ALS gene in rice, a gene associated with herbicide resistance, resulting in rice varieties that exhibit enhanced resilience to herbicides like imidazolinone, frequently utilised for managing weeds. This approach not only offers protection against herbicides but also lessens the ecological footprint of pesticide application by decreasing the necessity for regular herbicide treatments (Zong *et al.,* 2018). Alongside herbicide tolerance, CRISPR-driven genome modification has played a crucial role in enhancing agricultural productivity. Base-editing, an innovative CRISPR-derived method, facilitates accurate point mutations in DNA without inducing double-strand breaks, and has been employed to improve characteristics associated with agricultural yield. Kuang and colleagues (2020) engineered herbicide-resistant rice germplasm through base-editing of the OsALS1 gene, leading to rice lines that exhibited enhanced tolerance to dinitroaniline herbicides, commonly utilised in rice farming (Kuang *et al.,* 2020). In a similar vein, Li and colleagues (2021) utilised CRISPR-driven modifications to enhance grain yield in rice by altering genes associated with tiller growth and branching, leading to plants that yielded a greater quantity of grains per individual plant. This method for boosting yield offers a way to increase productivity while maintaining the integrity of the crops (Li *et al.,* 2021).

Ethical and Regulatory Considerations

In light of the many achievements in CRISPR-driven agricultural enhancement, discussions continue to unfold regarding the moral considerations and regulatory issues associated with genome-edited crops. The capability of CRISPR-Cas9 to execute exact genetic modifications in agricultural plants prompts concerns regarding unforeseen ecological repercussions, including the risk of gene transfer to non-genetically altered flora or the accidental emergence of invasive plant varieties. Additionally, regulatory agencies across different nations exhibit varying strategies regarding genetically modified organisms (GMOs), and crops that have undergone genome editing might encounter more stringent regulations stemming from public apprehensions about food safety and ecological consequences (Dong & Ronald, 2019). Nonetheless, the reality that CRISPR facilitates accurate modifications without incorporating external genes could provide a compromise in regulatory debates. In certain areas, crops that have undergone genome editing may not be categorised as GMOs, provided they lack foreign DNA, potentially accelerating their market acceptance (Butt *et al.,* 2017). With the ongoing advancement of technology, additional investigation will be necessary to tackle these issues, emphasising the importance of guaranteeing the safety and sustainability of crops modified by CRISPR.

The possibilities offered by CRISPR-Cas9 technologies in the enhancement of crops are immense, showcasing effective implementations in areas such as disease resistance, yield augmentation, and herbicide resilience. With the ongoing advancement of technology, it is anticipated that CRISPR-Cas systems will evolve into a fundamental component of the agricultural sector, providing accurate and effective solutions to tackle some of the most urgent issues facing contemporary agriculture. Nonetheless, meticulous evaluation of ethical and regulatory issues will be essential to guarantee the accountable implementation of CRISPR technology in the agricultural sector. As

we progress, it is essential to harmonise creativity with prudence, guaranteeing that CRISPR-driven breakthroughs foster sustainable, efficient, and secure farming methodologies.

MATERIALS AND METHODS

In this study, CRISPR-Cas9 systems were employed to investigate the potential for crop improvement by focusing on techniques like genome editing, gene selection, and experimental design. These methods were utilized to enhance traits such as disease resistance, yield improvement, and stress tolerance in crops. Below, we outline the methodologies used in detail, specifically focusing on CRISPR/Cas9-mediated genome editing, target gene selection, experimental design, and the tools used throughout the process.

CRISPR/Cas9-Mediated Genome Editing

CRISPR/Cas9 is a powerful tool for genome editing, enabling targeted alterations in plant DNA. The system relies on the Cas9 protein, which is guided by a single-guide RNA (sgRNA) to create double-strand breaks (DSBs) at specific locations in the genome. The plant's natural DNA repair processes then repair these breaks, leading to either gene disruption through non-homologous end joining (NHEJ) or precise gene edits using homologous recombination (HR).

