



CRISPR-Cas Genome Editing in Plants: Revolutionizing Precision Agriculture and Plant Biotechnology

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Abstract

Plant biotechnology has been transformed by CRISPR-Cas genome editing, which has improved crops with previously unheard-of accuracy and efficiency. Base editing, prime editing, and enhanced delivery systems are the most recent CRISPR applications for plant genome editing. Advances like CRISPR-Act3.0 and HDR-mediated precise insertions have expanded plant genetic engineering tools. We discuss successful applications in rice, wheat, maize, and woody species that have resulted in improvements in disease resistance, stress tolerance, and yield. Despite delivery efficiency and off-target effects, CRISPR technology suggests sustainable global agriculture and climate adaptation.

Keywords: CRISPR-Cas9, plant genome editing, crop improvement, precision breeding.

1 Introduction

CRISPR-associated (Cas) systems have transformed plant genome editing, offering researchers powerful

tools for precise genetic modifications. Since the initial demonstration of CRISPR-Cas9 in plant cells, this technology has quickly transformed from a lab curiosity to a key element of modern plant biotechnology, allowing for targeted enhancements in crop traits that were once unattainable through traditional breeding methods [12, 21]. Targeted gene knockout, sequence-specific mutagenesis/integration, and transcriptional control of target genes have revolutionized plant research with CRISPR. Cas9 targeting efficiency and capacity have been improved, gRNA expression optimized, and new Cas9 variants with high fidelity and alternative PAM specificities engineered (Figure 1). Bioinformatic tools facilitate the design of specific gRNAs and the assessment of off-target effects. The flexible CRISPR/Cas9 system allows precise crop breeding by engineering plant genomes [6]. Numerous CRISPR/Cas9 vectors are available for use in various plant species [6]. The technology allows targeted mutagenesis, base editing, HDR, and transcriptional regulation in plants, advancing basic plant science and crop improvement [25]. These advances have created new opportunities to tackle global issues like food security, climate adaptation, and sustainable agriculture. The advancement of CRISPR applications in plants is especially significant in woody species, where conventional breeding methods are constrained by



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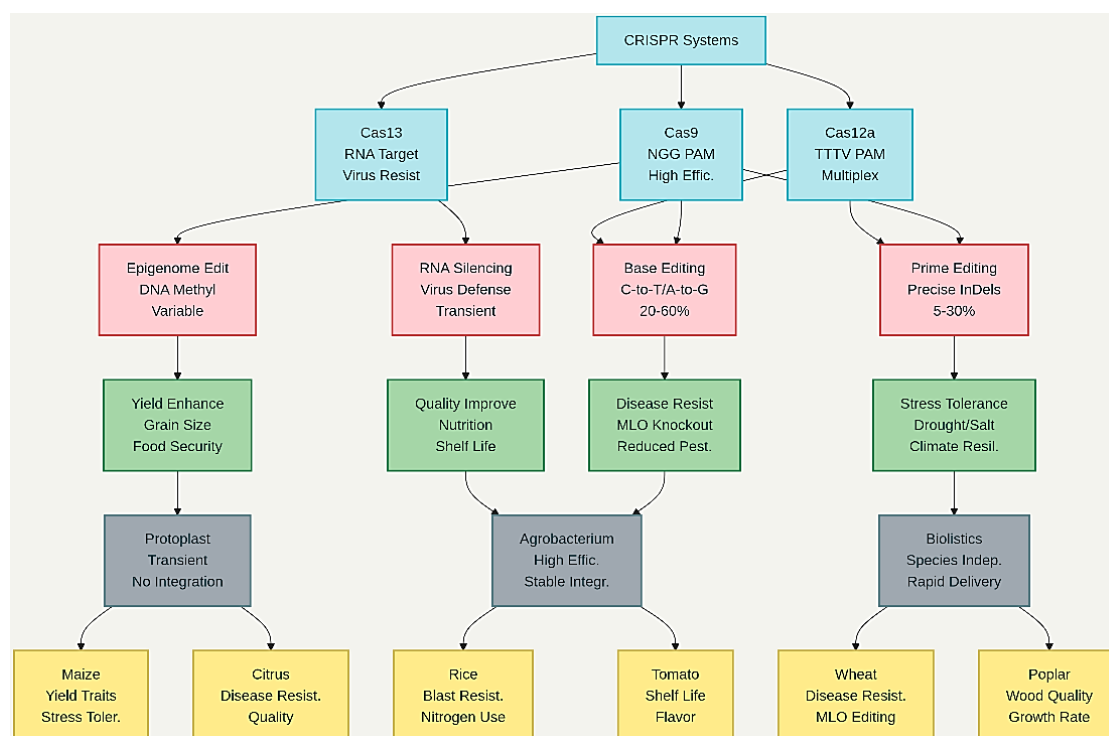


