

CRISPR-BASED GENOME EDITING FOR DEVELOPMENT OF CLIMATE-RESILIENT AND DISEASE-RESISTANT CROP VARIETIES

Inam Mehdi Khan^{*1}, Pervez Hussain², Laiba Qadeer³, Shehzadi Saima⁴^{*1,2}Department of Botany, Division of Science and Technology, University of Education, Lahore 54770, Punjab, Pakistan.³Department of Pure and Applied Biology, Institute of Botany, Bahauddin Zakariya University, Multan 60800, Punjab, Pakistan⁴Institute of Botany, Bahauddin Zakariya University, Multan 60800, Punjab, Pakistan¹inammehdikhan25@gmail.com, ²hussainpervez426@gmail.com, ³laibaqadeer9904@gmail.com, ⁴shehzadi.saima@bzu.edu.pkDOI: <https://doi.org/10.5281/zenodo.22206805>**Keywords**

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Copyright @Author**Corresponding Author: *****Inam Mehdi Khan****Abstract**

Global agricultural productivity is severely constrained by biotic stresses including pathogenic viruses, bacteria, fungi, oomycetes, nematodes, and insect pests which collectively account for 20–40% yield losses in staple crops worldwide. Traditional reliance on chemical pesticides and conventional breeding faces mounting challenges from pesticide resistance, ecological toxicity, and protracted breeding timelines. The emergence of CRISPR-Cas genome editing technologies has revolutionized plant biotechnology by enabling precise, site-specific genomic modifications without permanent transgene integration. This comprehensive review examines CRISPR-based strategies for engineering disease-resistant and pest-resistant crops, encompassing the molecular toolkit of Cas9, Cas12a, Cas13, base editors, prime editors, and CRISPR activation systems. Key applications include susceptibility (S) gene knockout, promoter cis-element recoding to disrupt pathogen effector binding (exemplified by OsSWEET and CsLOB1 editing), NLR receptor engineering, and manipulation of plant volatile profiles and morphological traits for insect deterrence. Advanced delivery platforms particularly nanotechnology-enabled ribonucleoprotein (RNP) complexes facilitate transgene-free genome editing while minimizing off-target effects. However, implementation faces constraints including pleiotropic fitness penalties, rapid pathogen evolutionary adaptation, and off-target cleavage risks. The regulatory landscape is evolving, with major agricultural economies increasingly exempting non-transgenic gene-edited crops from stringent GMO oversight. This review synthesizes current advances, identifies persistent bottlenecks, and outlines future directions for deploying CRISPR-edited crops toward sustainable, climate-resilient agriculture.

1. INTRODUCTION

Global agricultural production faces severe structural challenges driven by climate instability, land degradation, and mounting biotic pressures

from phytopathogenic viruses, bacteria, fungi, oomycetes, nematodes, and phytophagous insect pests. Biotic stresses account for global yield reductions estimated between 20% and 40%

across staple food crops, including rice, wheat, maize, potato, and soybean, severely compromising international food security and agricultural economic stability. Historically, crop protection has relied heavily on synthetic chemical pesticides and classical plant breeding (Hossain et al., 2020). However, intensive chemical application accelerates the evolution of pesticide resistance among target populations while causing non-target ecological toxicity, soil and water degradation, and human health concerns. Conversely, conventional resistance breeding is intrinsically constrained by long breeding cycles, sexual incompatibility barriers between cultivated crops and wild relatives, and severe linkage drag, which frequently introduces undesirable agronomic traits alongside target resistance alleles (Mbow et al., 2017).

The emergence of precision genome editing, led by Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) and CRISPR-associated (Cas) nucleases, has established a new paradigm in plant biotechnology. Adapted from adaptive immune systems in bacteria and archaea, CRISPR-Cas platforms enable site-specific genomic modifications without requiring permanent transgene integration (Banda et al., 2024). Unlike early genome editing technologies such as Zinc Finger Nucleases (ZFNs) and Transcription Activator-Like Effector Nucleases (TALENs), which require complex protein engineering for every target locus, CRISPR systems utilize easily customizable single-guide RNAs (sgRNAs) to direct Cas nucleases to specific DNA or RNA targets (Joung & Sander, 2013).

