Trends in Plant Science

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Review

Susceptibility reversed: modified plant susceptibility genes for resistance to bacteria

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Plants have evolved complex defence mechanisms to avoid invasion of potential pathogens. Despite this, adapted pathogens deploy effector proteins to manipulate host susceptibility (S) genes, rendering plant defences ineffective. The identification and mutation of plant S genes exploited by bacterial pathogens are important for the generation of crops with durable and broad-spectrum resistance. Application of mutant S genes in the breeding of resistant crops is limited because of potential pleiotropy. New genome editing techniques open up new possibilities for the modification of S genes. In this review, we focus on S genes manipulated by bacteria and propose ways for their identification and precise modification. Finally, we propose that genes coding for transporter proteins represent a new group of S genes.

Highlights

Plant pathogenic bacteria use host susceptibility (S) genes to promote disease development.

Many bacterial species use effector proteins to target S genes, resulting in effector-triggered susceptibility.

S genes are evolutionarily retained across plant species.

S genes can be identified and precisely mutated through new genome engineering tools.

Impairment of S genes can lead to recessively inherited broad-spectrum resistance to bacteria.

The fundamentals of plant immunity

Plants are constantly exposed to a multitude of potential pathogens, such as viruses, fungi, and bacteria. For microbes to become pathogenic, a high degree of adaptation is required to overcome the layers of defences plants have evolved [1,2]. Plants possess the ability to fight off the majority of invading microbes, making susceptibility the exception in plant–pathogen interactions [1]. In plants, a two-layered defence system is activated upon interaction of microbial molecules with extracellular and intracellular immune receptors. In the first layer, **pattern recognition receptors** (see Glossary) on the cell surface perceive conserved microbial elicitors called **'pathogen-associated molecular patterns' (PAMPs)**, leading to **PAMP-triggered immunity (PTI)**. Adapted pathogens can overcome PTI by deploying **effector** proteins, leading to **effector-triggered susceptibility (ETS)**. In the second layer of defence, plants counteract ETS through the evolution of **resistance (R) genes**. Inside the cell, pathogen effectors are directly or indirectly recognised by the products of corresponding dominant R genes, resulting in **effector-triggered immunity (ETI)** [3,4]. Effectors, however, can rapidly evolve to overcome ETI by avoiding recognition of R proteins, leading once again to ETS [3].

To secure a compatible interaction, pathogen effectors target plant factors encoded by **susceptibility (S) genes** to manipulate host processes to their advantage. Suppression of defences, nutrient acquisition, and transport of bacterial proteins in the host cell are some of the processes pathogens use to cause disease (Figure 1) [3–5]. Although S genes are exploited by pathogens to promote disease, their mutation can lead to durable, recessively inherited, and potentially broad-spectrum resistance in plants [6].

More than 200 species of bacteria can infect plants and cause diseases [7]. So far, management of bacterial diseases has been based mainly on the use of chemicals and host resistance [8]. For decades, resistance breeding has successfully relied on the introgression of major R genes that recognise microbial effectors and confer resistance in crops. Bacterial effectors are under strong negative selection when exposed to corresponding R genes, however, resulting in fast

