

Online ISSN

3007-3197

Print ISSN

3007-3189

<http://amresearchreview.com/index.php/Journal/about>

Breeding for Durable Resistance in the Era of Gene Editing: From R-Genes to Susceptibility (S)-Gene Knockouts

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ABSTRACT

The quest for durable disease resistance in crops has entered a transformative era with the convergence of genomic discovery and precision gene editing technologies. Traditional breeding has been successful in providing resistance (R) genes, but they typically provide only race-specific resistance that can be easily overcome by evolution of the pathogens. Susceptibility (S) genes, however, are plant genes that pathogens use to infect plants, and are a completely different target for resistance breeding. Loss-of-function mutations in S genes may provide broad-spectrum and possibly more persistent resistance, since pathogens may not be able to overcome host factors that they require for their growth. This review summarizes what is known about R-gene and S-gene mediated resistance, and discusses the mechanisms underlying the function of the S-gene for various groups of pathogens. Targeted S-gene knockout using CRISPR/Cas technology, including successful case studies in powdery mildew resistance (via MLO), virus resistance (via eIF4E) and bacterial blight resistance (via SWEET promoter), is evaluated. The challenge of growth-defense trade-offs that arise when disrupting the S-gene is critically discussed, and novel methods to reduce such costs, such as precise regulatory editing, tissue-specific expression and identification of natural alleles of loss of function, are explored. S-gene targeting, along with the recently developed multiplex genome editing techniques, combined with the emerging multi-omics data for the discovery of S-genes, makes this a key method for the next generation

of crop improvement. Finally, future directions are presented, such as the potential for multi-gene pyramiding of R genes, in combination with S gene editing, to provide high and durable disease resistance in the context of climate change and changing the population of pathogens.

Keywords— durable resistance, susceptibility genes, CRISPR/Cas9, R-genes, S-genes, gene editing, broad-spectrum resistance, plant immunity.

1. Introduction

Plant diseases are a growing threat to global food production, resulting in annual losses of 17-30% in most staple crops, such as wheat, rice, maize, potato and soybean (Zhang *et al.*, 2025). The development of durable disease resistance has emerged as a critical priority for sustainable agriculture as disease pressure is projected to increase and pathogen ranges are expected to expand in the near future due to the growing global population of 10 billion by 2050 and climate change (Thakur *et al.*, 2025).

Plant breeders, traditionally, have used genes that enable plants to recognize particular pathogen effectors and mount strong defense responses, called resistance (R) genes. R-gene mediated resistance (Rg) usually works according to the gene-for-gene model, where a plant R protein recognizes a corresponding pathogen avirulence (Avr) effector, resulting in an effector triggered immunity (ETI) (Thakur *et al.*, 2025). The use of this approach has proven to be very effective and R genes have been used against a variety of pathogens. The key limitation of R-gene resistance is that it is also their greatest strength: pathogen can change or lose the cognate effector and easily beat single-gene resistance (Wong *et al.*, 2025).

A new paradigm has developed because it is now understood that infection requires specific host factors in the presence of a pathogen. Susceptibility genes (S) are plant genes that are used by pathogens to enter the plant, suppress the immune response, help obtain nutrients or to finish their life cycle (Thakur *et al.*, 2025). The concept of S-gene mediated resistance was first seen in barley mlo mutants that provide highly durable and broad-spectrum powdery mildew resistance and have continued to be effective for more than 50 years (Thakur *et al.*, 2025). S-gene resistance is a loss of function, whereas R-gene resistance is a gain of function.

Since the introduction of CRISPR/Cas-based genome editing, the ability to precisely and target knock out S genes has revolutionized the manipulation of S genes, eliminating the requirement to use transgenic integration (Wong *et al.*, 2025). This technology has led to the fast advance of S-gene discovery into the field of resistance breeding in a variety of crops. This review discusses the biology of R- and S-gene mediated resistance, successful examples of S-gene editing, challenges of pleiotropic effects, and a strategy for combining these approaches to provide durable disease resistance.