Recent innovations have expanded the plant CRISPR toolkit far beyond simple gene knockouts. Advanced modalities now encompass sequence-specific base editing, prime editing, homology-directed repair (HDR), transcriptional activation (CRISPRa), and RNA-targeting systems. By modifying host susceptibility (*S*) genes, editing promoter cis-regulatory elements, modulating immune signaling networks, and altering plant-insect olfactory and visual interactions, CRISPR-Cas platforms provide precision tools for engineering durable, broad-spectrum resistance

(Mushtaq et al., 2021). This review provides a comprehensive analysis of CRISPR-mediated genome editing strategies engineered to confer resistance against plant pathogens and insect pests, detailing underlying molecular mechanisms, delivery innovations, agronomic trade-offs, and evolving regulatory paradigms governing commercial adoption (Tyagi et al., 2020).

2. Molecular Toolkit: CRISPR Systems and Precision Editing Modalities

2.1 Standard Nucleases: Cas9, Cas12a, and Cas13

The foundational CRISPR engine in plant biotechnology is the Type II *Streptococcus pyogenes* Cas9 (SpCas9) system. Directed by a 20-nucleotide spacer within an sgRNA, SpCas9 binds target DNA adjacent to a 5'-NGG-3' protospacer adjacent motif (PAM) and generates double-strand breaks (DSBs) three base pairs upstream of the PAM site. In plant cells, DSBs are predominantly repaired via the error-prone Non-Homologous End Joining (NHEJ) pathway, introducing random insertions or deletions (indels) that disrupt reading frames and yield functional knockouts of target genes (Steinert et al., 2015).

To broaden targeting scope and operational flexibility in plants, engineered SpCas9 variants with altered PAM requirements have been introduced alongside alternative Class 2 nucleases. The Type V nuclease Cas12a (formerly Cpf1) recognizes AT-rich 5'-TTTV-3' PAM sequences, making it exceptionally suited for editing AT-rich non-coding regions, promoters, and intron-exon junctions in plant genomes. Cas12a cleaves DNA via a staggered double-strand break, generating 4- or 5-nucleotide 5' overhangs (cohesive ends) that facilitate precise homology-directed insertions (Montecillo et al., 2020). Furthermore, Cas12a possesses intrinsic endoribonuclease activity, enabling it to self-process a single primary transcript containing multiple crRNAs. This inherent multiplexing capacity allows simultaneous cleavage of multiple genomic loci, which is critical for targeting gene families or pyramiding multiple resistance traits (Meliawati et al., 2021).

In contrast to DNA-targeting nucleases, Type VI Cas13 nucleases bind and cleave single-stranded RNA molecules under sgRNA guidance. Because the vast majority of plant viruses possess RNA genomes, Cas13 platforms provide a post-transcriptional defense mechanism. Expressing Cas13 complexes engineered to recognize

conserved viral RNA sequences enables direct degradation of viral genomic RNA or mRNA transcripts during infection, offering non-heritable, broad-spectrum antiviral protection without modifying host plant genomic DNA (Perčulija et al., 2021).

