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CRISPR-Cas Mediated Genome Editing for Disease Resistance in Crops: Advances and Challenges

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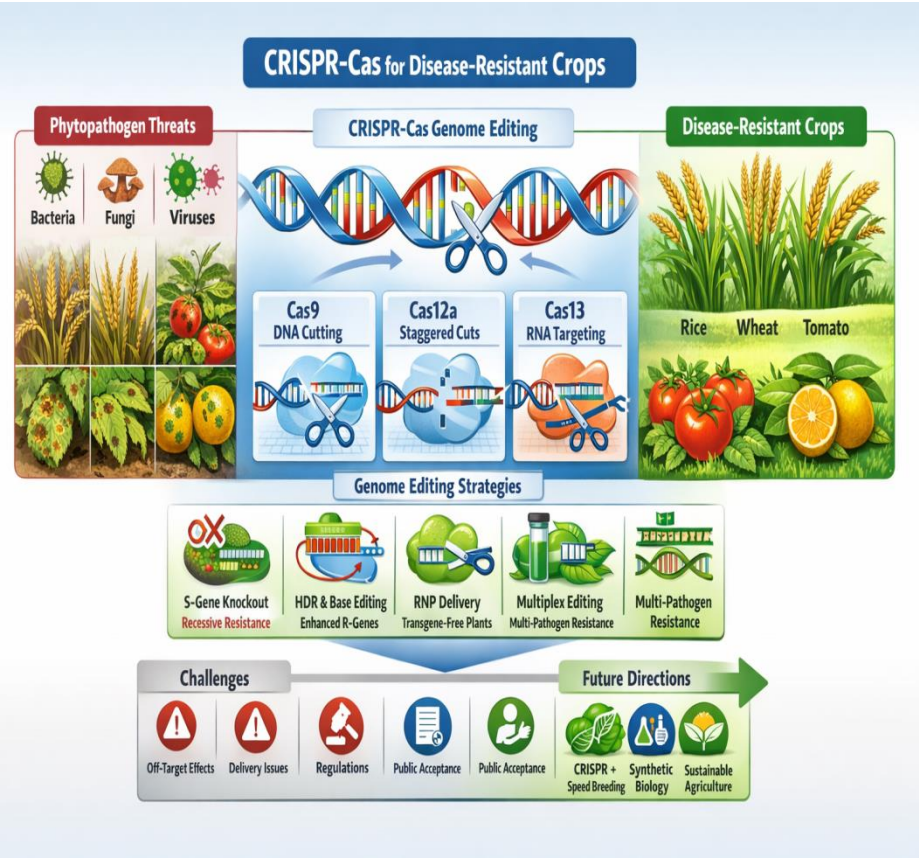
Abstract

Graphical Abstract



Abstract
The increasing menace of phytopathogens to food security in the world demands the creation of a type of crop with long-term and extensive disease resistance. CRISPR-associated protein (Cas) systems and Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) have transformed the way the genome of plants are engineered, providing unprecedented specificity, efficiency, and ease in the development of resistant crops. This review starts by describing the molecular processes of various CRISPR-Cas types (Cas9, Cas12a, and Cas13) and their use in plants. We then address some of the important strategies, such as targeted editing of host susceptibility (S) genes to generate recessive resistance, introducing broad-spectrum resistance (R) gene variants using homology-directed repair (HDR) and base editing, and producing transgene-free genome-edited plants by delivery of ribonucleoproteins (RNPs). Practical achievements are presented through successful case studies of major crops including rice, wheat, tomato and citrus. Moreover, we discuss novel technologies like CRISPR-Cas13 to interfere with viral RNA and multiplexed editing to overlay resistance characteristics against several pathogens. Though impressive advances have been made, there are still major issues to deal with, such as the effective transfer of CRISPR components to recalcitrant crop species, off-target effects and unwanted genomic modifications, regulatory barriers to transgene vs. transgene-free edited plants, and social acceptance. Lastly, we present future directions, including the combination of CRISPR and speed breeding, synthetic biology, and accelerated breeding technologies to create disease-resistant and robust crops to support sustainable agriculture

Keywords: CRISPR-Cas; Genome Editing; Disease Resistance; Susceptibility (S) Genes; Transgene-Free; Broad-Spectrum Resistance; Crop Improvement; Plant Pathogens; Sustainable Agriculture.