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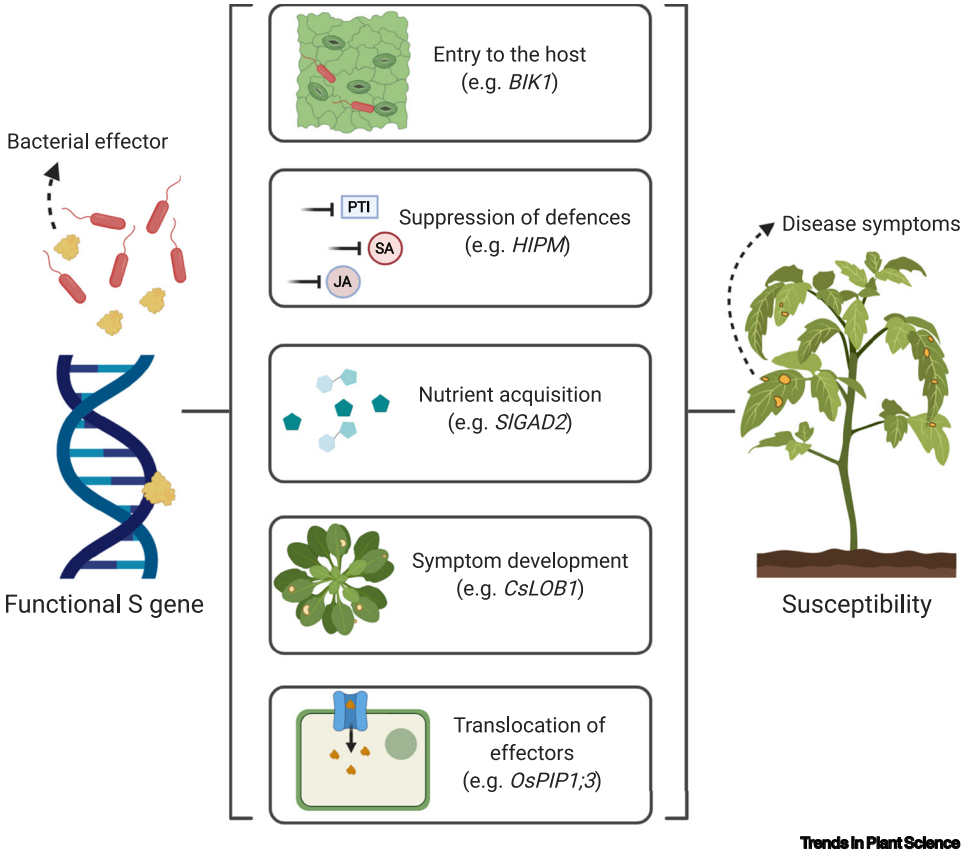

Figure 1. Manipulation of plant susceptibility (S) genes by bacterial pathogens. Plant pathogenic bacteria use effector proteins to target and manipulate plant S genes. Bacteria use functional S genes in order to complete processes such as entry into their host, suppression of defences, acquisition of nutrients, symptom development, and translocation of their effectors into host cells, resulting in plant susceptibility. JA, jasmonic acid; PTI, pathogen-associated molecular pattern–triggered immunity; SA, salicylic acid. This figure was created using BioRender (https://biorender.com/).

breakdown of resistance [9,10]. In addition, bactericide resistance can rapidly evolve through horizontal gene transfer between bacterial species [8,11]. Therefore, novel breeding strategies, such as the use of mutated plant S genes, for the control of bacterial diseases are needed. Here, we review S genes in different pathosystems, but we specifically focus on S genes recently shown to be involved in susceptibility to bacteria (Table 1). We propose ways in which S genes can be identified and modified to gain plant resistance to bacteria and suggest that genes coding for transporter proteins represent a new category of S genes.

Susceptibility genes…

Are defined as…

Any plant gene that facilitates a compatible interaction with the pathogen can be considered an S gene [12,13]. S genes belong to diverse gene families and have widely different functions (Figure 1). A first categorisation of S genes suggests that they largely fall into three categories [12]. The first includes genes that are involved in host entry. A well-known example in susceptibility to powdery mildew is *Mildew Locus O* (*MLO*). Inactivation of *MLO* prevents fungal penetration into host cells [14]. Genes that act as negative regulators of defences belong in the second category. An example is *Downey Mildew Resistance6* (*DMR6*), a putative 2(OG)-Fe(II) oxygenase

Glossary

Biotroph: an organism that derives its energy for its survival and multiplication from living cells.
Effector: small proteins secreted by pathogens into or around host cells. Effectors are usually crucial for the virulence of pathogens.
Effector binding element (EBE): specific DNA sequence on the promoter of genes on which TALEs bind to induce their expression.
Effector-triggered immunity (ETI): immune response triggered by the recognition of an effector by a corresponding R gene. ETI leads to resistance to the pathogen, and it is usually exhibited as cell death.
Effector-triggered susceptibility (ETS): successful infection of plants by pathogens through the secretion of effectors that interfere with plant processes.
Hemibiotroph: an organism that keeps the host alive during initial establishment in the host. In later stages, the pathogen turns into a necrotroph to derive nutrients.
Homology-directed repair (HDR): cell repair mechanism of double-stranded DNA breaks. The mechanism is activated in the presence of homologous pieces of DNA in the cell nucleus.
Hypersensitive response: fast localised cell death at the point of pathogen penetration that is mostly associated with plant resistance.
Necrotroph: an organism that actively kills the cells of its host in order to derive energy for its survival and multiplication.
Nonhost resistance (NHR): the resistance exhibited by the entirety of a plant species to nonadapted pathogens.
Pathogen-associated molecular pattern (PAMP): pathogen-derived molecules that are highly conserved within classes of microbes.
PAMP-triggered immunity (PTI): immune response triggered by the recognition of PAMPs by pattern recognition receptors.
Pattern recognition receptor: extracellular innate immune receptors that can recognise PAMPs.
Pleiotropic effects: effects caused by pleiotropy, a phenomenon in which one gene can influence two or more phenotypic traits.
Reactive oxygen species (ROS): highly reactive molecules derived from molecular oxygen. ROS act as signalling