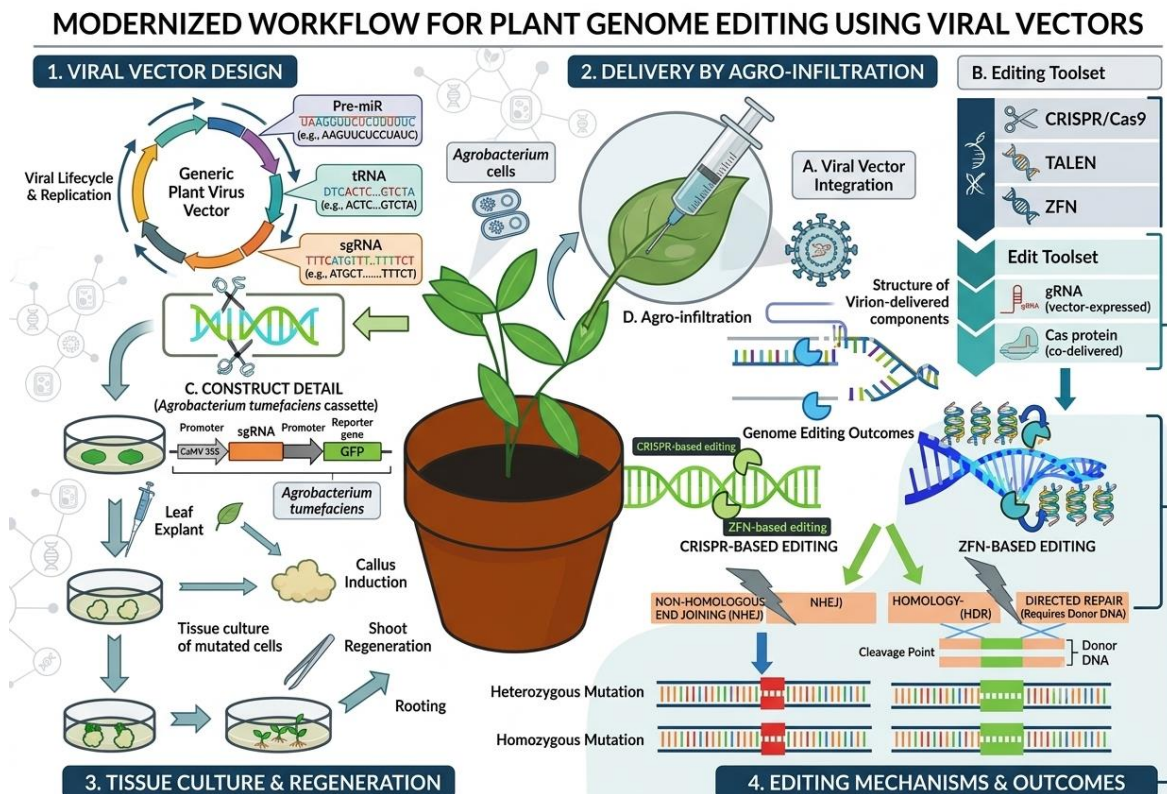


Figure 2: Plant Genome Editing Delivery Systems, Tissue Culture Regeneration, and Downstream Repair Mechanisms.

2.2 Advanced Non-DSB Precision Tools: Base Editing, Prime Editing, and Transcriptional Modulation

While DSB-induced knockouts via NHEJ are effective for gene inactivation, precise single-nucleotide alterations or sequence rewritings are often required to tune gene expression or alter protein interactions without inducing host DNA damage responses. Base editors solve this challenge by fusing catalytically impaired Cas nucleases such as Cas9 nickase (nCas9) or dead Cas9 (dCas9) to single-stranded DNA nucleoside deaminases (Martin et al., 2021). Cytosine Base Editors (CBEs) convert target

(C\$ \cdot GbasepairstoT \cdot A, whereas Adenine Base Editors (ABEs) convert A \cdot TbasepairstoG \cdot C within a narrow editing window near the PAM site. Base editing facilitates the introduction of specific amino acid substitutions in plant immune receptors or translation initiation factors without generating double-strand breaks, reducing insertion-deletion mosaicism and cytotoxic effects (Huang et al., 2021).

Prime Editing (PE) extends these capabilities by offering search-and-replace editing without requiring DSBs or donor DNA templates. The prime editor architecture consists of an nCas9

fused to an engineered reverse transcriptase, operating alongside a prime editing guide RNA (pegRNA). The pegRNA specifies the target genomic locus and contains an extension encoding the desired edit alongside a primer binding site (Zhao et al., 2023). Upon target site nicking, the reverse transcriptase copies the edit directly into the host locus, enabling all 12 base-to-base transitions and transversions, as well as targeted insertions and deletions. In plant breeding, prime editing allows precise introgressing of specific disease-resistant quantitative trait loci (QTLs) and beneficial single-nucleotide polymorphisms (SNPs) directly into elite commercial germplasm, bypassing

generation-long backcrossing pipelines (Mahmudul & TuskanGerald, 2020).

Beyond structural DNA changes, CRISPR activation (CRISPRa) modalities enable fine-tuning of endogenous defense gene expression. Advanced systems such as CRISPR-Act3.0 link dCas9 or dCas12a to transcriptional activator domains, allowing targeted upregulation of native defense-related transcription factors, secondary metabolite biosynthetic pathways, or pattern recognition receptors. Transcriptional modulation activates host systemic acquired resistance (SAR) dynamically, eliminating the risk of permanent gene disruption while overcoming underutilized host defense capacity (McLaughlin et al., 2025).