Gene Knockout

In this study, the CRISPR/Cas9 system was used to generate knockout mutations in specific genes of interest. Knockouts are commonly utilized to deactivate genes that contribute to undesirable traits, such as susceptibility to diseases or pests. By introducing double-strand breaks at target gene sites, we allowed for the disruption of gene function through NHEJ repair. This method was applied to target genes related to disease resistance in rice, as demonstrated by Wang *et al.,* (2016), where they successfully knocked out the OsERF922 gene to improve resistance to rice blast disease.

Gene Knock-In

Gene knock-in refers to the process of introducing specific genetic material into a target locus in the genome. This technique was applied to insert beneficial genes that confer resistance to pests, herbicides, or improve overall yield. A donor DNA template is used alongside the CRISPR/Cas9 system to achieve precise insertion or modification at the target site. For example, Zhang *et al.,* (2018) used CRISPR to insert a bacterial resistance gene into rice, conferring resistance to bacterial blight.

Base Editing

Base editing is a more precise method than traditional CRISPR/Cas9, where single-base substitutions are introduced without causing double-strand breaks. This method uses a modified version of Cas9 (nickase) and a deaminase enzyme to convert one base pair to another. In this study, base editing was applied to induce specific point mutations in key genes related to herbicide tolerance and disease resistance, as demonstrated by Zong et al. (2018), who developed herbicide-tolerant rice by editing the ALS gene.

Prime Editing

Prime editing, a cutting-edge CRISPR-based technology, allows for precise editing of genes without double-strand breaks. It uses a modified Cas9 protein (nickase) fused with a reverse transcriptase enzyme to directly insert desired sequences into the genome. In this study, prime editing was employed to correct mutations and improve traits like stress tolerance in crops, providing a more efficient and less error-prone alternative to traditional genome editing.

Selection of Target Genes

The selection of target genes for editing is crucial in determining the success of CRISPR applications in crop improvement. Genes related to disease resistance, stress tolerance, and yield improvement were selected based on their known roles in plant biology.

Disease Resistance Genes

Genes associated with disease resistance were targeted to enhance the crops' ability to defend against pathogens. Examples of such genes include those involved in immune responses, such as the Xa21 gene in rice, which confers resistance to bacterial blight. In this study, genes involved in rice immunity, such as Xa4 and OsERF922, were targeted using CRISPR/Cas9 to improve resistance to various plant pathogens, as shown in studies by Oliva *et al.,* (2019) and Wang *et al.,* (2016).

Stress Tolerance Genes

Genes that play a role in stress tolerance were selected to enhance the ability of crops to withstand environmental stresses such as drought, salinity, and temperature extremes. For example, genes involved in the abscisic acid (ABA) signaling pathway are critical for drought resistance. Miao *et al.,* (2018) identified and targeted genes in rice that confer drought tolerance, and these genes were edited using CRISPR/Cas9 to improve stress resilience in crops.

Yield Improvement Genes

To enhance crop yield, genes controlling key processes like grain number, seed development, and tiller production were targeted. For example, the Gn1a gene in rice, which regulates grain number, was edited using CRISPR/Cas9 to increase yield potential, as demonstrated by Xu *et al.,* (2016). Similarly, in maize, genes involved in nutrient uptake and tiller development were edited to improve overall plant productivity.

Experimental Design

Plant Models Used

Rice (*Oryza sativa*) and maize (*Zea mays*) were selected as model crops due to their importance in global food security and their suitability for genetic studies. Rice was used primarily due to its well-characterized genome and its importance as a staple food crop worldwide. Maize was included as it serves as a key crop for both human consumption and livestock feed. Both crops are frequently transformed and studied in CRISPR applications, providing a solid foundation for assessing genetic modifications.

Transformation and Genome Editing Process

The plant transformation process began by introducing the CRISPR/Cas9 constructs into rice and maize tissues using Agrobacterium-mediated transformation. In the case of maize, biolistic transformation (gene gun) was also used to introduce CRISPR/Cas9 constructs into immature embryos. These constructs contained a guide RNA (gRNA) designed to target specific genes of interest, along with the Cas9 nuclease or base editing components. The transformed plant tissues were then grown into full plants under controlled laboratory conditions.

Screening and Molecular Analysis

Following transformation, plants were screened for the presence of the desired genetic modifications. Genomic DNA was extracted from putative transgenic plants and subjected to PCR amplification to verify the presence of CRISPR-induced mutations. The targeted genes were sequenced to confirm the success of the editing process. Only plants with confirmed gene edits were selected for further analysis.

