Research Progress and Prospects of Gene Editing in Crop Pest-Resistant Molecular Breeding

Yujing Meng*
College of Agronomy, Sichuan Agricultural University, Chengdu, Sichuan, China
**Corresponding Author*

Abstrac: Plant diseases and insect pests are major threats to agricultural production and food security. Genome editing offers a precise, efficient way to breed resistant crops, addressing key limitations of traditional breeding methods. This paper reviews the latest advances in CRISPR-Cas9-mediated genome editing for disease and insect resistance in crops including strawberry, cucumber, tobacco, and cotton. It highlights representative cases of key target gene discovery worldwide, and discusses current technological developments, global regulatory frameworks, and future prospects of genome editing. Research confirms that this technology enables precise trait improvement without exogenous gene integration, providing critical support for food security and the green transformation of agriculture.

Keywords: Genome Editing; CRISPR-Cas9; Crop Resistance Breeding; Food Security; Molecular Breeding

1. Introduction

The Food and Agriculture Organization (FAO) points out in The Future of Food and Agriculture that global agriculture is under growing pressure from pests, diseases and climate change, making disease- and pest-resistant crops essential for sustainable food security [1]. Traditional breeding approaches have obvious drawbacks. Hybrid breeding is limited by reproductive isolation and long cycles; mutation breeding is random and inefficient; transgenic technology faces strict regulation due to exogenous gene insertion [2].

Genome editing represented by CRISPR/Cas9 overcomes these deficiencies. It achieves targeted modification without foreign DNA, improves crop resistance and shortens breeding cycles. CRISPR/Cas-based diagnostics enable early pathogen detection, supporting green prevention and control [3].

Although CRISPR/Cas9 has made progress in rice, maize, tomato and other crops, its application is restricted by unstable editing efficiency, insufficient key gene mining, incomplete technical integration and inconsistent global regulations [2-3]. This paper reviews advances in CRISPR/Cas9 for molecular breeding and pathogen detection, analyzes challenges and prospects future directions, providing references for crop resistance breeding and green pest management innovation.

2. Overview of Gene Editing

2.1 Early Editing Systems: ZFNs and TALENs

Early crop gene editing mainly relied on ZFNs and TALENs. Both recognize DNA via protein modules and function dependent on double-strand breaks. They suffer from cumbersome design and complicated construction, limiting large-scale application and being gradually replaced by CRISPR/Cas9 [4]. As protein-targeted systems, they are less accurate and flexible than CRISPR, base editing and in situ editing .

2.2 Revolutionary Breakthrough of CRISPR/Cas System

CRISPR/Cas9 adopts RNA-guided targeting with simple operation and high efficiency, which has been widely used in crop genetic improvement. Base editing and prime editing further break the limitation of double-strand breaks and achieve high-precision genome modification [5]. The CRISPR/Cas system lays a key technical foundation for base editing and crop in situ editing .

2.3 Development of Advanced Precision Editing Tools

Conventional CRISPR/Cas9 has limitations such as off-target effects, PAM dependence and genome instability. Various DSB-free precision

editing tools have been continuously developed. Different from ZFNs, TALENs, conventional CRISPR/Cas9 and base editing, in situ editing modifies endogenous genes precisely at native loci without introducing exogenous genes, which better meets the requirements of biosafety breeding [6] .

2.4 Application of Prime Editing in Crop In Situ Editing

In situ editing refers to precise modification at native crop gene loci without exogenous fragments, and prime editing is an ideal technique for this strategy. Li et al. (2026) used a prime editing library to perform in situ truncation at the native promoter locus of rice D53 gene. Gene expression was precisely regulated without exogenous DNA integration, providing a typical reference for in situ promoter modification and molecular breeding of crops [7].

3. Regulatory Mechanism Differences Between Crop Disease and Insect Resistance

There are distinct differences in molecular regulation mechanisms between crop disease resistance and insect resistance breeding. Disease resistance mainly relies on SA-JA-ethylene immune signaling crosstalk and pathogen recognition pathways. In contrast, insect resistance research focuses more on JA signal transduction, insect toxin receptors, plant detoxification genes, and pesticide resistance molecular markers.