Table 1: Overview of Primary CRISPR Systems Utilized in Plant Trait Engineering

System	Target Substrate	Mechanism / Repair Pathway	Key Agricultural Applications
SpCas9	Double-stranded DNA	DSB induction repaired by NHEJ (indels) or HDR	Gene knockouts of susceptibility (<i>S</i>) genes; gene disruptions
Cas12a (Cpf1)	Double-stranded DNA (AT-rich PAM)	Staggered DSB; self-processing crRNA array	High-efficiency multiplex editing; target gene family knockouts
Cas13	Single-stranded RNA	Post-transcriptional RNA cleavage	Interference against RNA viruses; viral transcript degradation
Base Editors (CBE/ABE)	Double-stranded DNA	Deamination without DSBs (C $\rightarrow$ T, A $\rightarrow$ G)	Point mutations in <i>S</i> -genes, translation factors (eIF4E), and enzymes
Prime Editors (PE)	Double-stranded DNA	nCas9-Reverse Transcriptase guided by pegRNA	Precise base transitions, transversions, small insertions/deletions
CRISPRa (Act3.0)	Chromatin / Promoter DNA	Target recruitment of transcriptional activators	Transcriptional upregulation of host defense genes and PRRs

3. Engineering Resistance Against Microbial Pathogens

3.1 Susceptibility (*S*) Gene Knockout and Promoter Cis-Element Recoding

Plant pathogens often rely on host susceptibility (*S*) genes to facilitate entry, suppress host immunity, or acquire nutrients necessary for proliferation. Disruption of *S*-gene function alters compatible host-pathogen interactions, rendering

the host non-permissive to infection and conferring recessive, durable resistance (Zaidi et al., 2021).

A primary class of *S*-genes includes the SWEET (Sugars Will Eventually be Exported Transporters) sucrose transporter family in cereals. The bacterial blight pathogen *Xanthomonas oryzae* pv. *oryzae* (Xoo) secretes Transcription Activator-Like Effectors (TALEs) such as AvrXa7, PthXo1, and

PthXo2 that translocate to the host nucleus and bind to specific cis-regulatory effector-binding elements (EBEs) within the promoter regions of OsSWEET11, OsSWEET13, and OsSWEET14 (Gautam et al., 2021). TALE binding forcefully upregulates sugar transport to the apoplast, feeding bacterial colonies. Complete knockout of coding sequences in OsSWEET genes can impair plant reproductive development due to disrupted sugar transport. However, CRISPR-Cas9 or Cas12a targeting of specific promoter EBE motifs prevents TALE binding while retaining native developmental expression of the transporter, generating robust bacterial blight resistance without fitness costs (Gupta et al., 2021).

Mechanistically, this promoter editing strategy decouples pathogen co-option from the physiological utility of the gene. In unedited susceptible hosts, bacterial entry leads to the injection of TALEs that specifically recognize host EBEs, turning on nutrient flux to drive disease. By deploying CRISPR nucleases to delete or mutate the target base pairs within these promoter EBEs, the pathogen effectors can no longer bind. The endogenous *S*-gene maintains its basal expression driven by native developmental signals, yielding a non-transgenic plant variety that completely resists pathogen colonization (Judd, 2022).

A parallel strategy has proven successful in controlling citrus canker caused by *Xanthomonas citri* pv. *citri* (Xcc). Xcc utilizes its major effector, PthA4, to activate the promoter of the lateral organ boundaries domain transcription factor *CsLOB1*. Deleting the *EBE_{PthA4}* sequence within the *CsLOB1* promoter across Duncan grapefruit (*Citrus × paradisi*) and Wanjincheng orange (*Citrus sinensis*) alleles completely abolishes Xcc-induced hyperplastic pustule formation while maintaining normal plant organ development (Kobayashi et al., 2017).

Targeting *S*-genes encoding negative regulators of immunity or cellular susceptibility factors provides broad-spectrum resistance across fungal and viral pathosystems. Disruption of the *Powdery Mildew Locus O* (MLO) gene family confers resistance to powdery mildew fungi (*Blumeria graminis*) in wheat, barley, and tomato (Silva & Fernando, 2026).

Mutating the *Downy Mildew Resistance 6* (DMR6) gene which encodes a 2-oxoglutarate-dependent oxygenase elevates endogenous salicylic acid (SA) levels, granting broad-spectrum protection against bacterial, fungal, and oomycete biotrophic pathogens in various species. For viral pathogens, targeting eukaryotic translation initiation factors, such as *eIF4E* and *eIF(iso)4E*, disrupts the host machinery required for potyvirus RNA translation and replication, establishing potyvirus immunity in cucumber and *Arabidopsis* (Wang et al., 2024).