Introduction

The world has never encountered an equivalent challenge in production of 50-70 percent more food by 2050 but reducing the losses due to more than 80,000 plant diseases that result in 20-40 percent annual yield losses (Savary *et al.,* 2019). Conventional disease control is based on the use of chemical pesticides that are hazardous to the environment and health, and resistant cultivars that are bred in a conventional manner. There is, however, a limitation to breeding due to a lack of genetic variation, linkage drag, and the long generation time (Doebley *et al*., 2006).

Plant biotechnology has been revolutionized by the discovery of CRISPR-Cas9 as an adaptive immune system in bacteria and its reuse as a genome editing tool (Jinek *et al*., 2012). In contrast to previous nucleases, including ZFNs, TALENs, CRISPR-Cas is simple, has a multiplexing ability, and is efficient (Gaj *et al.,* 2013). Importantly, CRISPR can be edited without transgene insertion by using ribonucleoprotein (RNP) delivery or transient expression, producing transgene-free edited crops, potentially circumventing GMO regulations in various locations (Woo *et al*., 2015).

The given review is devoted to the use of CRISPR-Cas to increase crop resistance to disease. We deconstruct molecular strategies, showcase breakthrough case studies, discuss current challenges, and suggest solutions to how the laboratory success can be achieved in the field.

Molecular Strategies for Engineering Disease Resistance

Three major CRISPR-Cas approaches have been utilized (1) knockout of susceptibility (S) genes, (2) targeted insertion of resistance (R) gene alleles, and (3) transcriptional control of defense pathways.

The research aims to identify genes associated with increased vulnerability to the condition, namely Susceptibility (S) Genes.

Host factors that are used by pathogens to facilitate infection are called S genes. S genes mutated in a loss-of-function fashion may result in recessive, and sometimes long-lasting, resistance (van Schie & Takken, 2014). Knockout of the knockout of the powdery mildew resistance gene, Mlo (Mildew resistance locus o), using the CRISPR-Cas9 system offers widespread powdery mildew resistance in wheat, barley, and tomato (Nekrasov *et al.*, 2017; Wang *et al*., 2014). On the same note, bacterial blight by the Xanthomonas oryzae pv. is inhibited by disruption of a promoter of OsSWEET (sugar transporters). *oryzae* (Xoo) in rice (Oliva *et al*., 2019; Li *et al*., 2012).

The second option involves improving the R Gene activity. The genes (R) are those that encode nucleotide-binding leucine-rich repeats (NLR) proteins that perceive the pathogen effectors (Dangl & Jones, 2001). CRISPR-Cas can either insert natural resistance alleles of wild relatives or generate new versions of the R genes via homology-directed repair (HDR), but HDR is low in plants (Puchta, 2017). Instead, it has been successful with promoter editing to increase the activity of already existing R genes (Gao *et al*., 2020). Multiplexed Broad-Spectrum resistance editing is used to cut the 40ppt band. Pathogens evolve rapidly.

Multiplex CRISPR systems (e.g., CRISPR-Cas12a) make it possible to edit multiple S genes or various promoter elements at the same time. In rice, the concomitant editing of three promoters of the OsSWEET family gave resistance against various strains of Xoo (Xu *et al*., 2019; Zsogoen *et al*., 2017).

CRISPR activation (CRISPRa) and interference (CRISPRi) represent additional CRISPR methods to inhibit gene expression. Fine-tuning of expression of defense genes can be achieved by catalytically silent Cas (dCas9) connected to transcriptional activators (CRISPRa) or repressors (CRISPRi) without cleaving DNA (Lowder *et al*., 2015). CRISPRa of WRKY transcription factors increased wheat resistance to Fusarium (Kim *et al.*, 2022).

Advances in Delivery and Regeneration

Efficient delivery of CRISPR components into plant cells and subsequent regeneration of whole plants are major bottlenecks.