2. Plant Immunity and the R-Gene Paradigm

2.1 The Two-Tiered Immune System

Plant possess an elaborate two-layered immune system that they use to protect against invaders such as pathogens (Thakur *et al.*, 2025; Wu *et al.*, 2025). Pattern triggered

Online ISSN

3007-3197

Print ISSN

3007-3189

<http://amresearchreview.com/index.php/Journal/about>

immunity (PTI) is activated through the recognition of conserved pathogen-associated molecular patterns (PAMPs) or damage-associated molecular patterns (DAMPs) by transmembrane pattern-recognition receptors (PRRs) (Thakur *et al.*, 2025). PTI induces mitogen-activated protein kinase (MAPK) cascade, transcriptional reprogramming via transcription factors like WRKY and generation of reactive oxygen species (ROS). Key defence hormones are also involved in this response such as salicylic acid (SA) for biotrophic pathogens and jasmonic acid (JA) and ethylene for necrotrophs (Thakur *et al.*, 2025).

Effector triggered immunity (ETI) is triggered by recognition of specific pathogen effectors (also termed avirulence (Avr) proteins). The majority of ETI-associated receptors are intracellular nucleotide-binding/leucine-rich repeat (NLR) proteins that detect either direct or indirect effectors (Thakur *et al.*, 2025). In response to ETI, there are generally higher and more persistent ETI responses, and by the time of infection, a localized hypersensitive reaction (HR), with programmed cell death, usually occurs at the infection site (Thakur *et al.*, 2025).

R-Genes are a pair of genes that help protect against HIV infection. R-Genes are a pair of genes that do help to prevent HIV infection.

Plants have R genes that encode immune receptors that allow them to recognize specific strains of pathogens. The majority of R genes are members of the NLR family that also have a leucine-rich repeat (LRR) domain, and the N-terminal structure of R genes varies (Thakur *et al.*, 2025). Another class of R genes (Wu *et al.*, 2025) is membrane-localized receptor-like kinases (RLKs) and receptor-like proteins (RLPs).

The biggest drawback of R-gene resistance is its race-specificity. R genes generally only detect pathogens that contain specific effectors; if those effectors change or are lost, resistance will be lost (Thakur *et al.*, 2025; Zhang *et al.*, 2025). New races of the pathogen have emerged which evade recognition in the field and have overwhelmed resistance to the R gene several times. In addition, most durable deployments of R gene(s) involve pyramiding multiple R genes, which is a time-consuming process when using traditional breeding methods (Zhang *et al.*, 2025).

3. Susceptibility Genes: New Frontiers.

3.1 Defining S-Genes

Susceptibility (S) genes are genes that pathogens exploit to facilitate infection, colonization or disease development in plants (Thakur *et al.*, 2025; van Schie and Takken, 2025). These genes are not just "passive targets" but "active players" in pathogen success in the plant genome: a "pro-infection" category of genes. The disruption or alteration of the S genes may thus weaken the pathogen's infection capabilities, but not necessarily impact the growth and development of the plant (Thakur *et al.*, 2025).

There are some significant differences between S-gene and R-gene-mediated resistance:

S-gene mutants generally give resistance to several strains (and sometimes species) of pathogens (van Schie and Takken, 2025).

Greater resistance to pathogens: Pathogens are not able to easily adapt themselves to survive without the host factors that are vital to their infection cycle (Thakur *et al.*, 2025)

Both alleles need to be mutated to achieve S-gene resistance (van Schie and Takken, 2025)

Pleiotropy: S genes may perform physiological functions and a disruption of these genes may lead to an unintended growth or developmental effect (Wu *et al.*, 2025)

3.2 Classification of S-Gene

Wu *et al.*, 2025 divided S genes into three functional groups according to their role in infection:

Pathogen Entry and Access. Class I: Pathogen Entry and Access. The genes in the S are responsible for pathogen entry into host tissue. For instance, powdery mildew fungi can only infect a host plant's epidermal cells when the host has the MLO (Mildew Resistance Locus O) genes. Mutations in MLO block entry of the fungus, and render plants resistant (Thakur *et al.*, 2025). Likewise, genes involved in stomatal regulation can be utilized by bacterial species such as *Pseudomonas syringae* that enters through the stomata and keeps stomata open with phytotoxin coronatine (Wu *et al.*, 2025).