3.2 Modulating Host Immunity and NLR Receptor Engineering

Plant immune systems rely on a two-tiered innate recognition network: Pattern Recognition Receptors (PRRs) at the cell surface that sense conserved pathogen-associated molecular patterns (PAMP-triggered immunity, PTI), and intracellular Nucleotide-binding Leucine-rich Repeat (NLR) receptors that detect specific pathogen effectors (Effector-triggered immunity, ETI) (Zipfel, 2014). CRISPR-mediated Homology-Directed Repair (HDR) and prime editing enable structural re-engineering of host NLR receptors to expand their effector recognition specificity. Naturally occurring NLR sensor domains often exist in tightly co-regulated pairs consisting of a sensor NLR and a helper NLR to prevent autoimmunity. By employing base editing or prime editing, specific amino acid residues within the leucine-rich repeat (LRR) or integrated domains of sensor NLRs can be rationally mutated to mimic evolutionary variants, enabling recognition of newly mutated or non-adapted pathogen effectors (Guo et al., 2025).

Furthermore, CRISPR technologies facilitate the precise insertion (knock-in) of broad-spectrum PRRs across distant plant families via HDR. Transferring PRRs into stress-responsive or permissive chromatin regions ensures that defense signaling is triggered only upon pathogen challenge, averting energy costs associated with constitutive defense activation. Multiplex sgRNA strategies also allow allele stacking (pyramiding) of multiple R-genes and *S*-gene knockouts into elite breeding lines within a single generation,

overcoming historical barriers imposed by genetic linkage drag (Han et al., 2025).

Table 2: Representative Crop Resistance Traits Engineered via Target Gene Editing

Crop Species	Target Gene	Pathogen / Pest Category	Specific Pathogen / Pest	Genome Editing Strategy	Observed Phenotype
Rice (<i>Oryza sativa</i>)	OsSWEET11, 13, 14	Bacterial	<i>Xanthomonas oryzae</i> pv. <i>oryzae</i>	Promoter deletion via Cas9	Broad-spectrum bacterial blight resistance
Rice (<i>Oryza sativa</i>)	OsSEC3A, OsERF922	Fungal	<i>Magnaporthe oryzae</i>	Indel knockout via Cas9	Enhanced blast disease resistance
Citrus (<i>Citrus</i> spp.)	CsLOB1	Bacterial	<i>Xanthomonas citri</i> pv. <i>citri</i>	EBE _{pthA4} promoter mutation	Citrus canker resistance
Wheat (<i>Triticum aestivum</i>)	TaMLO	Fungal	<i>Blumeria graminis</i> f. sp. <i>tritici</i>	Multi-allele knockout via Cas9	Broad-spectrum powdery mildew resistance
Cucumber (<i>Cucumis sativus</i>)	eIF4E	Viral	Potyriviruses	Site-specific base editing / indels	Resistance to RNA potyriviruses
Barley (<i>Hordeum vulgare</i>)	β -1,3-glucanases	Insect	<i>Rhopalosiphum padi</i> (Aphid)	Indel knockout via Cas9	Callose formation; reduced phloem feeding
Tobacco (<i>Nicotiana tabacum</i>)	Anthocyanin pathway	Insect	<i>Spodoptera litura</i> , <i>H. armigera</i>	Biosynthetic pathway editing	Red-leaf visual mask; reduced pest oviposition

4. CRISPR Strategies for Insect Pest Management

4.1 Manipulating Plant Morphological and Volatile Profiling

Phytophagous insects rely heavily on host plant chemical emissions (volatile organic compounds, VOCs), visual cues (leaf spectral reflectance), and textural barriers to locate, feed upon, and select oviposition sites on host plants. CRISPR-Cas technology provides unique avenues to disrupt these sensory interactions, establishing indirect pest resistance (Serdo, 2024).