4. Research Progress of Gene Editing in Molecular Breeding for Crop Disease and Pest Resistance

4.1 Research Progress of CRISPR-Cas9 in Crop Disease Resistance Breeding

4.1.1 Disease resistance breeding of cruciferous crops

CRISPR/Cas9 gene editing presents dual values in genetic improvement and disease resistance breeding of cruciferous crops, including standardized technical system establishment, functional characterization of key disease-resistant genes and creation of broad-spectrum resistance. Ahmad et al. established a standardized CRISPR/Cas9 editing workflow for Brassica juncea. Specific sgRNAs were designed for target genes and delivered into cotyledon explants via Agrobacterium-mediated transformation. The sgRNA guided Cas9 to recognize PAM sequences and induce DNA double-strand breaks (DSBs). DSBs were mainly repaired through error-prone non-homologous end joining (NHEJ), causing insertions, deletions and frameshift mutations to silence target genes. Transgene-free edited plants were finally obtained through progeny screening. This universal and repeatable system fills the technical gap of standardized CRISPR editing in allotetraploid mustard, supporting functional genomics, stress tolerance and quality improvement [8] .Wu et al. focused on clubroot disease, a destructive disease of cruciferous crops. They first identified the susceptible gene GSL5 and clarified its regulatory pathway. The pathogen Plasmodiophora brassicae secretes effector PbPDIa, which directly binds and stabilizes host GSL5 protein. Stable GSL5 suppresses jasmonic acid (JA) biosynthesis and signaling, reduces JA-Ile accumulation, downregulate defense genes such as PR1 and PR2, and inhibits callose and lignin synthesis, thereby promoting pathogen infection. CRISPR/Cas9-mediated GSL5 knockout blocked PbPDIa-GSL5 interaction, restored JA immune pathway, and conferred broad-spectrum high resistance to multiple P. brassicae pathotypes in rapeseed, Chinese cabbage and cabbage, without adverse effects on agronomic traits and yield. This study reveals a core molecular module of “effector-susceptible gene-JA immunity” and provides elite germplasms and breeding strategies for green prevention of clubroot disease [9] .

4.1.2 Disease resistance breeding of tomato

Shawky et al. systematically summarized the application, molecular mechanisms and regulatory networks of CRISPR/Cas9 in tomato biotic stress resistance. Combined with base editing (CBE), editing components were delivered by Agrobacterium transformation or biolistic bombardment. Cas9-induced DSBs were repaired by NHEJ to generate knockout mutations or by homology-directed repair (HDR) for precise modification. Targeting the salicylic acid (SA)-JA-ethylene crosstalk network, knockout of key repressors such as SlSAMT and SlJAZ2 enhanced disease resistance. Disruption of susceptible genes including SlMlo1, SlTOM1 and SlPelo effectively inhibited pathogen colonization and viral proliferation. The edited tomatoes exhibited improved resistance to bacterial spot, bacterial wilt, powdery mildew,

fusarium wilt, ToBRFV and TYLCV with normal growth. CRISPR editing shortens breeding cycles and avoids exogenous DNA integration, offering a safe and efficient strategy for tomato disease resistance improvement and sustainable agricultural development [10] .

4.1.3 Disease resistance breeding of wheat

Yuan et al. constructed a CRISPR/Cas9 system targeting wheat dwarf virus (WDV). sgRNAs were designed against viral overlapping coding regions and large intergenic segments. Cas9 directly cleaved viral genomes, caused irreversible DSB damage, and inhibited viral replication and assembly. This host-independent strategy conferred complete and heritable WDV resistance, providing a novel approach for viral disease control in gramineous crops [11] .Waites et al. summarized three major editing strategies for wheat disease resistance: susceptible gene knockout, resistance gene modification and NLR receptor engineering. NHEJ-mediated mutation of TaMLO1, TaHRC and TaeIF4E enhanced basal immunity and hormone signaling resistance to powdery mildew, fusarium head blight and wheat yellow mosaic virus. HDR and base editing further optimized resistance genes and expanded pathogen recognition spectrum. These two studies jointly improved the CRISPR-based breeding system and provided theoretical and technical support for wheat disease management [12] .