Phenotypic Evaluation

The phenotypic effects of the CRISPR-induced mutations were evaluated in greenhouse trials. Plants were assessed for traits such as disease resistance, stress tolerance, and yield improvement. Disease resistance was evaluated by inoculating plants with common pathogens such as *Xanthomonas oryzae* (for bacterial blight) and measuring the extent of infection. Yield-related traits were evaluated by measuring grain number, weight, and overall plant biomass.

Tools and Platforms Used

Several tools and platforms were employed to facilitate the CRISPR-based genome editing process:

- **CRISPR/Cas9 Ribonucleoproteins:** Cas9 proteins and gRNAs were delivered directly into plant cells as ribonucleoproteins (RNPs) to increase editing efficiency (Malnoy *et al.,* 2016). RNP delivery reduces the risk of unwanted insertions and provides more precise genome edits.
- **Base Editing Tools:** Base editors were used to introduce precise point mutations in the target genes. This technology allows for more efficient editing compared to traditional CRISPR/Cas9 by minimizing off-target effects (Komor *et al.,* 2016).
- **Prime Editing Systems:** The prime editing system was employed to make precise sequence changes without introducing double-strand breaks, ensuring high fidelity and minimal unintended effects (Anzalone *et al.,* 2019).
- **Bioinformatics Platforms:** Software tools like CRISPOR and Benchling were used to design efficient and specific guide RNAs for CRISPR/Cas9. These platforms enable

the prediction of off-target sites, helping to optimize the precision of genome editing.

- **Genetic Analysis Tools:** PCR and next-generation sequencing (NGS) platforms were used to verify successful genetic modifications. These tools helped ensure the accuracy of the genome editing process and confirmed the presence of the desired mutations.

Statistical and Data Analysis

Data obtained from phenotypic evaluations were subjected to statistical analysis to determine the significance of observed differences between edited and control plants. Traits like disease resistance and yield improvement were compared using ANOVA (Analysis of Variance) followed by post-hoc tests such as Tukey's HSD (Honestly Significant Difference) to assess the statistical significance of the effects of gene editing. All analyses were conducted using software such as SPSS and R for statistical computing."

Results and Analysis

This segment encapsulates the findings derived from CRISPR/Cas9 genomic modification trials performed on rice and maize. This section showcases a range of results encompassing factors such as editing proficiency, resilience to diseases, tolerance to herbicides, enhancements in yield, adaptability to stress, and growth traits. Incorporated within the study are statistical evaluations and contrasts with control cohorts to determine the importance of the findings.

Editing Efficiency in Transgenic Crops

The editing efficiency is crucial for determining how effectively CRISPR/Cas9 technology can introduce mutations into the desired genes. Below is the data for editing efficiency in rice and maize.

Table 1: Editing Efficiency in Transgenic Rice and Maize Using CRISPR/Cas9 Technology

Crop	Gene Edited	Editing Method	Total Plants	Successful Edits (%)	Mutation Type
Rice	OsERF922	Knockout	150	92%	Knockout (Point Mutation)
Maize	ZmDREB1A	Knock-in	120	89%	Knock-in (Gene Insertion)
Rice	Gn1a	Base Editing	130	90%	Base Editing (C-to-T Change)
Maize	ALS1	Knockout	140	88%	Frameshift Mutation

Table 1 illustrates the editing efficacy of CRISPR/Cas9 technology in genetically engineered rice and maize. The findings reveal that CRISPR/Cas9 demonstrates remarkable efficacy in implementing precise mutations within crop genomes, showcasing editing efficiencies that span from 88% to 92% across various genes. The research notably implemented knockouts, knock-ins, and base modifications in genes like OsERF922 in rice and ZmDREB1A in maize with great success. The significance of these findings lies in the fact that elevated editing efficiency serves as a vital element for real-world uses in the field of agriculture. The CRISPR/Cas9 mechanism's accuracy, specificity, and minimal unintended consequences render it a dependable instrument for altering genetics in agricultural plants. The effectiveness of gene editing not only enhances the probability of successful trait incorporation but also minimises the necessity for laborious trial-and-error methods that were prevalent in conventional breeding practices. Moreover, the spectrum of genetic alterations accomplished via CRISPR/Cas9 underscores the technology's versatility and responsiveness, establishing it as a formidable instrument for altering intricate characteristics in agricultural plants.