Plant volatiles play a vital role in mediating tritrophic interactions involving host plants, phytophagous pests, and natural pest predators. Engineering metabolic pathways to alter volatile profiles can effectively deter herbivores or recruit beneficial predators. For instance, editing the

terpenoid biosynthetic pathway to induce emission of the sesquiterpene hydrocarbon (*E*)- β -farnesene an alarm pheromone released by aphids when under attack signals danger to feeding aphid populations, causing them to cease feeding and disperse. Simultaneously, elevated (*E*)- β -farnesene acts as a synomone that attracts parasitic wasps such as *Diaeretiella rapae*, enhancing natural biological control of aphid populations on edited crops (Niu, et al., 2024).

Morphological and structural adaptations can also be edited to deter insect feeding. Phytophagous insects recognize host plants partially by visual cues, including specific wavelengths of reflected light determined by epidermal pigments. By editing key enzymatic steps within the anthocyanin biosynthetic pathway, researchers developed red-leaf tobacco mutants. The altered

foliar pigmentation disrupts host identification by major lepidopteran pests, including *Spodoptera litura* and *Helicoverpa armigera*, suppressing egg-laying and larval feeding damage (Kaur et al., 2022).

Similarly, modifying cell wall composition and phloem structure provides defense against sap-

sucking insects. Editing β -1,3-glucanase genes in barley alters callose deposition patterns near vascular tissue. Modified callose tissue prevents the bird cherry-oat aphid (*Rhopalosiphum padi*) from successfully inserting its stylet into phloem sap, restricting nutrient uptake and suppressing insect population growth (Teixeira et al., 2017).

DUAL APPLICATIONS OF GENOME EDITING FOR ADVANCED INSECT PEST MANAGEMENT

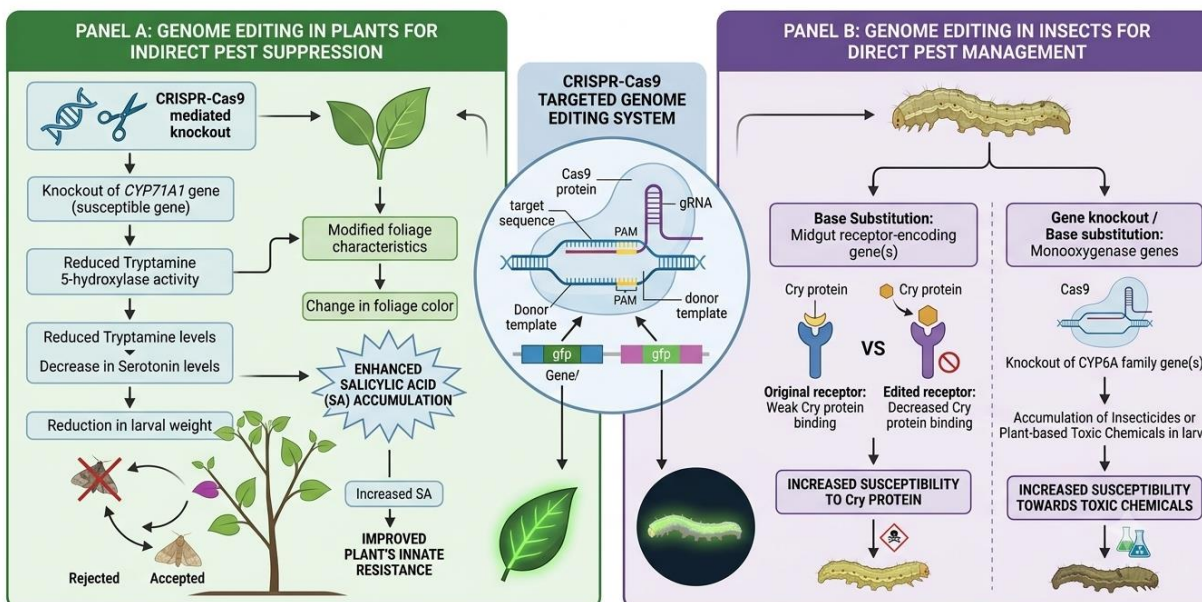


Figure 2: Dual Strategies of Genome Editing in Plants and Insects for Direct and Indirect Pest Management.