Table 1: Comparison of CRISPR-Cas Delivery Methods in Plants

Delivery Method	Advantages	Limitations	Representative Crops	Citation
***Agrobacterium* T-DNA**	High efficiency, stable integration	Genotype dependency, random insertion, transgene persistence	Rice, tomato, citrus	Nekrasov *et al*., 2017
Particle bombardment (DNA)	Species-independent, no bacterial vectors	Low efficiency, DNA fragmentation, high copy number	Wheat, maize	Wang *et al.*, 2014
Ribonucleoprotein (RNP)	No DNA integration, reduced off-	Difficult delivery to plant cells, RNP stability	Protoplasts, maize embryos	Svitashev *et al*., 2016; Woo et al., 2015

	target, transgene-free			
Viral vectors (e.g., TRV)	Fast, no stable transformation	Limited cargo size, virus spread concerns	*Nicotiana*, tomato	Ali *et al.*, 2015
PEG-mediated protoplast	High editing efficiency, transgene-free	Protoplast regeneration challenging, not routine for many crops	Rice, lettuce	Woo *et al*., 2015
Nanoparticle delivery	DNA-free, organelle targeting	Low efficiency in plants, optimization needed	Maize, wheat	Demirer *et al.*, 2019

Challenges Limiting Translational Impact

Although it has technical breakthroughs, there are a number of challenges that curtail its mass usage.

Off-Target Effects

CRISPR-Cas9 is also tolerant to mismatches and the PAM-distal site, which results in unintended mutagenesis (Fu et al., 2013). Whole-genome sequencing of edited rice has shown the off-target events are rare (Tang *et al*., 2018), whereas off-targets are reduced with high-specificity variants (e.g., SpCas9-HF1, eSpCas9) (Kleinstiver *et al.,* 2016). The structure of gRNAs is complicated in designing unique gRNAs in crops with polyploid genomes (Zhu *et al.,* 2019). Low Homology-Directed Repair (HDR) efficiency is low. The majority of CRISPR edits in plants are based on non-homologous end joining (NHEJ) to knock out genes. HDR remains inefficient (usually, less than 2-percent of edited events) in somatic cells when it comes to precise gene replacement or insertion (Puchta, 2017). NHEJ chemical inhibitors (e.g., SCR7) or geminivirus replicons to provide donor templates are emerging approaches (Wang *et al*., 2017; Čermák *et al*., 2015).

Genotype Dependency and Regeneration Bottlenecks

Numerous elite varieties of crop are recalcitrant to transformation and regeneration. An example is that variety Fielder of wheat is editable, yet agronomically inefficient; editing elite lines has a high failure rate (Ishida et al., 2015). The new haploid inducer-mediated editing (HI-Edit) or in planta particle bombardment (iPB) techniques avoid using tissue culture but still need optimization (Kelliher *et al.,* 2019; Kausch et al., 2019).

Unintended Pleiotropic Effects

S genes can be knocked out to the detriment of other agronomic traits. Mlo mutants are more resistant to mildew but occasionally have faster senescence or yield loss (Piffanelli *et al.,* 2004). Trade-offs might be alleviated by promoter editing (instead of total knockout) or tissue-specific CRISPR expression (Rodriguez-Leal *et al*., 2017).

Regulatory Landscape

The genome-edited crops regulatory classification is radically different across the world. The USDA has approved numerous transgene-free edits, with the EU now considering all CRISPR-edited plants GMOs, very restrictive of field trials (Callaway, 2018). Japan and Australia have less restrictive systems and China is working on case-by-case systems (Schmidt *et al.*, 2020). Global policies are summarised in the following table.