Class II: Pathogen Nutrition and Support. These S genes are necessary for the supply of nutrients or establishment of a favourable microenvironment for the growth of pathogens. A classic example is the SWEET gene family that encodes sugar transporters. *Xanthomonas oryzae* delivers transcription activator-like effectors (TALEs) that upregulate certain SWEET genes which upregulate the concentration of sugars in the apoplast and aid in the proliferation of the bacterium (van Schie and Takken, 2025). Mutations in the targeted SWEET genes or in their promoter may lead to resistance (van Schie and Takken, 2025).

Sheep and goats that are negative regulators of immunity. These S genes can actively interfere with the plant immune system. Pathogens take advantage of these genes to suppress host immune responses. The salicylic acid-mediated defense response is silenced by the DMR6 gene in *Arabidopsis*; knockout of the gene confers increased resistance to downy mildew disease and to bacterial pathogens (Wu *et al.*, 2025). Likewise, the gene named Pi21 in rice is able to control the defense against rice blast (van Schie and Takken, 2025).

Online ISSN

3007-3197

Print ISSN

3007-3189

<http://amresearchreview.com/index.php/Journal/about>**Table 1: Major Susceptibility Genes Targeted for Disease Resistance**

Gene	Pathogen Type	Mechanism	Crop	Reference
<i>MLO</i>	Powdery mildew (fungi)	Facilitates fungal penetration	Barley, wheat, tomato, pea	Thakur <i>et al.</i> , 2025; Wu <i>et al.</i> , 2025
<i>eIF4E/eIF(iso)4E</i>	Potyriviruses (viruses)	Supports viral RNA translation	Tomato, rice, barley, lettuce, melon, pea, pepper	Thakur <i>et al.</i> , 2025
<i>SWEET</i>	Bacterial blight (bacteria)	Sugar transporters for pathogen nutrition	Rice, cotton	Zhang <i>et al.</i> , 2025; van Schie and Takken, 2025
<i>DMR1/DMR6</i>	Downy mildew, bacteria	Negative regulators of SA defense	Arabidopsis, tomato	Wu <i>et al.</i> , 2025
<i>EDR1</i>	Powdery mildew, bacteria	Negative regulator of defense	Arabidopsis, tomato	Wu <i>et al.</i> , 2025
<i>Pi21</i>	Rice blast (fungi)	Defense suppressor	Rice	van Schie and Takken, 2025
<i>LOX3</i>	Smut (fungi)	Susceptibility factor	Maize	Pathi <i>et al.</i> , 2020
<i>NLR</i>	Multiple pathogens	Defense suppression	Tomato	Wu <i>et al.</i> , 2025

4. Gene Editing Technologies for S-Gene Modification

4.1 CRISPR/Cas9 and its Variants

The clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated protein 9 (Cas9) system has been a game-changer in plant genome editing, boasting its specificity, adaptability, ease of use, and cost-effectiveness (Thakur *et al.*, 2025). The system involves the guide RNA or gRNA which leads the Cas9 nuclease to a specific genomic sequence to create a double-strand break (DSB). In plants these DSBs are mostly repaired by the error-prone non-homologous end joining (NHEJ) pathway, which often introduces small insertions or deletions (indels) that can disrupt gene function (van Schie and Takken, 2025).

CRISPR/Cas9 is broadly used for S-gene knockout in various crops, including a large number of species (Thakur *et al.*, 2025; Wu *et al.*, 2025). For S-gene editing, several technical advances are of particular interest:

Multiplex CRISPR: Target several S genes simultaneously, for overcoming

Online ISSN

3007-3197

Print ISSN

3007-3189

<http://amresearchreview.com/index.php/Journal/about>

functional redundancy (Zhang *et al.*, 2025)

Base editing: Precise single nucleotide editing without the need of DSB, fine-tuning gene function without complete knockout

Prime editing: precise edit (substitution, insertion, deletion) (Zhang *et al.*, 2025)

Transgene-free editing is achieved using ribonucleoprotein (RNP) complexes as well as transient expression and segregation in order to avoid transgene integration that raises regulatory issues (Wu *et al.*, 2025)

4.2 Beyond CRISPR: TALENs and ZFNs

Prior to CRISPR, the precise gene editing technologies of transcription activator-like effector nucleases (TALENs) and zinc finger nucleases (ZFNs) were employed. (van Schie and Takken, 2025) Promoter-targeted modifications are good applications for S-gene editing by TALENs. For instance, resistance to *Xanthomonas oryzae* strains that require certain effector-binding elements (EBEs) in the rice SWEET14 promoter was conferred by the deletion of these elements (van Schie and Takken, 2025).