4.1.4 Disease resistance breeding of strawberry

Luo et al. explored the function of magnesium chelatase subunit gene FvCHLI during Xanthomonas fragariae infection. FvCHLI positively regulated abscisic acid (ABA) biosynthesis and signaling. Pathogen infection significantly upregulated FvCHLI, promoted ABA accumulation, activated downstream SnRK2 cascades, and induced stomatal closure to prevent bacterial invasion. CRISPR-generated Fvchli mutants showed decreased ABA content, impaired stomatal immunity, and higher susceptibility to bacterial angular leaf spot. Defects were stably inherited from T_1 to T_3 generations. In addition, chlorophyll synthesis was inhibited in mutant leaves. This study demonstrates the dual roles of FvCHLI in growth and immunity, and provides a key target gene for strawberry disease resistance breeding [13] .

4.1.5 Disease resistance breeding of cucumber

Fidan et al. performed CRISPR/Cas9 editing on the eukaryotic translation initiation factor gene eIF4E in cucumber. As a core susceptible gene for potyviruses, eIF4E interacts with viral VPg protein to support viral replication. Two sgRNAs targeting different exons of eIF4E were constructed and transformed into cucumber inbred lines. NHEJ-mediated small deletions caused frameshift mutation and premature termination, blocking VPg-eIF4E interaction. Homozygous mutants exhibited complete resistance to WMV, ZYMV and PRSV with negative viral detection. Even partial mutants showed reduced viral accumulation. All edited lines maintained normal plant height, fruit traits and yield. This study confirmed the distinct antiviral effects of different eIF4E mutations and established a scalable gene editing system for cucumber viral disease resistance [14] .

4.1.6 Disease resistance breeding of citrus

Khadgi et al. applied CRISPR/Cas9 to combat citrus Huanglongbing and citrus canker. Single and multi-gene mutants of SWEET family members were created in sweet orange, lemon and citrange. Pathogen inoculation, qPCR and transcriptome analysis confirmed that SWEET15 was markedly induced by pathogens. SWEET15 knockout significantly reduced pathogen titer and disease symptoms. Double or triple mutations of SWEET10/12/15 further enhanced broad-spectrum resistance. Mechanistically, pathogens hijack SWEET15 to acquire sugar nutrition for colonization. Disruption of sugar transport restricts pathogen growth and activates host immune responses. CRISPR-mediated SWEET15 editing generates new citrus germplasms with dual disease resistance, overcoming the shortage of resistant citrus resources [15] .

4.2 Research Progress of CRISPR-Cas9 in Crop Insect Resistance Breeding

4.2.1 Insect resistance breeding of tobacco

Based on the research platforms of the Chinese Academy of Agricultural Sciences, Southwest University and Sichuan Academy of Agricultural Sciences, Li et al. adopted multiple techniques, including transcriptome sequencing (RNA-seq), RT-qPCR, CRISPR/Cas9 gene editing, JA-Ile content determination, insect feeding bioassay and growth index analysis. They systematically analyzed the transcriptomic characteristics of the compatible interaction between tobacco and herbivorous insects, and clarified the molecular mechanism of jasmonoyl-L-isoleucine hydrolase 1 (JIH1) in regulating the growth-defense trade-

off in tobacco.In the compatible interaction induced by insect feeding, the JA-Ile signaling pathway was significantly activated, and JIH1, a key degrading enzyme of JA-Ile, exhibited dynamic expression changes with feeding duration. Molecularly, JIH1 negatively regulated insect defense by specifically hydrolyzing JA-Ile to weaken JA signaling, while positively modulating the expression of growth-related genes to maintain the dynamic balance between growth and defense. CRISPR/Cas9-mediated JIH1 knockout markedly increased endogenous JA-Ile accumulation, upregulated defense genes such as PR, LOX and AOS, and enhanced insect resistance, accompanied by reduced plant height, biomass and photosynthetic efficiency. In contrast, JIH1 overexpression lines showed weakened defense but accelerated growth. Transcriptome analysis further confirmed that JIH1 coordinated the expression of defense and growth genes by regulating the downstream transcription factor network of JA signaling, acting as a core regulator of the growth-defense trade-off. This study first revealed the pivotal role of JIH1 in tobacco-insect interaction and the molecular basis of JA-Ile metabolism in balancing plant growth and defense, providing crucial target genes and theoretical references for insect-resistant crop breeding [16] .