Table 2: Global Regulatory Stance on CRISPR-Edited Crops (2024-2025)

Country/Region	SDN-1 (Small indels, no transgene)	SDN-2 (Template-directed, small change)	SDN-3 (Insertion of foreign DNA)	Reference
United States	Not regulated (if no foreign DNA)	Not regulated (if plant-pest risk not increased)	Regulated as GMO	USDA-APHIS, 2021; Waltz, 2018
European Union	Regulated as GMO	Regulated as GMO	Regulated as GMO	ECJ Case C-528/16; Callaway, 2018
United Kingdom	Not regulated (if similar to conventional)	Not regulated (under 2023 Act)	Regulated	DEFRA, 2023
Japan	Not regulated (notification only)	Not regulated (notification only)	Regulated	Ohama, 2022; Tsuda *et al.*, 2019
China	Exempted (under 2023 guidelines)	Exempted (if no exogenous DNA)	Regulated	MoA, 2023; Li *et al.*, 2020
Brazil	Not regulated (based on CONABIA)	Case-by-case	Regulated	CONABIA, 2018; Whelan & Lema, 2019
Australia	Not regulated (if no novel combination)	Regulated as GMO	Regulated as GMO	OGTR, 2019

Emerging CRISPR Systems and Future Directions

Base Editing and Prime Editing

Base editors (BE4, ABE) correct single bases in the absence of the double-strand break, which allows the base of the pathogen effector binding site to be accurately modified. An example is that the binding of TAL effectors to the promoter of the OsSWEET14 gene was abolished by the conversion of a C to T in the promoter (Hua et al., 2019; Zong *et al.*, 2017). Prime editing (PE) is a process that allows any of the 12 base substitutions and deletions, as well as small inserts. Though PE efficiency in plants is becoming more efficient (1-10%), delivery is problematic (Lin *et al.*, 2020; Anzalone *et al.*, 2019).

CRISPR-Cas12a (Cpf1)

Cas12a identifies T-rich PAMs (TTTV), increases the range of targets, and cleaves its own crRNA array, best suited to multiplex editing (Zetsche *et al.*, 2015). Simultaneously, in rice, the multiplex Cas12a editing of three genes of the OsSWEET group resulted in the generation of lines resistant to several Xoo pathovars (Xu *et al.*, 2019; Tang *et al.*, 2019).

In this section, the authors explain that dominant resistance can be formed by CRISPR. CRISPR may also confer dominant resistance through pathogen effector target insertion (decoys), or through R protein design. As an illustration, engineering of the Xanthomonas TAL effector binding site to an executor R gene (Xa27) via CRISPR-HDR resulted in rice that exhibits dominant and broad-spectrum resistance (Huang *et al.*, 2021; Hummel *et al.*, 2018).

The synthetic biology and gene drives represent a significant area of investigation in the field of gene editing. CRISPRa and CRISPRi synthetic genetic circuits can dynamically react to infection by a pathogen (Khakhar *et al.*, 2018). CRISPR-based gene drives (notably controversial in plants) have the theoretical capability to disseminate resistance alleles via populations but have ecological concerns (Esvelt *et al.*, 2014; Neve, 2018).

Table 3: Summary of Successful CRISPR-Cas Applications for Disease Resistance in Major Crops

Crop	Pathogen	Target Gene	Editing Strategy	Resistance Outcome	Field Validation	Citation
Rice	*Xanthomonas oryzae* (bacterial blight)	*OsSWEET14* promoter	Knockout of EBE via NHEJ	Broad-spectrum resistance	Yes (confined trials)	Oliva *et al.*, 2019
Rice	*Magnaporthe oryzae* (blast)	*OsERF922*	Knockout	Enhanced resistance (50% lesion reduction)	Yes	Wang *et al.*, 2016
Rice	*Xanthomonas oryzae*	*OsSWEET11/13/14*	Multiplex Cas12a	Resistance to multiple Xoo strains	Yes	Xu *et al.*, 2019
Wheat	*Blumeria graminis* (powdery mildew)	*TaMLO-A/B/D*	Multiplex knockout	Durable resistance	Limited (growth penalty)	Wang *et al.*, 2014