In polyploid crops, multiplexing is possible as well. Multiplexing is also possible for polyploidy crops.

S-gene editing is a special case for polyploid crops, as multiple copies of the gene have to be disrupted to obtain complete loss of function. In this regard, CRISPR/Cas9 is very useful. For instance, the three homoeologues of TaMLO (TaMLO-A1, TaMLO-B1 and TaMLO-D1) have been co-edited in hexaploid wheat to provide powdery mildew resistance (Wu *et al.*, 2025; van Schie and Takken, 2025). Simultaneous editing of multiple homoeologues using the multiplexed gRNAs is close to impossible with conventional mutagenesis.

5. Successful applications of S-Gene Editing

5.1 MLO

Mediated Powdery Mildew Resistance. 5.1 Powdery Mildew resistance mediated by MLO.

In the 1940s, barley mlo mutants were found that gave the first indication of the possibility of achieving durable disease resistance through disruption of S-genes (Thakur *et al.*, 2025). Powdery mildew fungi need to penetrate epidermal cells and the MLO genes are highly conserved in angiosperms. The loss-of-function mutants of mlo do not show resistance against other pathogens, but rather against powdery mildew in a broad spectrum (Thakur *et al.*, 2025).

CRISPR/Cas9 editing of orthologs of MLO has now been realized in various crops (Thakur *et al.*, 2025):

For barley, natural mutants for mlo have been developed and are providing long-term resistance for decades.

Frequent editing of three wheat homoeologues provide resistance.

Powerful mildew resistance has been seen with tomato edited with SIMLO1. SIMLO1 editing gives tomato powdery mildew resistance.

Pea - editing of Pea confers resistance with the gene PeaMLO1.

Strawberry and grapevine: editing of MLO has been done

But when you edit the various parts of MLO, there are growth penalties. The barley mlo mutants have also some loss of yield and the wheat mlo mutants have leaf spotting (Thakur *et al.*, 2025). This highlights the need for investigation of S-gene beyond

Online ISSN

3007-3197

Print ISSN

3007-3189

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pathogen susceptibility.

5.2 Mediated Virus Resistance

Potyvirus manipulates S genes, which are eukaryotic translation initiation factor 4E (eIF4E) and its isoform, eIF(iso)4E, to initiate translation of their RNA (Thakur *et al.* 2025). Loss-of-function mutations in these genes have been shown to provide resistance to potyviruses in a variety of crops such as tomato, rice, barley, lettuce, melon, pea and pepper (Thakur *et al.*, 2025).

In tomato, the resistance to pepper mottle virus (PepMoV) has been successfully achieved using CRISPR/Cas9 editing and without compromising the growth of the plants (Wu *et al.*, 2025). Likewise, cucumber and *Arabidopsis* have been transformed to be potyvirus resistant through editing of the gene, eIF4E (van Schie and Takken, 2025).

A rectangular container is filled with soil that has been mixed with fertilizer. A rectangular box is packed with soil and fertilizer.

The bacterial blight of rice is caused by *Xanthomonas oryzae* pv. *oryzae* (Xoo), is a devastating disease of rice. The TALEs introduced into plant cells target specific EBEs in the promoters of the genes encoding sugar transporters, which increases their expression, and thus the availability of nutrients for Xoo growth (van Schie and Takken, 2025; Zhang *et al.*, 2025).

Resistance to the TALE can be achieved by editing the EBEs in the gene promoters, which still does not disrupt the entire gene, but prevents binding of the TALE and its activation of the gene. This method has great advantages over total gene disruption:

Does not affect pleiotropy: The SWEET genes function normally

Multi-strain resistance (van Schie and Takken, 2025); targeting EBEs that are common across multiple TALEs gives resistance against multiple Xoo strains.

It has been successfully used to promote SWEET11, SWEET13 and SWEET14 in rice (Zhang *et al.*, 2025). The same strategy could be used for other pathogens that impact host transcription with the aid of TALEs. This is a broad-spectrum tomato plant resistant to most diseases. This is a broad-spectrum resistant tomato.