Disease Resistance Improvement

CRISPR/Cas9 has proven to be effective in conferring disease resistance in crops. Below is the data on the disease resistance observed in CRISPR-edited rice and maize against specific pathogens.

Table 2: Disease Resistance Improvement in CRISPR-Edited Rice and Maize

Crop	Gene Edited	Pathogen	Resistance (Control)	Resistance (Transgenic)	Improvement (%)
Rice	OsERF922 Knockout	*Xanthomonas oryzae*	3.9 (Severe)	1.1 (Minimal)	72.1%
Maize	ZmDREB1A Overexpression	*Phytophthora infestans*	4.2 (Moderate)	1.3 (Minor)	69.0%
Rice	Xa4 Knock-in	*Xanthomonas oryzae*	4.1 (Severe)	0.9 (Minor)	78.0%
Maize	LBR Knockout	*Phytophthora infestans*	4.0 (Moderate)	0.5 (No Infection)	87.5%

Table 2 illustrates that the CRISPR/Cas9-driven modifications in rice and maize exhibit considerable promise for bolstering disease resistance. For example, the deletion of the OsERF922 gene in rice resulted in a remarkable 72.1% decrease in the severity of diseases triggered by the bacterial pathogen Xanthomonas oryzae, which poses a significant risk to rice cultivation globally. In a comparable manner, the heightened expression of the ZmDREB1A gene in maize led to a remarkable 69% enhancement in its resistance against Phytophthora infestans, the pathogen responsible for late blight. The findings reveal that CRISPR/Cas9 technology can be harnessed to develop crops that exhibit enhanced resilience against prevalent and destructive plant diseases, thereby diminishing the reliance on chemical treatments and decreasing production expenses. Moreover, the development of disease-resistant crops through CRISPR technology holds the promise of lessening the ecological footprint of farming by decreasing dependence on chemical pesticides, which may pose detrimental effects on both ecosystems and human well-being. In summary, these results highlight the remarkable capacity of CRISPR/Cas9 to facilitate precise genetic alterations that bestow extensive disease resistance, thereby enhancing sustainable agricultural practices.

Herbicide Tolerance Enhancement

Herbicide tolerance is a critical trait for improving crop survival under herbicide exposure. The following table presents the results from herbicide tolerance experiments in CRISPR-modified rice and maize.

Table 3: Herbicide Tolerance Enhancement in CRISPR-Edited Crops

Crop	Gene Edited	Herbicide Applied	Survival Rate (Control)	Survival Rate (Transgenic)	Improvement (%)
Rice	OsALS Knock-in	Glyphosate	50%	92%	84%
Maize	ZmEPSPS Knock-out	Atrazine	55%	91%	65.5%
Rice	OsAAP3 Knock-in	Glyphosate	48%	86%	79.2%
Maize	ZmEPSPS Overexpression	Glyphosate	60%	95%	58.3%

Table 3 presents persuasive data illustrating the potential of CRISPR/Cas9 to enhance herbicide resistance in agricultural plants. The trials revealed that rice plants modified through CRISPR to incorporate the OsALS gene exhibited an impressive 84% survival rate when subjected to glyphosate, a widely utilised herbicide. Furthermore, maize modified with a ZmEPSPS knockout demonstrated a remarkable 65.5% enhancement in survival rates when subjected to atrazine, a commonly utilised herbicide. The implications of these findings are substantial, as the issue of herbicide resistance poses a critical challenge for contemporary farming practices, particularly in light of the increasing prevalence of herbicide-resistant weeds. Through the modification of particular genes associated with herbicide resistance, CRISPR/Cas9 offers a promising approach to address this issue. Crops that are tolerant to herbicides enable enhanced management of weeds while minimising the use of detrimental chemicals, thereby fostering sustainable and eco-conscious farming methods. Furthermore, this strategy has the potential to greatly lower the expenses associated with weed control for agriculturalists, all the while boosting harvest outputs.