that is involved in the catalysis of the defence-associated hormone salicylic acid (SA). Loss of function of *DMR6* leads to resistance to pathogens via induction of SA levels [15,16]. The third category includes genes that allow sustained compatibility with the host, such as genes that assist with nutrition and metabolic processes of the pathogen. For instance, *Sugars Will Eventually Be Exported Transporter* (*SWEET*) genes, which act as effector targets, are involved in sugar transport to the apoplast, where bacteria reside. During infection, they are upregulated by **transcription activator-like effectors (TALEs)** and provide nutrients to the bacteria [17,18].

As S genes are researched further, it is becoming clear that more functional categories of genes are involved in susceptibility. As discussed in the 'To translocate effectors' subsection of this review, it was recently shown that genes coding for transporter proteins are targeted by bacteria for the translocation of their effectors. Thus, transporter proteins represent an important new S gene category.

Are often involved in physiological processes of plants

It might seem counterintuitive that plant genes which promote plant susceptibility to pathogens have been evolutionarily retained. Many S genes, however, are required in physiological processes of plants. Transporter *OsSWEET11* is involved in pollen development and grain filling in rice (*Oryza sativa*). During infection, upregulation of the gene also supports the growth of the bacterium *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) *in planta* [19,20]. Due to their dual role in physiological processes and susceptibility, inactivation of S genes might lead to resistance along with **pleiotropic effects**. Yet, the extent of fitness costs is dependent on the plant species and growing conditions. *mlo* barley (*Hordeum vulgare* L.) mutants exhibit broad-spectrum powdery mildew resistance accompanied with autonecrosis and early leaf senescence. In tomato (*Solanum lycopersicum*), the naturally occurring *ol-2* mutant, an ortholog of barley *MLO*, confers broad-spectrum resistance to powdery mildew without any fitness costs observed [21]. Downregulation of the gene *Defence No Death1* (*DND1*) results in autonecrosis and severe stunting in tomato. In potato (*Solanum tuberosum*), silencing of the ortholog causes only mild autonecrosis that is dependent on the plant growing conditions [22].

Are often conserved

Orthologs of S genes are often present across species, most probably due to their involvement in biological functions of plants. *SWEET* gene orthologs involved in seed development have been identified in arabidopsis (*Arabidopsis thaliana*), rice, and soybean (*Glycine max*) [20,23,24]. The auxin transporter *Walls Are Thin1* (*WAT1*), which is involved in secondary cell wall biosynthesis, is a functional S gene to vascular pathogens in cotton (*Gossypium hirsutum*) and arabidopsis [25,26]. Since its discovery in barley, *MLO* has been identified in species such as arabidopsis, tomato, pea (*Pisum sativum*), cucumber (*Cucumis sativus* L.), eggplant (*Solanum melongena*), tobacco (*Nicotiana benthamiana*), grapevine (*Vitis vinifera*), and apple (*Malus domestica*) [14,21,27–31]. Orthologs of S genes, such as *DMR1*, *DMR6*, *Powdery Mildew Resistance4* (*PMR4*), *PMR6, Cellulose Synthase A catalytic subunit 3* (*CESA3*), and *DND1*, have been identified and functionally characterised in arabidopsis, tomato, and potato [32–34]. The conservation of S genes is an important feature for their applicability in breeding. In the postgenomics era, discovery of S genes in model species makes the identification and functional characterisation of orthologs in crops a relatively easy and rather straightforward task (Figure 2).

S genes are exploited by bacteria…

To enter the host

Entry to the host is a critical step for bacterial infections. Natural openings such as the stomata or hydathodes are important entry portals for bacteria [35]. Stomata closure upon pathogen

molecules that trigger the activation of pathways in response to different stresses.