Wheat	*Puccinia striiformis* (stripe rust)	*TaPsIPK1*	Promoter editing	Broad-spectrum resistance	Yes	Zhang *et al*., 2021
Wheat	*Fusarium graminearum*	*TaEDR1*	Knock out	Enhanced Fusarium resistance	Greenhouse	Li *et al.*, 2021
Tomato	*Oidium neolycopersici* (powdery mildew)	*SlMlo1*	Knock out	Complete resistance	Yes (greenhouse)	Nekrasov *et al.*, 2017
Tomato	*Phytophthora infestans* (late blight)	*SlDMR6-1*	Knock out	Enhanced resistance	Greenhouse	de Toledo Thomazella *et al*., 2021
Citrus	*Xanthomonas citri* (canker) & HLB	*CsLOB1*	Knock out	Canker resistance, HLB tolerance	Field trial ongoing	Peng *et al.*, 2021; Jia *et al*., 2017
Cassava	*Cassava brown streak virus*	*eIF4E isoforms*	Multiplex knock out	Reduced virus titer	Greenhouse	Gomez et al., 2019
Maize	*Fusarium verticillioides* (ear rot)	*ZmFUS3*	Knock out	Reduced fumonisin accumulation	Greenhouse	Liu et al., 2020
Soybean	*Phytophthora sojae*	*GmRpp1*	Promoter editing	Enhanced R gene expression	Greenhouse	Zhang et al., 2020

Commercial Products and Near-Market Examples

A number of disease-resistant crops edited with CRISPR have been or are on the verge of commercialization. The commercialization of the first CRISPR-edited crop (a GABA-enriched tomato) happened in Japan in 2021 (Waltz, 2022). To overcome disease resistance, a powdery mildew-resistant tomato (edited) has been and continues to be the subject of regulatory discussions in the US and Japan. By 2026, Pairwise predicts its gene-edited mustard greens will be disease-resistant (Ledford, 2023).

CRISPR-edited rice resistant to bacterial blight has gone through confined field trials and is awaiting registration as a variety in China (Chen *et al.*, 2023; Cohen, 2019).

Table 4: Comparison of CRISPR-Cas with Other Breeding Techniques for Disease Resistance

Parameter	Conventional Breeding	Transgenic (GM)	CRISPR-Cas (Transgene-free)
Time to develop resistant variety	5-10 years	3-5 years	1-2 years
Precision	Low (linkage drag)	Moderate (random insertion)	High (targeted)
Introgression of wild relative genes	Yes	Yes	Yes (via HDR)
Off-target risk	Minimal	Low	Low to moderate (can be mitigated)
Regulatory burden (varies by country)	Low	High	Variable (low in US, high in EU)
Consumer acceptance	High	Low	Moderate to high (if transgene-free)
Durability of resistance (single gene)	Moderate	Moderate	Can be enhanced via multiplexing
Cost per product (USD million)	10-30	30-50	5-15
Citations	Doebley *et al*., 2006	Gaj *et al*., 2013	

Socioeconomic and Ethical Considerations

There are significant concerns about the use of CRISPR-edited crops. In the case of the low-income countries, smallholder farmers can be deprived of proprietary CRISPR technologies because of intellectual property (IP) limitations (Egelie *et al*., 2016). CRISPR patents (US 9,790,490) by the Broad Institute encompass numerous uses, although the humanitarian use is royalty-free through the CRISPR for Agriculture program (Ledford, 2023).

Moreover, transgene-free edited crops are likely to avoid GMO regulations, but there is still divided opinion. European consumers have been skeptical, but Asian and American consumers are more tolerant towards gene editing when the benefits (e.g., less use of pesticides) are evident (Ishii and Araki, 2016).

The possible risks linked to ecology involve the possibility of gene flow of edited crops to wild relatives, which may have selective benefits (Neve, 2018). Nevertheless, since the majority of S gene knockouts are not associated with herbicide tolerance, these risks are reduced compared to conventional transgenics (Nadakuduti and Enciso-Rodriguez, 2021).

Future perspectives

The future of CRISPR-Cas–mediated genome editing in crop disease resistance will rely on technological precision, interdisciplinary integration, and alignment with global sustainability and health priorities. Emerging tools such as base editing and prime editing are expected to enable highly accurate nucleotide modifications with minimal off-target effects, thereby improving biosafety and functional stability. This emphasis on safety and biological compatibility aligns with recent advancements in nutritional and biosafety assessments of novel food systems (Butt et al., 2025a), as well as quality and safety evaluations in food products (Butt et al., 2024), highlighting the growing need to ensure that genome-edited crops meet both agronomic and health standards.