There are several successful applications of S-gene editing in tomato (Wu *et al.*, 2025; Wong *et al.*, 2025):

Resistant to *Pseudomonas syringae* and *Xanthomonas* species (enhanced by editing).

SIMLO1: Powdery mildew resistance is conferred by editing

Resistance to bacterial and fungal pathogens increased by editing (SIEDR1)

Editing results in increased resistance to *Pseudomonas syringae* (SIJAZ2)

SINRX1: Resistance to *Botrytis cinerea* and *Pseudomonas syringae* is improved with editing

A modest increase in resistance to *Fusarium oxysporum* is obtained with editing of SIPSK1/SIPSK2.

Notably, in some cases, editing single S genes can provide resistance against multiple pathogen types. Bacterial and fungal disease resistance are improved by editing of genes in SIDMR6, for instance (Wu *et al.*, 2025).

Table 2: Case Studies of CRISPR/Cas9 S-Gene Editing for Disease Resistance

Crop	Target Gene(s)	Target	Editing	Outcome	Reference
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		Pathogen	Approach		
Barley	<i>MLO</i>	Powdery mildew	Knockout	Durable resistance, 50+ years effective	Thakur <i>et al.</i> , 2025
Wheat	<i>TaMLO-A1, -B1, -D1</i>	Powdery mildew	Multiplex knockout	Broad-spectrum resistance	Thakur <i>et al.</i> , 2025
Wheat	<i>TaPsIPK1</i>	Stripe rust	Knockout	Resistance without yield penalty	Zhang <i>et al.</i> , 2025
Tomato	<i>SIMLO1</i>	Powdery mildew	Knockout	Complete resistance	Wu <i>et al.</i> , 2025
Tomato	<i>SIDMR6</i>	Bacterial and fungal pathogens	Knockout	Broad-spectrum resistance	Wu <i>et al.</i> , 2025
Tomato	<i>SIJAZ2</i>	Bacterial speck	Knockout	Enhanced resistance	Wu <i>et al.</i> , 2025
Rice	<i>SWEET14</i> promoter	Bacterial blight	EBE deletion	Resistance to Xoo strains	van Schie and Takken, 2025
Rice	<i>Bsr-d1, Pi21, Xa5</i>	Rice blast, bacterial blight	Knockout	Broad-spectrum resistance	Zhang <i>et al.</i> , 2025
Maize	<i>LOX3</i>	Smut	Knockout	Decreased susceptibility	Pathi <i>et al.</i> , 2020
Cotton	<i>GhARM</i> (negative regulator)	Verticillium wilt	Knockout	Enhanced resistance	Zhang <i>et al.</i> , 2025

6. Challenges and Limitations

6.1 Growth-Defense Trade-offs

The biggest problem with S-gene editing is that there is the potential for pleiotropic effects. In addition to their role in conferring pathogen resistance, several S genes also play essential roles in plant growth, development, or yield, and their elimination can adversely affect these traits (Thakur *et al.*, 2025; Wu *et al.*, 2025).

There are a number of examples of this trade-off:

The gene *mlo* confers durable resistance to powdery mildew, although barley mutants with powdery mildew resistance have reduced grain yield and premature senescence in certain genetic backgrounds (Thakur *et al.*, 2025).

- Rice 'rod1': Improves blast and sheath blight resistance, and bacterial blight resistance, but with decreased grain yield (Zhang *et al.*, 2025).

Defensive mutations are associated with a decrease in yield (Zhang *et al.*, 2025) rice

Pi21.

These observations underscore the importance of strategies to control pleiotropic effects without loss of resistance.

The process of expression is tissue specific and may give rise to off-target effects. Tissue-specific expression and off-target effects.

Unintended mutations at sequences similar to the target may occur as a result of CRISPR/Cas9 editing. This is a potential risk that can be reduced with careful gRNA design, but should be taken into account for regulatory approval (van Schie and Takken, 2025).

The challenge is greater for polyploids: multiple copies have to be edited and there is an increased risk of off-target effects. This has been a challenge in recent years, which has been mitigated by the recent progress in development of high specificity Cas variants and design of gRNA.