Resistance (R) gene: genes that primarily code for intracellular receptors that consist of a nucleotide-binding (NB) domain and a leucine-rich repeat (LRR) domain, commonly referred to as 'NB-LRRs.' In the presence of corresponding pathogen effectors, R genes confer resistance to the pathogen strain carrying the effector.

Susceptibility (S) gene: any plant gene that can lead to a compatible host–pathogen interaction.

Transcription activator-like effectors (TALEs): effectors found primarily in *Xanthomonas* spp. that bind to promoter sequences to activate the expression of genes.

Translocon: a complex of proteins containing a channel through which bacterial effectors are passed into host cells.

Table 1. Identified susceptibility genes manipulated by bacteria, their function, and interacting effectors in different plant species

Susceptibility gene	Effector	Pathogen	Plant species	S gene category	Refs
BIK1	XopR	*Xanthomonas oryzae* pv. *oryzae* (*Xoo*)	Arabidopsis	Suppression of defences/ entry to the host	[38]
TaNCED_5BS, TaNCED_5DS	tal8	*Xanthomonas translucens* pv. *undulosa Xtu*	Wheat	Suppression of defences	[44]
SAM-MT1, SAM-MT2	AvrXccB	*Xanthomonas campestris* pv. *campestris (Xcc)*	Arabidopsis	Suppression of defences	[41]
HIPM	HrpN	*Erwinia amylovora*	Apple	Suppression of defences	[40,62]
SWEET11	PthXo1	*Xanthomonas oryzae* pv. *oryzae* (*Xoo*)	Rice	Nutrient acquisition	[18,48]
SWEET13	PthXo2	*Xanthomonas oryzae* pv. *oryzae* (*Xoo*)	Rice	Nutrient acquisition	[18,48]
SWEET14	AvrXa7 PthXo3 TalC TalF	*Xanthomonas oryzae* pv. *oryzae* (*Xoo*)	Rice	Nutrient acquisition	[18,48]
GhSWEET10	Avrb6	*Xanthomonas citri* pv. *malvacearum (Xcm)*	Cotton	Nutrient acquisition/ symptom development	[17]
MeSWEET10a	$TAL20_{xam668}$	*Xanthomonas axonopodis* pv. *manihotis (Xam)*	Cassava	Nutrient acquisition	[49]
CsLOB1, CsLOB2, CsLOB3	Any effector of Xcc and Xfa, PtXa4	*Xanthomonas campestris* pv. *campestris (Xcc), Xanthomonas fuscans* pv. *aurantifolii (Xfa)*	Sweet orange, Grapefruit	Symptom development	[53–55]
OsPIP;3	Hpa1	*Xanthomonas oryzae* pv. *oryzae (Xoo)*	Rice	Translocation of effectors	[57,59,60]
OsImpα1a, OsImpα1b	TALEs of Xoo and Xoc	*Xanthomonas oryzae* pv. *oryzae (Xoo), Xanthomonas oryzae* pv. *oryzicola (Xoc)*	Rice	Translocation of effectors	[5]
Osaba1	Unknown	*Xanthomonas oryzae* pv. *oryzae (Xoo), Xanthomonas oryzae* pv. *oryzicola (Xoc)*	Rice	(Entry to the host) Suppression of defences	[36]
LPT3, LPT4	Unknown	*Pseudomonas syringae*	Arabidopsis	Suppression of defences	[45]
WAT1	Unknown	*Ralstonia solanacearum*	Arabidopsis	Suppression of defences	[25,26]
Upa20	AvrBs3	*Xanthomonas campestris* pv. *vesicatoria*	Pepper	Nutrient acquisition/ symptom development	[51]
CaMLO6	Unknown	*Ralstonia solanacearum*	Pepper	Suppression of defences	[46]
AtGAD1, AtGAD2, AtGAD4, NdGAD4, SlGAD2	RipI	*Ralstonia solanacearum*	Arabidopsis, tobacco, tomato	Nutrient acquisition	[50]

challenge is a well-known basal defence mechanism that limits pathogen entry [35,36]. For instance, the negative immune regulator *RPM1-interacting protein4* (*RIN4*) acts together with H^+-ATPases AHA1 or AHA2 to control stomata reopening during *Pseudomonas syringae* DC3000 invasion [37]. Similarly, effector XopR of bacterium *Xoo* targets *Botrytis-induced Kinase1* (*BIK1*) to suppress PAMP-induced stomata closure [38]. New insights suggest that stomata-regulated transpiration is a novel mechanism restricting bacterial growth and spread. Bacterial pathogen growth and spread appear to be favoured by high humidity and water soaking of leaves. In rice, abscisic acid (ABA) mutant *Osaba1* exhibits broad-spectrum resistance to *Xoo*. The stomata of the mutant plant remain open after infection, leading to higher water loss and limited spread of the bacterium [36].