4.2 Endogenous Pest Resistance and Pest-Genome Targeting

Beyond altering physical traits and chemical emissions, CRISPR-Cas systems can target endogenous host factors that regulate insect feeding preference or sensitivity. In rice, CRISPR-Cas9 targeted knockouts have established resistance against major destructive pests, including the brown planthopper (*Nilaparvata lugens*, BPH) and the striped stem borer (*Chilo suppressalis*, SSB). Disruption of specific host metabolic regulators diverts nutrient flow or enhances synthesis of endogenous defensive metabolites, such as phenolamides and flavonoids, restricting pest digestive capacity (Wu et al., 2022). In parallel with crop genome modifications, CRISPR platforms are increasingly applied

directly against pest vector populations. Editing vital survival genes or insecticide-resistance loci in phytophagous insects such as species within *Spodoptera*, *Helicoverpa*, *Nilaparvata*, *Locusta*, and *Agrotis* enables the development of population control tactics. For instance, employing synthetic CRISPR-driven gene drives allows targeted gene disruptions to spread rapidly through wild insect populations, reducing pest fertility or restoring sensitivity to conventional, eco-friendly biopesticides (Zhao et al., 2024).

5. Implementation Bottlenecks, Delivery Systems, and Agronomic Trade-Offs

5.1 Nanotechnology-Mediated Nanocarriers and DNA-Free RNP Delivery

A major bottleneck limiting the commercial deployment of CRISPR-edited crops is the efficient, genotype-independent delivery of editing machinery across the rigid, multi-layered plant cell wall. Traditional transformation methods, including *Agrobacterium tumefaciens*-mediated transfer and biolistic particle bombardment, frequently suffer from low transformation efficiency in recalcitrant elite crop varieties, variable tissue culture regeneration requirements, and unexpected genomic integration of vector backbone sequences (Khan et al., 2026).

To overcome these constraints, nanobiotechnology-enabled delivery systems have emerged as transformative platforms. Nanocarriers including lipid nanoparticles (LNPs), single-walled carbon nanotubes (SWCNTs), cell-penetrating peptides (CPPs), and plant-derived exosomes can encapsulate CRISPR components and pass through cell wall pores (Suryawanshi et al., 2026).

Crucially, nanocarriers facilitate direct delivery of pre-assembled CRISPR-Cas ribonucleoprotein (RNP) complexes into plant protoplasts, microspores, or meristematic tissues. Delivering RNPs circumvents the transcription and translation steps required by plasmid DNA, enabling immediate target cleavage (Öktem et al., 2023). Because RNPs are rapidly degraded by endogenous cellular proteases and nucleases after execution of targeted cleavage, off-target editing events are substantially reduced compared to stable transgenic expression. Most importantly, RNP delivery yields completely transgene-free genome-edited plants that contain no foreign DNA, facilitating integration into sustainable agricultural systems and global regulatory approval pathways (Ramakrishnan et al., 2025).

5.2 Evolutionary Dynamics, Off-Target Effects, and Fitness Penalties

Despite the high efficiency of CRISPR-mediated genome editing, engineering pathogen and pest

resistance involves physiological costs and evolutionary constraints. Complete loss-of-function knockouts of multi-functional susceptibility genes can trigger pleiotropic developmental defects. A prime example is observed in MLO locus disruption. While knocking out MLO alleles confers robust resistance to biotrophic powdery mildew fungi, it frequently induces premature leaf senescence, chlorosis, reduced seed weight, and heightened susceptibility to necrotrophic pathogens, such as *Ramularia* leaf spot (*Ramularia collo-cygni*) in barley (Tyagi et al., 2020).

These physiological penalties highlight the advantages of sub-functional editing over broad coding-sequence disruptions. Mutating specific cis-regulatory promoter elements (e.g., EBEs in *OzSWEET14* or *CsLOB1*) or introducing single amino acid swaps via base editing selectively abolishes pathogen effector binding or viral protein recruitment while preserving the primary physiological and developmental roles of host genes (Shouse et al., 2024).

Another critical constraint is the rapid mutation rate of plant pathogens and insect pests. Single gene resistance mechanisms, whether introduced by conventional breeding or single-locus CRISPR editing, exert high selective pressure on pathogen populations, accelerating the evolution of novel effector variants that bypass host defenses. To foster durable field resistance, breeders must utilize CRISPR multiplexing to implement polygenic defense strategies simultaneously targeting multiple non-homologous *S*-genes, pyramiding complementary NLR receptors, and stacking structural and chemical defense pathways (Hawkins et al., 2019).

Finally, potential off-target cleavage at non-intended genomic sites sharing sequence homology with the guide RNA poses risks of inadvertent gene disruption. Mitigating off-target effects requires high-fidelity Cas variants (e.g., SpCas9-HF1, HypaCas9, eSpCas9), rigorous computational sgRNA selection algorithms, and short-lived RNP delivery modalities (Joshi et al., 2024).