Figure 1. CRISPR-Cas Systems and Applications in Plant Genome Editing. Comprehensive overview showing different CRISPR technologies, advanced editing methods, plant-specific applications including disease resistance and stress tolerance, and delivery systems used in plant genome editing. The figure illustrates the versatility of CRISPR systems (Cas9, Cas12a, Cas13) and their specific applications in crop improvement.

extended generation times and intricate genetics. Recent research has shown effective CRISPR-mediated genome editing in poplar trees, reaching precise homology-directed repair (HDR) efficiencies of up to 48% by concurrently enhancing HDR factors and inhibiting non-homologous end joining pathways [16]. This advancement has substantial ramifications for forestry and biomass production, illustrating the extensive applicability of CRISPR technology among plant species.

2 CRISPR Systems and Mechanisms in Plant Genome Editing

2.1 CRISPR-Cas9: The Foundation Technology

The most popular genome editing tool in plants is still the CRISPR-Cas9 system, which is favored for its simplicity, high efficiency, and broad compatibility across plant species. The system comprises a single guide RNA (sgRNA) that guides the Cas9 endonuclease to create targeted double-strand breaks at specific genomic loci in plants. Implementing Cas9-mediated editing has been accomplished across a diverse range of plant species, from model organisms like *Arabidopsis* to major crops including rice, wheat, maize, and tomato [17, 21]. Recent advancements in Cas9 engineering have addressed a primary concern

in plant genome editing applications by producing high-fidelity variants that exhibit diminished off-target activity. The enhanced guide RNA design algorithms, in conjunction with superior Cas9 variants, have significantly enhanced the precision of plant genome editing, increasing HDR efficiency, decreasing total polymorphisms while preserving high on-target efficiency (Figure 2) [16].

2.2 Advanced CRISPR Systems: Cas12a and Cas13

The diversification of the CRISPR toolkit beyond Cas9 has afforded plant researchers supplementary alternatives for targeted applications. Cas12a systems recognize AT-rich PAM sequences, self-process guide RNAs, and improve multiplexing. These properties make Cas12a ideal for simultaneous gene editing, which is essential for complex trait engineering in crops [22]. Cas13 systems, which target RNA instead of DNA, offer new potential for plant virus resistance and gene expression regulation post-transcriptionally. Cas13's RNA-targeting ability has helped establish resistance to RNA viruses, which threaten global crop production. Targeted genome degradation has been shown to defend against multiple plant RNA viruses [1].

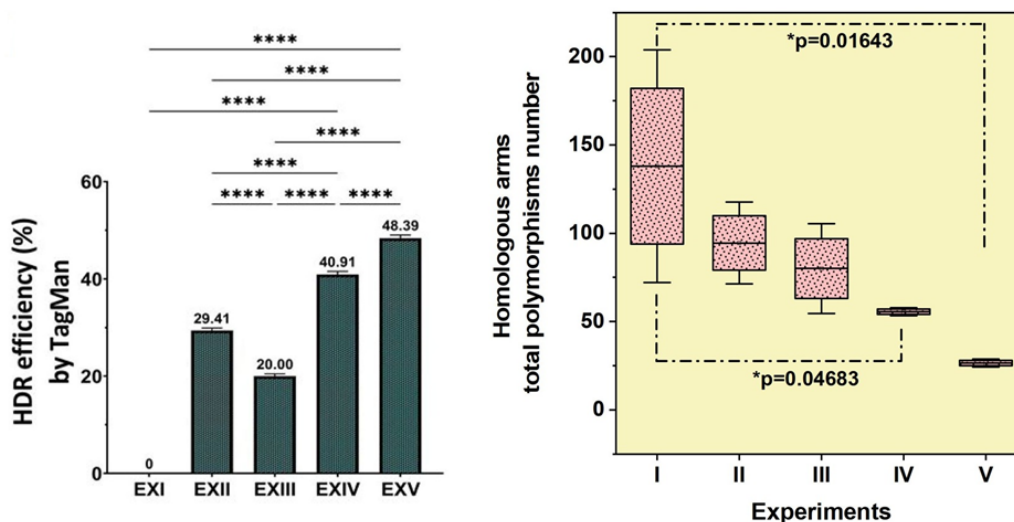


Figure 2. HDR efficiency was calculated as a percentage based on the fully edited replicate numbers. Events that occurred at the ExV were found to have substantially higher HDR efficiency (percentage) than events that occurred elsewhere, at approximately 48%. The error bars indicate the standard deviation; **** $P \leq 0.0001$. The box-and-whisker plot indicated that most polymorphisms occurred in the homology arms via ExI, with a greater frequency than those in ExV and IV. Error bars denote the standard error [16].