To supress immune responses

To fight off invading pathogens, plants activate complex defence responses, such as the generation of **reactive oxygen species (ROS)**, cell wall modifications, and the production of antimicrobial compounds [12]. The contribution of these responses to resistance is dependent on the lifestyle

of the pathogen. For example, ROS generation and **hypersensitive response** limit the growth of **(hemi)biotrophs**. Inversely, to successfully infect the host, **necrotrophs** stimulate ROS production to induce susceptibility-associated cell death [39]. In apple, the necrogenic bacterium *Erwinia amylovora* targets the gene *HIPM* (*HrpN-interacting protein from Malus spp.*) to stimulate ROS generation and establish infection [40]. In contrast, effector AvrXccB of *Xanthomonas campestris* pv. *campestris* (*Xcc*) targets the putative methyltransferase complex SAM-MT1/SAM-MT2 to suppress ROS production and callose deposition to induce susceptibility [41].

To further modulate immune responses, plants have evolved tightly regulated networks of hormones [42]. The relationships between hormonal pathways can be antagonistic or synergistic [43]. Thus, genes involved in changes in the balance of hormones are prime targets for bacteria. Genes *TaNCED_5BS* and *TaNCED_5DS*, which are involved in the catalysis of the hormone ABA, are upregulated by the pathogen *Xanthomonas translucens* pv. *undulosa* (*Xtu*) in wheat. ABA-induced *Lipid Transfer Protein* (*LPT*) genes *LPT3* and *LPT4* are upregulated by *P. syringae* pv. *tomato* in arabidopsis during infection. In both cases, induction of expression of the genes leads to susceptibility through antagonism between the ABA and SA pathways [44,45]. In arabidopsis, mutation of *WAT1* enhances broad-range resistance to vascular pathogens, including *Ralstonia solanacearum*, via altered cross-regulation of auxin and SA pathways [25]. In transgenic pepper (*Capsicum annum*) plants, silencing of gene *CaMLO6* decreases the development of wilting symptoms caused by *R. solanacearum*, possibly due to blocking of SA- and jasmonic acid–dependent signalling [46].

Impairment of S genes involved in the regulation of hormonal pathways targeted by bacteria can lead to resistance to pathogens. Nevertheless, resistance obtained in mutants to a group of pathogens due to antagonistic relationships between hormones can lead to increased susceptibility to pathogen groups with contrasting lifestyles [47].

To acquire nutrients and cause symptoms

After pathogens have entered the host and suppressed immune responses, they must sustain their compatible interaction. To do so, bacteria use host genes to acquire nutrients, proliferate, and cause symptoms. Sugar transporters of the *SWEET* family are manipulated by bacteria to fulfil their nutritional needs. *SWEET* genes are upregulated during infection through binding of TALEs to their promoters. Upregulation of their expression increases the efflux of sugars that bacteria use as carbon sources in the apoplast where they reside [17,18]. In rice, three *SWEET* genes – *OsSWEET11, OsSWEET13*, and *OsSWEET14* – are targeted by *Xoo* TALEs [18,48]. Cotton gene *GhSWEET10* is targeted by Avrb6 of *X. citri* pv. *malvacearum* (*Xcm*). Silencing of *GhSWEET10* leads to reduced development of water-soaking symptoms [17]. Similarly, $TAL20_{Xam668}$ carried by *X. axonopodis* pv. *manihotis* binds and upregulates *MeSWEET10a* in cassava (*Manihot esculenta*) [49].