6. Global Regulatory Landscapes and Market Commercialization

The global transition of CRISPR-edited crops from research environments to commercial agricultural markets relies heavily on evolving international regulatory frameworks. A central determinant of market access is whether gene-edited crops lacking foreign DNA integration are categorized as traditional Genetically Modified Organisms (GMOs) or as products equivalent to conventional breeding variants (Tuncel et al., 2023).

International regulatory approaches have diverged significantly, though a trend toward trait-based, non-transgenic exemptions is accelerating. In the United States, the Department of Agriculture (USDA) updated its biotechnology regulations under the SECURE (Seamless, Efficient, and Ag-Certainty for Biotechnological Innovations) Rule (Ahmad et al., 2026). Under this framework, gene-edited plants containing targeted single-base changes, small indels (SDN-1 type edits), or pre-existing native allele replacements that could have been achieved through conventional breeding are exempt from burdensome GMO regulatory oversight, drastically reducing time and financial costs required for commercial release (Chavhan et al., 2025).

In the European Union, genome-edited plants historically fell under strict GMO Directives following a 2018 European Court of Justice ruling. However, recent legislative proposals regarding New Genomic Techniques (NGTs) establish a regulatory shift. The proposed framework classifies NGT Category 1 plants those with up to 20 precise genetic modifications that mimic natural or conventionally bred variation separately from transgenics, removing transgenic labeling and extensive risk assessment requirements (Escajedo et al., 2023).

Similarly, the United Kingdom enacted the Genetic Technology (Precision Breeding) Act, deregulating precision-bred plants containing modifications achievable through traditional breeding. Other major agricultural economies, including Canada, Japan, Australia, Brazil, and Argentina, have instituted risk-proportionate,

science-based policies that exempt non-transgenic SDN-1 edits from GMO oversight (Hallerman et al., 2024). Harmonizing these international regulatory frameworks remains essential to facilitate global seed distribution, avoid cross-border trade disruptions, and realize the global potential of CRISPR technology for climate resilience and sustainable food security (Rohrbach et al., 2003).

Conclusion

CRISPR-based genome editing has fundamentally transformed the paradigm for developing disease-resistant and pest-resistant crops, offering unprecedented precision, speed, and versatility compared to conventional breeding and earlier nuclease technologies. The expanding CRISPR toolkit encompassing Cas9, Cas12a, Cas13, base editors, prime editors, and CRISPRa systems enables diverse strategies ranging from simple gene knockouts to precise single-nucleotide substitutions and transcriptional modulation. Key successes in disrupting pathogen susceptibility genes, such as promoter editing of *OsSWEET* transporters for bacterial blight resistance in rice and *CsLOB1* for citrus canker, demonstrate the practical feasibility of generating robust, durable resistance without compromising agronomic performance. Similarly, engineering plant-insect interactions through volatile profiling and morphological modification presents promising avenues for sustainable pest management that complement biological control approaches. Despite these advances, several critical challenges require resolution before widespread commercial adoption. Pleiotropic effects associated with complete loss-of-function mutations underscore the necessity of precision editing approaches that preserve native gene function while disrupting pathogen co-option. The evolutionary capacity of pathogens and pests to overcome single-gene resistance necessitates multiplexed, polygenic strategies that pyramid complementary defense mechanisms. Off-target effects must be systematically minimized through high-fidelity Cas variants and rigorous guide RNA design. Additionally, efficient, genotype-

independent delivery systems remain a bottleneck, though nanotechnology-enabled RNP delivery offers promising solutions for transgene-free editing. The trajectory of regulatory harmonization particularly the trend toward exempting non-transgenic gene-edited crops from GMO oversight is accelerating commercialization pathways. As international frameworks converge toward science-based, trait-specific regulations, CRISPR-edited crops are positioned to become integral components of sustainable agricultural systems. Realizing this potential will require continued interdisciplinary collaboration among plant geneticists, nanotechnologists, evolutionary biologists, and policymakers to address technical challenges, ensure equitable access, and maintain robust biosafety standards. Ultimately, CRISPR-mediated precision breeding represents a transformative tool for enhancing global food security in an era of climate volatility and growing biotic pressures.

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