2.3 Base Editing: Precision Without Double-Strand Breaks

Base editing, which allows precise single-nucleotide changes without double-strand breaks, is a major CRISPR advancement. CBEs convert C to T, while ABEs convert A to G. These systems are particularly useful in plant applications that require precise point mutations to change plant traits [11]. Base editing has achieved C-to-T substitutions with nitrogen use efficiency greater than 35% in rice by targeting the NRT1.1B gene [3]. Base editing has also modified disease-resistant quantitative trait loci (QTLs) in wheat, offering new ways to strengthen crops against pathogens.

2.4 Prime Editing: The Search-and-Replace Technology

Prime editing is a versatile CRISPR-based technique that allows targeted insertions, deletions, and all base-to-base conversions without double-strand breaks or donor DNA templates. Cas9 nickase linked to reverse transcriptases are directed by a prime editing guide RNA (pegRNA) with targeting and template data [2]. Recently implemented prime editing in plants has shown accurate trait alteration. Prime editing has modified the Waxy gene in rice with 15 to 30% efficiency, demonstrating the system's potential for precise starch quality enhancement [14]. Prime editing is ideal for correcting agricultural mutations or incorporating beneficial variants from

wild crop relatives because of its adaptability.

3 Applications in Major Crop Species

3.1 Rice: A Model for CRISPR Innovation

Rice has functioned as a principal model for the development and optimization of CRISPR applications in plants, with numerous successful implementations showcasing the technology's potential for enhancing crops. Enhancing disease resistance has been a primary objective, successfully targeting SWEET genes to improve resistance to bacterial blight induced by *Xanthomonas oryzae* (Table 1). These applications have attained editing efficiencies of 25 to 40% and exhibited substantial enhancements in pathogen resistance in field conditions [27]. The enhancement of nutritional quality constitutes a notable application domain in rice. The CRISPR-mediated enhancement of nitrogen use efficiency via base editing of the NRT1.1B gene has shown potential for the sustainable intensification of rice production (Table 1). Analogously, alterations to starch biosynthesis genes have facilitated the creation of rice varieties with enhanced processing attributes and nutritional qualities.

3.2 Wheat: Addressing Complex Polyploid Genomes

Wheat poses distinct challenges for genome editing owing to its polyploid structure, with most cultivated varieties possessing six gene copies (allohexaploid).

Table 1. Comprehensive Overview of CRISPR Applications in Major Plant Species. This table presents successful CRISPR applications across 18 different crop species, showing target genes, editing systems, delivery methods, efficiencies, and agricultural impacts. The data show the broad applicability of CRISPR technology across diverse plant species and traits.