During infection, the highly conserved RipI effector of *R. solanacearum* physically interacts with plant glutamate decarboxylase (GAD) proteins to promote their biochemical activation. In return, *GAD*s catalyse the biosynthesis of γ-aminobutyric acid, an amino acid used by *R. solanacearum* as a nutrient [50]. Coimmunoprecipitation (co-IP) assays have confirmed the interaction of RipI with GAD proteins in different plant species. In arabidopsis, RipI physically interacts with proteins AtGAD1, AtGAD2, and AtGAD4. In *Nicotiana benthamiana* and tomato, genes *NbGAD4* and *SlGAD2*, respectively, are targeted by RipI. Mutation of *AtGAD1* and *AtGAD2* genes in arabidopsis leads to compromised bacterial growth and delayed symptom development. Likewise, downregulation of *SlGAD2* in tomato roots leads to reduced wilting symptoms [50].

In pepper, effector AvrBs3 of *X. campestris* pv. *vesicatoria* (*Xcv*) upregulates the expression of basic helix–loop–helix transcription factor *Upa20*, resulting in cell hypertrophy. Xcv likely exploits cell hypertrophy for increased nutrient production of enlarged cells [51]. Transcription factor C*sLOB1* is a major S gene in citrus species [52]. *CsLOB1* is targeted by all the major TALE effectors carried by *X. citri* spp. *citri* (*Xcc*) and *X. fuscans* pv. *aurantifolii* (*Xfa*) strains that cause citrus canker [53]. Upregulation of *CsLOB1* in citrus species promotes bacterial growth and pustule formation [53,54]. Additionally, two more homologues of *CsLOB1* – *CsLOB2* and *CsLOB3* – are targeted by TALEs and have been shown to contribute to pustule formation in citrus [55].

To translocate effectors

Many aspects of plant physiology are dependent on membrane transport processes. Transporter proteins, such as channels, pumps, and other carriers, are involved in important processes, such as nutrient acquisition, osmoregulation, and stress responses [56]. Recent reports have identified new roles of such genes in plant immunity. As an example, aquaporins that act as intracellular channels for the transport of water and small substrates across membranes [57] have been involved in both resistance and susceptibility to bacteria. In arabidopsis, aquaporin *AtPIP1;4* transports pathogen-induced H_2O_2 (a core component of ROS) to the cytoplasm for the activation of PTI pathways [58]. By contrast, aquaporin *OsPIP1;3* in rice is an S gene to bacterium *Xoo.* To deliver effector proteins, Gram-negative bacteria use a type III **translocon** that is assumed to be assembled by interacting bacterial translocators and eukaryotic proteins [59]. The Hpa1 translocator of *Xoo* physically interacts with *OsPIP1;3* to deliver effector PthXo1 into the cytoplasm. Inactivation of *OsPIP1;3* leads to resistance to *Xoo* through disruption of the translocation of the effector into the cytosol during infection [57,59,60]. Exciting examples of genes that can confer broad-spectrum resistance to TALEs carrying bacteria are *OsImpα1a* and *OsImpα1b* in rice. These two genes code for nucleocytoplasmic transporters of the importin family. In their study, Hui *et al*. [5] identified five conserved amino acids on the nuclear localisation signal of all TALEs of the *Xoo* and *X. oryzae* pv. *oryzicola* (*Xoc*) strains they studied that interact with the OsImpα1a/OsImpα1b proteins. Downregulation of *OsImpα1a* and *OsImpα1b* disables the translocation of TALEs in the nucleus, where they target *SWEET* genes, leading to broad-spectrum resistance [5]. Hence, genes involved in transport processes might represent a new category of S genes. Depending on the conservation of the interacting sites of the proteins, suppression of such genes may provide a new strategy to gain broad-spectrum resistance to bacteria.

S genes…

Can be identified

Most S genes have been identified through forward genetic studies. Screenings of wild germplasm or mutagenised populations have yielded a number of recessive alleles that confer resistance [13]. Here, we explore alternative options for the identification of S genes (Figure 2).

A recurrent feature of S genes is their conservation among species. Several S genes have been identified in the model species *A. thaliana*. The abundance of available sequencing and transcriptomics data of crops greatly eases the identification of crop S genes through phylogenetic analyses [12]. After identification of a crop ortholog, functional analyses should follow to confirm the function of the gene as a susceptibility factor.