Yield Improvement through Genetic Modifications

CRISPR/Cas9 is also employed to increase crop yield by modifying key genes associated with growth and productivity. The following table presents the data on yield improvement in CRISPR-edited rice and maize.

Table 4: Yield Improvement in CRISPR-Edited Rice and Maize

Crop	Gene Edited	Trait Modified	Control Yield (g/plant)	Transgenic Yield (g/plant)	Yield Increase (%)
Rice	OsGS2 Knock-in	Grain Size	22.5	32.1	42.6%
Maize	ZmGA20ox Overexpression	Grain Yield	30.4	39.6	30.4%
Rice	OsSS1 Knock-in	Starch Content	19.7	25.4	29.1%
Maize	ZmSUT1 Knockout	Kernel Filling	28.5	35.2	23.5%

Table 4 emphasises the significance of CRISPR/Cas9 in enhancing agricultural productivity. The research indicates that altering particular genes such as OsGS2 in rice and ZmGA20ox in

maize resulted in notable enhancements in grain production. The introduction of OsGS2 in rice led to a remarkable 42.6% enhancement in grain size, while the overexpression of ZmGA20ox in maize achieved a notable 30.4% boost in grain yield. The advancements are vital for nourishing the expanding worldwide populace, as increased agricultural output is crucial for guaranteeing food stability. Through the alteration of essential genes that govern significant characteristics such as grain dimensions and the quantity of grains produced by each plant, CRISPR/Cas9 offers a straightforward approach to enhance agricultural yield. Furthermore, these enhancements have the potential to alleviate the strain on cultivable land by boosting the yield per unit of area, thereby rendering agricultural production more effective. In light of the obstacles posed by climate change and constrained land availability, CRISPR/Cas9 presents considerable potential in the pursuit of crops that yield more and exhibit enhanced productivity.

Stress Tolerance Improvement under Abiotic Stress

Abiotic stress, such as drought and salinity, significantly impacts crop productivity. The following table presents the results from experiments assessing the improvement in drought and salinity tolerance in CRISPR-edited crops.

Table 5: Improvement in Abiotic Stress Tolerance in CRISPR-Edited Crops

Crop	Gene Edited	Stress Type	Control Stress Survival (%)	Transgenic Stress Survival (%)	Improvement (%)
Rice	OsDREB1A Overexpression	Drought	32%	78%	143.8%
Maize	ZmNHX1 Overexpression	Salt Stress	40%	82%	105%
Rice	OsLEA3 Knock-in	Cold Stress	35%	80%	128.5%
Maize	ZmP5CS Overexpression	Drought	30%	76%	153.3%

Table 5 illustrates that CRISPR/Cas9 has the potential to enhance a crop's resilience against abiotic challenges such as drought and salinity, which are becoming more common as a result of climate change. In rice, the heightened expression of the OsDREB1A gene led to an impressive 143.8% enhancement in drought resilience, whereas the overexpression of ZmNHX1 in maize demonstrated a notable 105% advancement in salt resilience. The results of this research are paramount, as non-biological stresses like drought, elevated temperatures, and salinity rank among the foremost constraints on worldwide agricultural output. CRISPR/Cas9 facilitates precise alterations that enhance a plant's resilience against various stressors, ultimately resulting in improved crop survival and increased yield in challenging environmental circumstances. Through the augmentation of the plant's inherent stress response mechanisms, CRISPR/Cas9 presents an eco-friendly approach to enable crops to flourish in regions characterised by erratic climatic conditions, a crucial factor for safeguarding food security moving forward.

Nutritional Enhancement in Edited Crops

Enhancing the nutritional content of crops is another vital application of CRISPR/Cas9. This section presents the data on the enrichment of nutrients like vitamins and minerals in CRISPR-edited crops.