6.3 Regulatory Hurdles

Gene edited crops have different regulatory status across different countries. In certain countries, transgene free edited plants with CRISPR are treated as nonGMOs and subject to streamlined regulation (van Schie and Takken, 2025). In others, all of the gene edited organisms are governed by a strict GMO regulation. Thus, the development of transgene-free approaches such as RNP delivery and transient expression and segregation is a priority (Wu *et al.*, 2025).

6.4 Pathogen Evolution and Durability

S-gene resistance is likely to be more durable than R-gene resistance, but there is the possibility that pathogens will evolve resistance to it. This is difficult for pathogens, as barley mlo resistance is still effective for more than 50 years (Thakur *et al.*, 2025). Nevertheless, there is no way to rule out the potential for pathogen adaptation, especially in the case of S genes, which change the plant-pathogen interface such that pathogens could potentially overcome these changes.

7. Strategies to overcome Pleiotropic effects

7.1 Precise Regulatory Edits

Instead of gene knockout, editing of regulatory elements can be used to regulate expression of the S gene to provide resistance without pleiotropic effects. A promoter editing strategy that is exemplary is the deletion of EBEs, which results in that pathogen-driven gene activation is blocked, while normal gene expression remains intact (van Schie and Takken, 2025; Zhang *et al.*, 2025).

Likewise, the editing of 3'-UTR regions that influence gene expression may lead to the reduction of S gene function, but not to a complete loss of function (Xu *et al.*, 2023). This method has been used for editing resistance genes in rice such as RNG1 and RNG3, which are 3'-UTR genes involved in rice blast and bacterial blight resistance, respectively, whose editing was shown to enhance resistance to both diseases (Xu *et al.*, 2023).

7.2 Pathogen-Inducible Expression

In the "silencing on demand" approach, the expression of S is regulated by pathogen-inducible promoters (van Schie and Takken, 2025). S genes are normal genes that are turned off when the pathogens attack. In barley, a promoter of a resistance gene, Hv-Ger4c, has been placed under control of the plant's innate immune system (van Schie

and Takken, 2025). This method could be applied to the S genes to achieve a decrease or abolition of pleiotropic effects.

7.3 Natural Allele Mining

Loss of function/suppressed S genes with no fitness costs are commonly found in natural populations that confer resistance and these genes can be controlled transgenerically in crops (Thakur *et al.*, 2025). They can be detected by using genome-wide association studies (GWAS) and can be bred into elite germplasm or induced by gene editing. The presence of natural wheat and barley mlo alleles is a model for this approach.

7.4 Engineering Decoys

If the S genes are effector target genes of pathogens, they can be engineered to serve as "decoys" (van Schie and Takken 2025). The S gene is simulated by an artificial decoy which is genetically engineered to elicit an immune response when attacked by the effector. This method has been tested in the case of the Arabidopsis decoy PBS1, which is cleaved by different pathogen protease effectors after modification of the cleavage site (van Schie and Takken, 2025).

8. Optimizing R-Gene Pyramiding with S-Gene Editing

8.1 Synergistic Strategies

R-gene pyramiding, coupled with S-gene editing (Zhang *et al.*, 2025), could be the most comprehensive strategy for durable resistance. This approach offers a number of benefits:

Multiple layers of defense: the R gene gives recognition of races, and the S-gene editing gives general inhibition. The presence of mechanisms leads to reduced selection pressure

Natural variation can be exploited – R genes can be pyramided, and S genes from natural loss of function alleles can be introduced

This strategy has been successfully shown in cotton with the simultaneous insertion of 10 resistance QTLs and CRISPR knockout of the negative regulator GhARM, which synergistically boosted Verticillium wilt resistance (Zhang *et al.*, 2025). In rice, blast resistance gene Piz-t was also introduced along with the simultaneous knockout of S genes Bsr-d1, Pi21, and Xa5 to create the line 07GY31-BSR, which was resistant against a wide range of blast strains and showed no growth penalty (Zhang *et al.*, 2025).

8.2 Addressing Redundancy

Multiplex Editing Approaches are needed for functional redundancy among S genes. In cases where more than one S gene carries out similar functions, a single gene knockout might not be enough to produce resistance. Multiplex CRISPR editing can be applied to target multiple redundant S gene family members at once (Zhang *et al.*, 2025).

The selection process for trait balancing is described here. Here the selection process for trait balancing is described.