Bacterial pathogens secrete effectors that can induce susceptibility. Knowledge on the range of effectors carried by pathogens can enable their use as molecular tools for the discovery of S genes [61]. Physical interaction between effectors and S gene proteins has been demonstrated multiple times

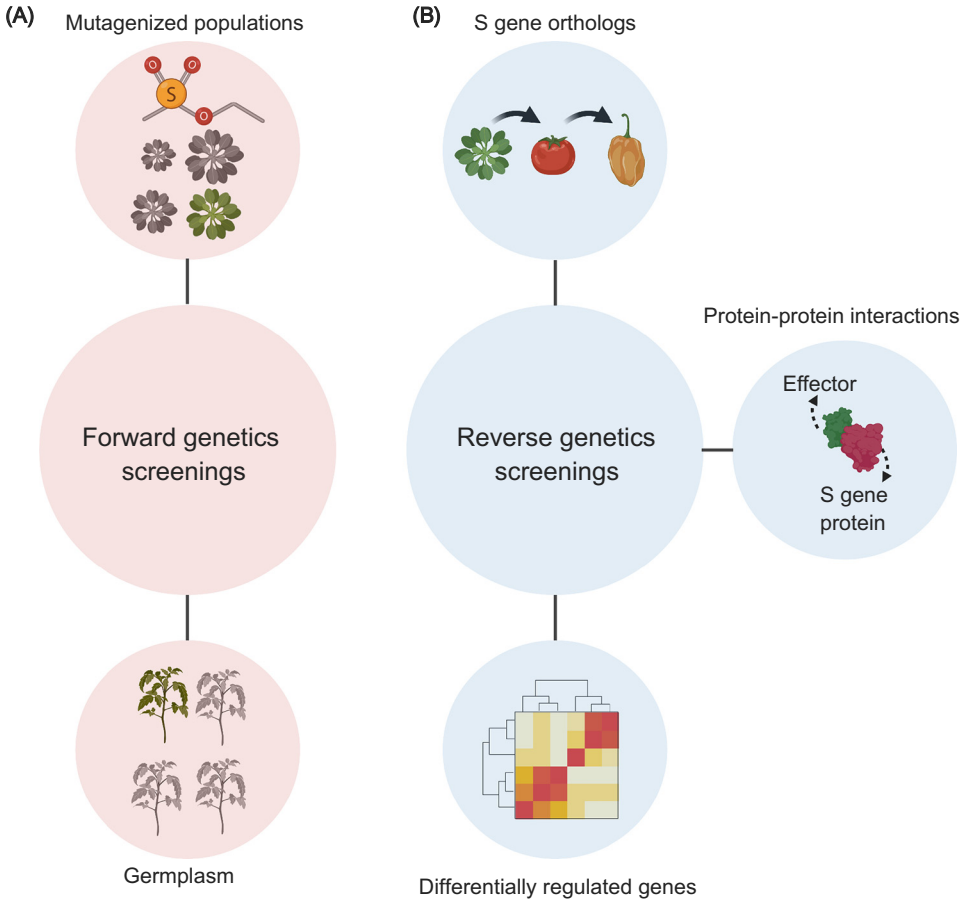

Figure 2. Identification of plant susceptibility (S) genes. (A) A number of plant S genes have been identified through forward genetics screenings, such as screening of mutagenised populations or screening of germplasm. (B) Here, we propose reverse genetics screens for the identification of S genes. A characteristic of S genes is their conservation between species. Phylogenetic analyses of known S genes between species can lead to the mining of S gene orthologs in different plant species. Physical interaction between bacterial effectors and proteins encoded by S genes has been demonstrated multiple times. The use of protein–protein interaction assays using bacterial effectors as molecular probes can lead to the identification of novel S genes. A common pathogen strategy seems to be the upregulation of S genes. The generation of transcriptomics data can aid in the identification of differentially expressed genes between infected and mock-treated plants. Differentially regulated genes might represent candidate S genes. This figure was created using BioRender (https://biorender.com/).

[5,57,62]. The use of protein–protein interaction assays, such as yeast two-hybrid assays, co-IP, or proximity labelling using the effector as a probe, can assist the identification of novel S genes [61].

Upregulation of S genes is a common pathogen strategy. *PME3*, a gene involved in susceptibility of arabidopsis to nematode *Heterodera schachtii*, is upregulated upon pathogen challenge [63]. Similarly, *MLO* homologs in grapevine, cucumber, and tomato are upregulated during powdery mildew infection [29,31,64]. Bacterial pathogens use effectors to upregulate corresponding S genes [51,65,66]. The generation of transcriptomics data using high-throughput techniques, such as RNA sequencing, can be a valuable tool in the identification of classes of differentially regulated genes. Differentially upregulated genes between infected and mock-treated plants may represent S genes. Using this approach, three *CsLOB* homologues were identified in citrus [55].