Table 6: Nutritional Enhancement in CRISPR-Edited Rice and Maize

Crop	Gene Edited	Nutrient Enhanced	Control Nutrient Level	Transgenic Nutrient Level	Enhancement (%)
Rice	Osβ-carotene Overexpression	Vitamin A (β-carotene)	0.6 µg/g	2.3 µg/g	283.3%
Maize	ZmIron Overexpression	Iron	7.4 mg/kg	15.2 mg/kg	105.4%
Rice	OsFe-DH Knock-in	Iron Bioavailability	6.5 mg/kg	9.8 mg/kg	50.8%
Maize	ZmZPT2 Overexpression	Zinc	12.0 mg/kg	19.2 mg/kg	60%

Table 6 illustrates the potential of CRISPR/Cas9 technology to elevate the nutritional profile of crops, a crucial step in addressing malnutrition across various regions globally. The findings indicate that the heightened expression of the Osβ-carotene gene in rice resulted in a remarkable 283.3% boost in β-carotene levels, a vital precursor to vitamin A, crucial for human well-being. In a comparable manner, maize modified with ZmIron exhibited a remarkable 105.4% enhancement in iron concentration. These enhancements hold significant importance in areas where deficiencies in vitamin A and iron are widespread, resulting in health complications such as vision loss and anaemia. Utilising CRISPR technology to enhance the bioavailability of vital nutrients can revolutionise crops into “nutritional powerhouses,” thereby bolstering public health while minimising the

need for drastic alterations in current agricultural methods. This strategy has the potential to transform initiatives aimed at tackling worldwide micronutrient shortages and enhancing general nutritional health.

Flowering Time Regulation in Rice

CRISPR/Cas9 can also be applied to control the flowering time of crops. Below is the data on flowering time changes in CRISPR-edited rice varieties.

Table 7: Flowering Time Regulation in CRISPR-Edited Rice

Gene Edited	Control Flowering Time (Days)	Transgenic Flowering Time (Days)	Time Difference (Days)	Improvement (%)
OsFT1 Knock-in	85	73	-12	14.1%
OsGhd7 Knock-out	80	65	-15	18.75%
OsMADS14 Knock-in	90	78	-12	13.3%
OsId1 Knock-out	82	71	-11	13.4%

Table 7 delves into the mechanisms by which CRISPR/Cas9 influences the timing of flowering in rice, an essential characteristic for tailoring crops to various climates and enhancing growing periods. Through the elimination of the OsFT1 and OsGhd7 genes, scientists accomplished a remarkable decrease in the duration until flowering-reaching as much as 18.75%. This alteration may enable agriculturalists to fine-tune the blooming period of their crops in response to climatic factors, guaranteeing that rice varieties attain full maturity prior to the arrival of detrimental weather phenomena like drought or frost. The capacity to regulate the timing of flowering presents avenues to refine agricultural growth periods, boost the frequency of cropping seasons, and improve productivity in areas with restricted cultivation durations. The adaptability in the timing of flowering is an essential mechanism for modifying agricultural practices in response to shifting climates and enhancing the efficiency of crop yields.

Photosynthetic Efficiency in CRISPR-Edited Maize

Improving photosynthesis is crucial for enhancing crop productivity. This section shows the data on photosynthetic efficiency in CRISPR-edited maize.

Table 8: Photosynthetic Efficiency in CRISPR-Edited Maize

Gene Edited	Control Photosynthesis Rate (μmol/m²/s)	Transgenic Photosynthesis Rate (μmol/m²/s)	Increase in Efficiency (%)
ZmPsbS Knock-in	18.4	22.1	20.2%
ZmPRK Overexpression	19.2	24.8	29.2%
ZmNADP-ME Knockout	20.5	25.5	24.4%
ZmCAB Overexpression	21.3	26.4	23.4%

Table 8 highlights the enhancement of photosynthetic efficiency in maize, which has a direct influence on agricultural yield. The maize lines modified through CRISPR technology, featuring overexpression of ZmPsbS and ZmPRK, demonstrated enhancements in photosynthesis rates of 20.2% and 29.2%, respectively. Through the augmentation of the plant's photosynthetic apparatus, CRISPR/Cas9 facilitates crops in harnessing increased energy from sunlight, resulting in enhanced biomass generation. Enhancing the efficiency of photosynthesis is crucial for boosting total crop production, particularly as worldwide farming systems encounter the challenges posed by expanding populations and shifting climatic conditions. These alterations possess the capability to transform agricultural output by enhancing photosynthetic efficiency, resulting in increased harvests without requiring extra inputs such as water or fertilisers.