Crop Species	Target Gene/Trait	CRISPR System	Editing Type	Delivery Method	Editing Efficiency (%)	Phenotypic Outcome	Agricultural Impact	Reference Example
Rice (Oryza sativa)	OsSWEET13/14 (Bacterial blight resistance)	Cas9	Gene knockout	Agrobacterium	25-40	Enhanced resistance to Xanthomonas	Reduced pesticide use	[27]
Rice (Oryza sativa)	OsNRT1.1B (Nitrogen efficiency)	Base editing (ABE)	C-to-T substitution	Agrobacterium	35-50	Improved nitrogen utilization	Sustainable intensification	[14]
Rice (Oryza sativa)	Waxy gene (Starch quality)	Prime editing	G-to-A substitution	Agrobacterium	15-30	Altered starch properties	Improved processing quality	[14]
Wheat (Triticum aestivum)	TaMLO-A1/B1/D1 (Powdery mildew resistance)	Cas9	Multiplex knockout	Biolistics	20-35	Broad-spectrum mildew resistance	Chemical-free disease control	[23]
Wheat (Triticum aestivum)	TaGW2 (Grain weight)	Cas9	Gene knockout	Agrobacterium	30-45	Increased grain size and weight	Enhanced yield potential	[26]
Maize (Zea mays)	ZmTMS5 (Male sterility)	Cas9	Gene knockout	Agrobacterium	40-60	Cytoplasmic male sterility	Hybrid seed production	[5]
Maize (Zea mays)	ZmIPT2 (Drought tolerance)	Cas12a	Gene knockout	Biolistics	25-35	Enhanced drought tolerance	Climate resilience	[20]
Tomato (Solanum lycopersicum)	SIMLO1 (Powdery mildew resistance)	Cas9	Gene knockout	Agrobacterium	70-85	Complete mildew resistance	Organic farming compatibility	[17]
Tomato (Solanum lycopersicum)	STAY-GREEN (Shelf life)	Cas9	Gene knockout	Agrobacterium	45-65	Extended shelf life	Reduced food waste	[24]
Potato (Solanum tuberosum)	StDMR6-1 (Late blight resistance)	Cas9	Gene knockout	Agrobacterium	20-35	Improved late blight resistance	Sustainable disease management	[10]
Soybean (Glycine max)	GmFATB1A/1B (Oil composition)	Cas9	Gene knockout	Agrobacterium	30-50	Modified fatty acid composition	Healthier oil profile	[7]
Citrus (Citrus sinensis)	CsLOB1 (Citrus canker resistance)	Cas9	Gene knockout	Agrobacterium	15-25	Reduced canker susceptibility	Citrus industry protection	[18]
Poplar (Populus tremula)	MKK2 (Stress responses)	Cas9	Gene insertion (HDR)	Agrobacterium	48 (HDR)	Enhanced stress responses	Forestry applications	[16]
Grapevine (Vitis vinifera)	VvMLO7 (Powdery mildew resistance)	Cas9 RNP	Gene knockout	Protoplast	15-May	Potential mildew resistance	Premium wine quality	[19]
Cucumber (Cucumis sativus)	eIF4E (Virus resistance)	Cas9	Gene knockout	Agrobacterium	60-80	Virus resistance	Virus-free production	[4]
Cassava (Manihot esculenta)	Cassava mosaic disease resistance	Cas9	Gene knockout	Agrobacterium	35-50	Reduced viral symptoms	Food security in Africa	[13]
Barley (Hordeum vulgare)	HvMLO (Powdery mildew resistance)	Cas9	Gene knockout	Agrobacterium	40-60	Durable mildew resistance	Sustainable barley production	[8]
Sugarcane (Saccharum officinarum)	ScSPS1 (Sugar metabolism)	Cas9	Gene knockout	Biolistics	20-30	Altered sugar accumulation	Bioenergy applications	[9]

CRISPR technology has effectively resolved these challenges through multiplexed editing methods that concurrently target all gene copies. The most significant achievement has been the targeting of MLO genes to confer powdery mildew resistance, wherein the concurrent editing of all six alleles yielded broad-spectrum disease resistance [23]. Yield enhancement applications in wheat have concentrated on the determinants of grain size and weight. The CRISPR-mediated knockout of the TaGW2 gene has led to enhanced grain size and weight, exhibiting editing efficiencies of 30-45% and notable advancements in yield potential. These applications underscore the

capability of CRISPR technology to tackle intricate agronomic characteristics in polyploid crop species.

3.3 Woody Plants: Overcoming Long Generation Times

Woody plants pose specific challenges for genetic enhancement owing to their extended juvenile periods and intricate genetic structures. The ability of CRISPR technology to accelerate genetic improvements that would otherwise take decades to achieve through traditional breeding methods has been demonstrated by recent advances in CRISPR applications for woody species [16]. Poplar has proven to be an amenable species for CRISPR-mediated genome editing. Stress

response genes have been successfully targeted in this species, and the results have demonstrated that HDR-mediated insertions can be accomplished with impressive precision and efficiency. The simultaneous overexpression of HDR enhancer factors, such as CtIP and MRE11, and the suppression of NHEJ cofactors, like XRCC4, have resulted in HDR efficiencies of up to 48%. This represents a significant breakthrough in the field of precise gene targeting in woody species [16]. Integrating the BleoR-MKK2 fusion protein has been shown to improve poplar's response to salt stress, which provides an increased resilience to environmental stresses.

4 Advanced Techniques and Emerging Technologies

4.1 CRISPR Activation Systems

CRISPR activation (CRISPRa) systems enhance gene expression without modifying DNA. CRISPR-Act3.0 employs augmented activation domains and guide RNA scaffolds to facilitate gene activation, indicating its potential applications in plants [15]. Issues of construct instability and variable activation in polyploid genomes persist; however, CRISPR-Act3.0 demonstrates potential for application in plant biotechnology. The proposed solutions include the implementation of modular Golden Gate assembly systems to enhance construct stability and the utilization of chromatin accessibility factors to increase activation efficiency across complex plant genomes.