A critical bottleneck remains the efficient and genotype-independent delivery of CRISPR components into plant systems. Future innovations such as nanoparticle-mediated delivery, ribonucleoprotein (RNP)-based editing, and viral vectors can draw parallels from functional food and bioactive delivery systems, where stability, bioavailability, and targeted functionality are key considerations (Ahmed et al., 2024; Butt et al., 2025b). These cross-disciplinary insights suggest that integrating delivery science from food and biological systems can accelerate the development of transgene-free, efficient genome editing platforms.

Beyond disease resistance, CRISPR technologies are expected to play a transformative role in developing nutritionally enhanced and health-promoting crops. With increasing global interest in functional foods and metabolic health, genome editing can be utilized to biofortify crops and regulate bioactive compounds, supporting findings from studies on probiotic functionality (Ahmed et al., 2024), metabolic health interventions (Rashid et al., 2026), and epigenetic regulation through diet (Butt et al., 2026b). Furthermore, targeted genetic modifications may enhance micronutrient bioavailability and physiological performance, as suggested by research on nutrient-gene interactions such as zinc-mediated IGF-1 expression (Butt et al., 2026a).

The integration of CRISPR with speed breeding, synthetic biology, and multiplex genome editing will enable the stacking of multiple desirable traits, including disease resistance, improved nutrition, and environmental resilience. These advancements align with broader sustainability and ESG-driven frameworks, where agricultural systems are increasingly evaluated based on environmental impact and social responsibility (Khurshid et al., 2026). Additionally, the role of emerging technologies and artificial intelligence in optimizing biological systems and decision-making processes (Kamal & Butt, 2026) can further enhance precision breeding and predictive modeling in genome editing applications.

From a translational perspective, successful deployment of CRISPR-edited crops will require addressing regulatory challenges and improving public acceptance. Insights from food science and nutrition research—particularly studies evaluating dietary fats and health outcomes (Khan et al., 2024), food safety and consumer product quality (Butt et al., 2024), and sustainable protein innovations (Butt et al., 2025b; Butt et al., 2025c)—highlight the importance of aligning technological innovation with consumer expectations, safety standards, and environmental stewardship. Moreover, interdisciplinary evidence from health and performance studies (Mahmood et al., 2026) reinforces the need for long-term evaluation of biological interventions, which is equally critical for genome-edited crops.

Finally, the convergence of CRISPR-Cas systems with advanced breeding technologies, functional food science, and sustainability-driven innovations offers a promising pathway toward resilient and nutritionally optimized crops. By integrating insights

from diverse research domains—including food science, human health, artificial intelligence, and sustainability—future genome editing strategies can contribute significantly to global food security, environmental sustainability, and improved human well-being.

Conclusion

CRISPR-Cas has undoubtedly spurred the creation of disease-resistant crops. With the capability to specifically silence susceptibility genes, design promoters, and multiplex edits, a potent toolkit is available to counteract fast-evolving pathogens. Notable developments are transgene-free editing through RNP delivery, single-nucleotide accuracy base/prime editing, and regulatory exemptions in major agricultural economies (Zhu *et al*., 2019; Hua *et al.*, 2019).

However, the obstacles are still daunting: enhancing the efficiency of HDR, eliminating the dependence of genotypes in the process of regeneration, and overcoming the obstacles of international regulation. Future advances are likely to include (i) tissue-culture-free editing techniques in plants, (ii) CRISPR delivery into perennial crops using viruses, (iii) computational design of gRNAs with reduced off-targets in polyploid genomes (Kleinstiver *et al.*, 2016), and (iv) machine-learning-based methods to predict S gene interactions (Savary *et al*., 2019)

CRISPR-edited disease resistance is not the luxury, but the need in the light of the growing climate crisis, which broadens the areas of pathogen distribution and appearance. This technology will support the next generation of the sustainable, resilient agricultural systems with responsible stewardship, transparent regulation, and equitable IP access.

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