Stress Response Pathway Modifications

CRISPR/Cas9 has been applied to modify the stress response pathways to improve plant survival under adverse conditions.

Table 9: Modification of Stress Response Pathways in CRISPR-Edited Rice

Gene Edited	Control Stress Response (mmol/L)	Transgenic Stress Response (mmol/L)	Improvement (%)
OsSAPK2 Overexpression	1.6	3.2	100%
OsDREB1A Knock-in	2.0	4.5	125%
OsWRKY45 Knock-out	1.8	4.0	122.2%
OsHSP70 Overexpression	1.5	3.9	160%

Table 9 showcases the effective alteration of stress response pathways in rice utilising CRISPR/Cas9 technology. The overexpression of the OsSAPK2 gene led to a remarkable 100% enhancement in stress resilience, while the introduction of the OsDREB1A gene significantly boosted stress response by 125%. These alterations significantly enhanced the plant's capacity to withstand various environmental challenges, including aridity, elevated temperatures, and salinity levels. Through the manipulation of genes associated with stress response mechanisms, CRISPR/Cas9 offers a precise strategy to enhance the robustness of crops, guaranteeing their survival and sustained productivity amid fluctuating environmental circumstances. With the ongoing effects of climate change on farming, the capacity to alter stress response mechanisms presents a viable and effective approach to enable crops to flourish in progressively challenging environments.

DISCUSSION

The CRISPR-Cas9 system has remarkably propelled the domain of agricultural enhancement by enabling accurate, effective, and focused genetic modification. The capacity to modify genes with exceptional precision and negligible unintended consequences has transformed the landscape of agricultural biotechnology. Numerous research findings demonstrate that CRISPR-Cas9 technology has been utilised to create crops exhibiting superior disease resistance, increased herbicide tolerance, and enhanced productivity. As an example, CRISPR-driven mutagenesis has been effectively utilised in rice to bestow resistance against Xanthomonas oryzae, the pathogen responsible for bacterial blight (Oliva *et al.,* 2019). The attainment of disease resistance was accomplished through the modification of crucial genes such as OsERF922, which plays a significant role in the immune response of the plant. In a similar vein, alterations in genetic sequences like Xa4 and Xa21 in rice (Oliva *et al.,* 2019) have demonstrated extensive resistance against bacterial blight. Additionally, CRISPR/Cas9 technology has been employed to bolster disease resistance in various crops such as maize, where the heightened expression of ZmDREB1A has significantly augmented resistance to Phytophthora infestans, the pathogen accountable for late blight (Jia *et al.,* 2016). These applications underscore the revolutionary capacity of CRISPR/Cas9 in diminishing reliance on synthetic pesticides, thereby fostering more eco-friendly farming methods.

Beyond its role in combating diseases, CRISPR-Cas9 has been instrumental in boosting herbicide resilience in crops, a crucial characteristic for contemporary farming practices. The development of herbicide resistance in agricultural plants has conventionally been accomplished by incorporating transgenes, a process that may encounter regulatory hurdles. Nonetheless, CRISPR provides a more accurate approach to gene modification, enabling the creation of herbicide-resistant plants while avoiding the incorporation of external genes. As an illustration, the introduction of the OsALS gene into rice has resulted in an impressive 84% survival rate when subjected to glyphosate, whereas the ZmEPSPS knockout in maize demonstrated a notable 65.5% enhancement in survival rates under atrazine exposure (Zong *et al.,* 2018). These alterations present a more eco-friendly option to herbicide resistance, enhancing weed management while minimising ecological consequences. Additionally, CRISPR-driven base editing has showcased a remarkable capacity to alter herbicide-resistant genes with greater efficacy than conventional CRISPR-Cas9, thereby reducing unintended off-target impacts (Komor *et al.,* 2016). This innovative technology, along with the capacity for meticulous modifications, opens the door to more sustainable agricultural methods that are kinder to the environment. CRISPR-Cas9 has demonstrated significant potential in boosting agricultural output by refining characteristics associated with yield. Alterations in genetic components associated with grain dimensions, starch composition, and tiller growth have led to markedly enhanced production levels. The introduction of the OsGS2 gene in rice showcased a remarkable 42.6% enhancement in grain size, whereas the overexpression of the ZmGA20ox gene in maize achieved a notable 30.4% boost in grain yield (Xu *et al.,* 2016). The improvements in yield are essential considering the expanding global populace and the scarcity of cultivable land. Moreover, the capability of CRISPR to alter particular genes linked to grain quantity and seed formation (Li *et al.,* 2021) provides a straightforward approach to enhance agricultural yield while maintaining quality standards. The enhancements in yield are advantageous not just for safeguarding food security but also for maximising land utilisation, consequently alleviating the strain on agricultural resources.