4.2.2 Insect resistance breeding of maize

With the support of Nanjing Agricultural University and the University of Arizona, Wu et al. used CRISPR/Cas9 editing, insect virulence bioassay, genetic linkage analysis, Sf9 heterologous expression and Xenopus oocyte patch-clamp technology to clarify the differential toxic pathways and functional redundancy of Bt proteins Cry1Ab and Cry1Fa against Ostrinia furnacalis.The toxicity of Bt proteins relies on specific binding to midgut membrane receptors and subsequent pore formation. Cadherin, ABCC2 and ABCC3 are core receptors mediating Bt toxicity in O. furnacalis. Cry1Ab possesses two redundant toxic pathways: pathway 1 depends on ABCC2 independent of cadherin and ABCC3, while pathway 2 requires the coordination of cadherin and ABCC3. By comparison, Cry1Fa only relies on the ABCC2-dominated pathway. Six homozygous knockout lines, including single and double mutants, were constructed via CRISPR-Cas9. Bioassays showed that simultaneous disruption of two redundant pathways generated high-level resistance to Cry1Ab, whereas ABCC2 knockout alone led to extreme resistance to Cry1Fa. Heterologous expression and electrophysiological tests further verified the receptor-specific pore-forming mechanism. This study demonstrated the functional redundancy of Cry1Ab toxic pathways and the distinct dose effect of resistance-related genes, offering key strategies for Bt resistance management and durable insect-resistant maize breeding [17] .

4.2.3 Insect resistance breeding of cotton

Helicoverpa armigera is a destructive global agricultural pest, and Cry1-type Bt toxins are widely applied for its control, with field resistance becoming a major limiting factor. Fang et al. employed CRISPR/Cas9 editing, embryo microinjection, Sanger sequencing, insect bioassay, RT-qPCR and RNA-seq to explore the function and molecular mechanism of midgut cadherin gene HaCad1 in mediating Cry1 resistance in Helicoverpa armigera conferta.HaCad1 acts as a critical receptor for Cry1 toxins. After activation in the alkaline midgut environment, Cry1Ac binds to the membrane-proximal domain of HaCad1, triggering toxin oligomerization, membrane insertion and cation channel formation, which eventually causes midgut cell lysis and larval death. Loss-of-function mutation of HaCad1 blocks toxin binding and reduces larval susceptibility to Cry1 toxins. A homozygous HaCad1 knockout line with an 11 bp deletion was generated by CRISPR/Cas9, showing a 44-fold resistance ratio to Cry1Ac and 16-fold resistance to Cry1A.105, but no obvious resistance to Cry1F. Transcriptome data revealed significant upregulation of immune response and P450 detoxification pathways in mutant larvae. This study confirmed that HaCad1 knockout confers Cry1 resistance in Australian cotton bollworms, explaining the low frequency of field Cry1Ac resistance and optimizing regional Bt resistance management strategies [18] .

4.2.4 Insect resistance breeding of soybean

Cai et al. conducted research at Nanjing Agricultural University and the National Key Laboratory of Crop Genetics and Germplasm Innovation. Combining CRISPR/Cas9 editing, Agrobacterium-mediated transformation, multi-omics analysis and field insect resistance identification, they illustrated the regulatory mechanism of oil body calcium-binding protein gene GmCLO1 in soybean resistance to Spodoptera litura.GmCLO1 functions as a negative regulator of the JA signaling pathway,

which represses the biosynthesis of JA and bioactive JA-Ile to weaken insect defense responses. GmCLO1 knockout relieved such inhibition, activated downstream defense genes, and enhanced insect resistance. Insect feeding significantly induced GmCLO1 expression. CRISPR-generated knockout lines exhibited elevated JA accumulation and strong resistance, while overexpression lines showed weakened defense capacity. Combined transcriptomic and metabolomic analysis identified a series of differentially expressed defense-related genes and secondary metabolites involved in JA-mediated resistance. Moreover, GmCLO1 modification had no adverse impacts on major agronomic traits and yield. This study identified a novel negative regulatory gene in the JA cascade, providing valuable genetic resources for high-yield and insect-resistant soybean molecular breeding [19] .