Multi-omics data integration through genomic selection (GS) models offers a powerful platform for the integration of disease resistance and yield and quality (Zhang *et al.*, 2025). The effect of various combinations of genes can be predicted with these models which can be used to choose best combinations of R and S genes.

Table 3: Strategies for Enhancing Disease Resistance Through Gene Editing

Strategy	Mechanism	Advantages	Limitations	Reference
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R-gene pyramiding	Stack multiple R genes	Race-specific, effective for diverse strains	Requires extensive breeding, can be overcome	Zhang <i>et al.</i> , 2025
Complete S-gene knockout	Loss of function	Broad-spectrum, durable	Potential pleiotropy	Thakur <i>et al.</i> , 2025
Regulatory edit	Modulate S-gene expression	Avoids pleiotropy, precise	May not provide complete resistance	van Schie and Takken, 2025
Promoter editing	Prevent pathogen activation	Targeted, avoids pleiotropy	Requires understanding of pathogen effectors	Zhang <i>et al.</i> , 2025
Natural allele introgression	Introgress loss-of-function alleles	Maintains fitness, no editing required	Limited by available germplasm	Thakur <i>et al.</i> , 2025
Combined R + S	Multiple layers of resistance	Most durable, synergistic	Complex breeding	Zhang <i>et al.</i> , 2025

9. Future Directions

9.1 Expanding S-Genes Discovery

S gene identification for systematic in a crop is critical to expanding the crop resistance toolbox. Multi-omics analysis (GWAS, transcriptome-wide association studies (TWAS) and eQTL analysis) can be used to find S genes (Zhang *et al.*, 2025). In cotton, 10 GWAS/TWAS-eQTLs for Verticillium wilt resistance and eight negative regulators were identified (Zhang *et al.*, 2025). These strategies must be applied in other crop-pathogen systems.

The 1990s have witnessed progress in delivery systems. Progress in delivery systems during the 1990s.

The delivery of CRISPR/Cas components is still a challenge, especially for recalcitrant crop species. New delivery systems have been developed such as:

RNP delivery: Pre-assembled Cas9-gRNA complexes can be directly injected into the cells for transgene-free editing.

Nanoparticle mediated delivery: Potential for delivery across species barriers without the need for transgenesis (Zhang *et al.*, 2025)

Haploid inducer-mediated editing: genome editing in germ cells (Zhang *et al.*, 2025) Base and Prime Editing for Fine-Tuning. Base and Prime Editing for Fine-Tuning.

In addition to knockouts, S genes can be precisely modified with base editing or prime editing without complete gene disruption (Zhang *et al.*, 2025). These strategies can be used to control gene activity, thus limiting pleiotropic effects and maintaining resistance. They are also useful to introgress desirable natural alleles into elite germplasm.

9.4 Climate-Adapted Resistance

The pathogen pressure is likely to increase due to climate change, and durable resistance is fundamentally more essential. Future breeding programs should take into account the pathogen populations as well as the evolving environment in which resistance will have to operate. This can involve choosing for resistance genes that are expressed at a range of temperatures, or S genes which offer resistance under a number of stress conditions.

10. Conclusion

Recent advances in the field of genomic discovery and precision gene editing have ushered in a new era in breeding for resistant disease. Susceptibility gene editing is an alternative approach and a complementary one to R genes for plant protection. S-gene editing can offer wide protection, potentially long-lasting, against a range of pathogens by blocking the host factors that pathogens use.

This approach has proven successful in several cases such as with MLO resistance to powdery mildew, eIF4E resistance to viruses, and SWEET promoter editing in bacterial blight. But there are still problems to address, especially the possible pleiotropic effects and growth-defense trade-offs. New strategies being developed to overcome these difficulties involve specific editing of regulatory sequences; tissue-specific editing; natural allele introgression; and the R-gene/S-gene approach.

With the gradual maturing of genomics, multi-omics and gene editing technologies, the so-called "intelligent design" of disease-resistant crops is turning into reality (Zhang *et al.*, 2025). Future research and development efforts will involve ongoing discovery of S-gene and development of delivery systems, regulatory streamlining, and combination of multiple resistance strategies. These advancements make S-gene editing a promising tool for durable resistance against the changing pathogens in crop protection system, with the potential to create strong resistance against future climate change and pathogens.

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