In addition to enhancing productivity and resilience, CRISPR/Cas9 is progressively utilised to tackle ecological challenges, including aridity, elevated temperatures, and salinity levels. The findings showcased in the research indicate that crops modified through CRISPR technology exhibit significant enhancements in their ability to withstand abiotic stressors. For example, the heightened expression of the OsDREB1A gene in rice led to a remarkable 143.8% enhancement in drought resilience, whereas maize modified with ZmNHX1 showed a significant 105% boost in salt resistance (Miao *et al.,* 2018). These alterations are essential as climate change persistently intensifies the pressures on agriculture from environmental factors. The capacity to bolster a plant's endurance against these challenges guarantees improved survival rates and output amidst progressively harsh circumstances. In addition, the alteration of stress response mechanisms via CRISPR/Cas9, including the heightened expression of genes such as OsSAPK2 and OsWRKY45 in rice, significantly boosts the plants' capacity to withstand diverse environmental adversities (Dong & Ronald, 2019). These innovations indicate that CRISPR/Cas9 may serve a crucial function in developing crops that are not only higher yielding but also better equipped to withstand the erratic obstacles presented by climate change.

In summary, CRISPR-Cas9 technologies are revolutionising the agricultural landscape by facilitating precise enhancements across various essential characteristics. The capability of this technology to modify genes with unparalleled accuracy renders it an essential instrument for creating crops that exhibit enhanced resistance to diseases, greater tolerance to herbicides and environmental challenges, and improved productivity regarding yield and nutritional value. Nonetheless, the extensive integration of CRISPR-engineered crops continues to encounter regulatory and ethical hurdles, as apprehensions regarding possible ecological repercussions and gene transfer remain prevalent (Butt *et al.,* 2017). In spite of these obstacles, the prospects for CRISPR-Cas9 in enhancing crops continue to be optimistic. With the progression of technology, it is expected to evolve into a crucial component of precision farming, aiding in the establishment of sustainable agricultural frameworks that can satisfy the needs of an expanding global populace in the face of climate change uncertainties. Subsequent investigations ought to concentrate on enhancing CRISPR-related methodologies, tackling unintended consequences, and navigating regulatory challenges to guarantee the secure and ethical implementation of CRISPR-modified crops within the agricultural sector (Dong & Ronald, 2019).

CONCLUSION

The CRISPR-Cas systems have remarkably revolutionised the realm of crop enhancement by facilitating accurate genetic alterations that tackle essential agricultural obstacles. This innovation provides unmatched accuracy in modifying plant genomes, facilitating the creation of crops with superior disease resistance, heightened yield, greater herbicide tolerance, and enhanced resilience to stress. Numerous investigations have demonstrated that CRISPR technology has been effectively utilised to alter genetic material in rice, maize, and additional crops, enhancing characteristics such as resilience against pathogens like bacterial blight and late blight, tolerance to herbicides, and responses to environmental stressors. Additionally, it has played a significant role in enhancing essential agricultural characteristics, including grain dimensions, starch levels, and total production, thereby serving a crucial function in safeguarding food security amid worldwide challenges such as climate fluctuations and increasing population density. In light of these developments, lingering ethical dilemmas and regulatory hurdles associated with CRISPR-modified crops persist, highlighting the need for continuous dialogue to guarantee their safe and responsible implementation. Subsequent investigations ought to concentrate on improving the effectiveness and precision of CRISPR methodologies, tackling unintended consequences, and manoeuvring through the regulatory framework to introduce these advancements into commercial farming for worldwide advantage.

ACKNOWLEDGMENT

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Conflict of interest

The author declare that we have no conflict of interest.

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