4.2.5 Insect resistance breeding of rice

Based on research institutions in Jiangsu Province, Zhang et al. utilized CRISPR/Cas9-mediated homology-directed repair (HDR) knock-in, bioassay, genetic hybridization and fitness evaluation to verify the causal relationship between the CHS1 G932C mutation and buprofezin resistance in Nilaparvata lugens.As a chitin synthesis inhibitor, buprofezin disrupts insect growth and development by blocking chitin production. The G932C substitution in chitin synthase 1 alters protein spatial conformation and enzyme activity, reducing target sensitivity and conferring pesticide resistance. The site-specific G932C mutation was precisely knocked in via embryo microinjection, generating a homozygous mutant line with 94.9-fold resistance to buprofezin. This resistance was inherited in an incomplete dominant manner, and mutant insects displayed obvious fitness costs including reduced survival rate, emergence rate and fecundity. This study directly validated the function of the key resistance mutation in hemipteran insects, providing specific molecular markers for field resistance monitoring and theoretical support for rational pesticide rotation and resistance management [20] .

4.2.6 Insect resistance breeding of potato

Sonawala et al. from the University of Cambridge and the James Hutton Institute established a standardized CRISPR-Cas9 targeted long-read sequencing protocol for the two major potato cyst nematodes, Globodera pallida and Globodera rostochiensis.This in vitro Cas9 targeted enrichment workflow designed high-specific sgRNAs flanking effector genes such as HYP1 and HYP3. High-molecular-weight genomic DNA extracted from second-stage juveniles was cleaved by pre-assembled Cas9 ribonucleoprotein complexes. Target fragments were further processed and sequenced via Nanopore platforms. This method dramatically improved target region coverage without PCR amplification, eliminating amplification bias and enabling accurate variation detection. Critical technical parameters, including high-purity DNA extraction and high-efficiency sgRNA design, were systematically optimized. This versatile technical system is suitable for genetic variation analysis and functional research of plant-parasitic nematodes, breaking the limitation of low target coverage in conventional whole-genome sequencing [21] .

4.2.7 Insect resistance breeding of rosaceae fruit trees

Focusing on the increasing abamectin resistance of Grapholita molesta, Su et al. (2025) screened and functionally verified the core detoxification gene GmGSTs2 using toxicology detection, in vitro enzyme activity assay, CRISPR/Cas9 knockout and Drosophila heterologous overexpression.The resistant strain showed 99.75-fold abamectin resistance with significantly elevated GST activity. GmGSTs2 was markedly upregulated in resistant larvae and specifically expressed in midgut and abdominal detoxification tissues. In vitro recombinant GmGSTs2 protein directly degraded abamectin and eliminated pesticide-induced ROS to alleviate oxidative damage. CRISPR-mediated GmGSTs2 knockout sharply reduced resistance levels and aggravated ROS accumulation, while heterologous overexpression in Drosophila enhanced pesticide tolerance. In summary, GmGSTs2 mediates high-level metabolic resistance through dual pathways of xenobiotic degradation and oxidative stress scavenging. This study clarified the molecular mechanism of metabolic resistance in fruit borer pests, providing molecular markers for field resistance monitoring and new targets for green pest control [22] .

5. Current Challenges and Regulatory Status of Gene-Edited Crops

Gene editing is a core innovation in agricultural biotechnology, and its industrialization is closely

tied to global regulation. Driven by differences in scientific understanding, industrial needs and social values, global regulatory frameworks for gene-edited crops are diverse and dynamic.Three major regulatory models exist internationally. The EU applies strict process-oriented regulation, classifying gene-edited crops as GMOs with full risk assessment [23-25] .The US and Canada adopt product-oriented regulation, simplifying approval for transgene-free edited products.Australia and Japan implement classified regulation, exempting SDN-1 products from GMO oversight[24] .Global regulatory coordination is gradually strengthening.China has established a risk-based regulatory system under the Biosafety Law, but industrialization faces challenges in approval, intellectual property and public perception [23,25] .Qin & Su pointed out key constraints including poor industry-university-research collaboration, inefficient approval, insufficient IPR protection and public bias, putting forward targeted regulatory suggestions.Although unified global standards have not been formed, classified, risk-adapted and innovation-friendly regulation has become an international consensus [22-24] .Future efforts will focus on scientific regulation and global policy alignment to support sustainable agricultural development [23-24] .

6. Conclusions and Perspectives

Gene editing, especially CRISPR-Cas9, has boosted crop pest and disease resistance breeding, yet faces challenges including low editing efficiency, limited resistance genes, divergent global regulations and low public acceptance.

Future research will focus on mining resistance genes, optimizing editing systems, and pyramiding elite traits. Improved regulations and public acceptance will facilitate its agricultural application, supporting food security and green agricultural development.

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