4.2 Multiplexed Genome Editing

The ability of CRISPR to edit multiple genes simultaneously is one of its biggest advantages over previous genome editing methods. Multiplexed editing has successfully engineered complex traits in many crop species. This involves modifying multiple disease resistance genes simultaneously for broad-spectrum pathogen resistance. Recent guide RNA multiplexing advances allow targeting up to twenty genomic loci in a single transformation event. These methods are useful for metabolic pathway engineering. This field requires many enzymatic modifications to achieve secondary metabolite synthesis or nutritional improvement.

4.3 Delivery Systems and Transformation Methods

Genome editing success depends on efficient CRISPR delivery to plant cells. For dicotyledonous plants, *Agrobacterium*-mediated transformation is the most common method for CRISPR construct integration

and expression. Optimizing bacterial strain selection, transformation conditions, and construct design for editing efficiency has improved *Agrobacterium*-based delivery. For monocotyledonous crops and species resistant to *Agrobacterium* transformation, biolistic delivery, and protoplast transfection have been optimized. CRISPR-mediated genome editing is now possible in more species thanks to improved tissue culture and regeneration methods. Recently developed transient delivery methods allow CRISPR without stable transgene integration. Biolistics or electroporation of ribonucleoprotein (RNP) complexes has edited genomes without transgenic crop regulatory issues.

5 Applications in Disease Resistance

5.1 Bacterial Disease Resistance

The advancements in CRISPR technology have led to significant improvements in the resistance of bacteria to disease through various approaches. The focus on susceptibility genes has shown significant efficacy, as evidenced by the alteration of SWEET genes in rice to improve resistance against bacterial blight. The findings indicate that the precise knockout of certain members of the SWEET family can markedly diminish pathogen virulence while maintaining plant growth and development [27]. In citrus, the use of CRISPR to target the LOB1 susceptibility gene has shown promising results in conferring resistance to citrus canker induced by *Xanthomonas citri*. The modification of CsLOB1 has reached resistance levels similar to those of conventional resistant varieties, all while preserving fruit quality traits, highlighting the promise of CRISPR technologies in enhancing perennial crops [18].

5.2 Fungal Disease Resistance

A notable success of CRISPR technology for fungal disease control is powdery mildew resistance. The strategic targeting of MLO genes in various crop species has consistently resulted in extensive resistance to powdery mildew pathogens. CRISPR-mediated knockout of SIMLO1 in tomato resulted in total resistance to *Oidium neolyopersici*, with editing efficiencies exceeding 70% [17]. In wheat, the simultaneous targeting of all six MLO alleles resulted in the development of varieties with long-term resistance to powdery mildew. MLO-mediated resistance's extensive capabilities make it particularly useful for long-term disease management, as it protects a wide range of pathogen races without relying on

specific resistance genes.

5.3 Viral Disease Resistance

CRISPR can directly target viral genomes and modify host susceptibility factors to confer viral disease resistance. CRISPR-mediated knockout of eIF4E genes in cucumbers increases resistance to cucumber vein yellowing virus, suggesting that targeting RNA viruses is particularly effective [4]. Direct targeting of viral genomes using CRISPR-Cas systems may provide resistance to DNA virus infections. The successful development of geminivirus resistance in various crop species using CRISPR technology has shown its efficacy in combating viral diseases that are difficult to control.

6 Conclusion

The advent of CRISPR-Cas genome editing has significantly revolutionized plant biotechnology, offering remarkable possibilities for enhancing crops and promoting sustainable agricultural practices. From gene knockout to precise base substitutions, targeted insertions, and complex multiplexed modifications, technology has advanced greatly. Advanced base editing systems, prime editing technology, and improved delivery methods are expanding CRISPR's plant science applications. The effective use of CRISPR technology in various crop species, ranging from annual grains to perennial woody plants, highlights its significant potential in tackling global issues related to food security, climate adaptation, and environmental sustainability. The creation of varieties that exhibit greater disease resistance, resilience to stress, and superior nutritional quality highlights how precision genome editing can play a significant role in promoting sustainable agricultural intensification. Recent studies in woody species [15, 16] illustrate that ongoing technological advancements and the refinement of CRISPR systems will broaden their applications and enhance their efficiency. The combination of CRISPR technology with cutting-edge breeding programs and innovative digital agriculture strategies holds the potential to speed up the creation of climate-resilient, high-yielding, and nutritionally improved crop varieties. The future of CRISPR technology in plant sciences looks highly promising, as advancements in precision, efficiency, and applicability are expected to foster ongoing innovation in crop enhancement and sustainable agricultural practices. With the ongoing evolution of regulatory frameworks and the increasing acceptance among the public, crops edited with

CRISPR technology are set to become vital in tackling the global challenges of the 21st century.

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Conflicts of Interest

The authors declare no conflicts of interest.

Ethical Approval and Consent to Participate

Not applicable.

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