Can be modified

Nonhost resistance (NHR) is the resistance exhibited by the entirety of a plant species against nonadapted pathogens [67,68]. Hallmarks of NHR are its durability and broad spectrum [69]. The inability of a pathogen to infect a nonhost plant has been proposed to be based largely on host resistance, with both PTI and ETI being involved [70,71]. To successfully infect a host plant, pathogens use effector proteins to target host genes coding for susceptibility factors [12]. Failure of effectors to successfully manipulate their host target could lead to NHR [71]. In our view, the absence of either an evolved or functional host target or an effector evolved to manipulate the host target could maintain NHR of a plant species. Loss of function of S genes, such as *MLO* and *Eukaryotic Translation Initiation Factor 4E* (*EIF4E*), provide durable and

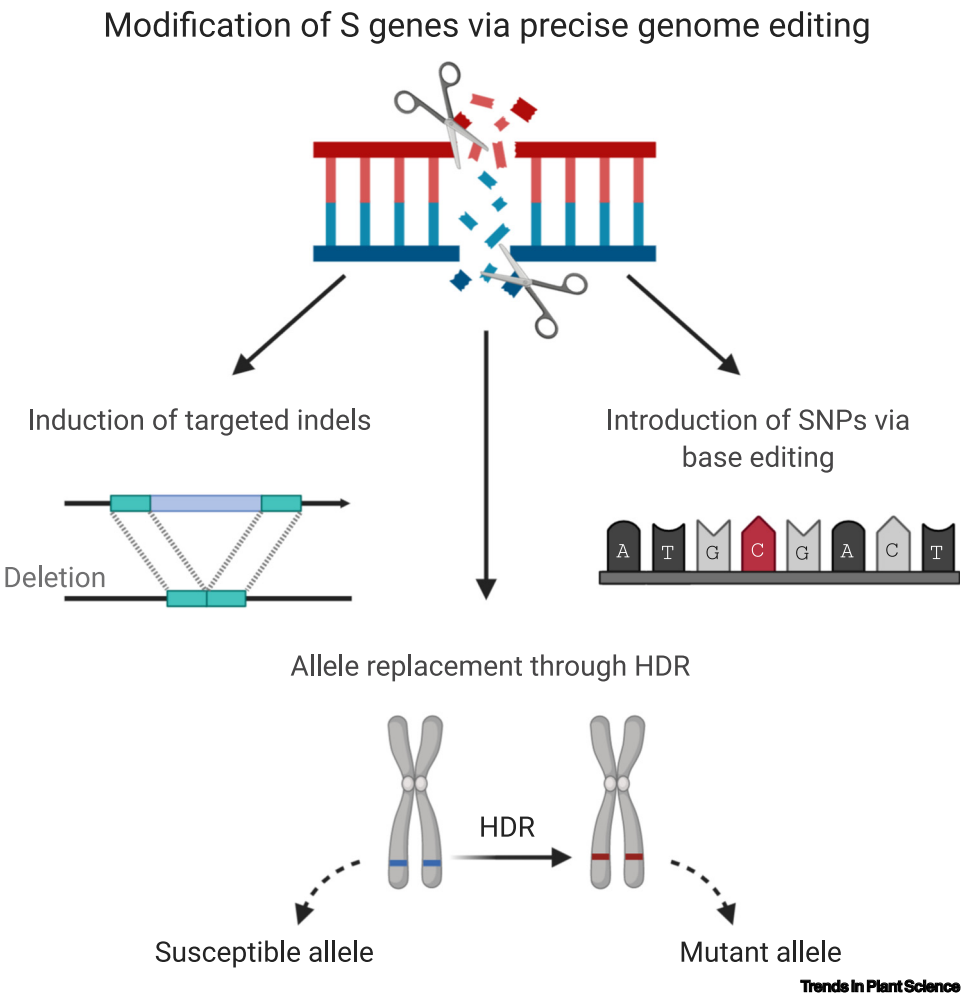

Figure 3. Modification of plant susceptibility (S) genes via precise genome editing. The potential pleiotropic effects observed upon mutation of a plant S gene may hinder their use in the breeding of resistant crops. However, the use of precise genome editing tools is offering new possibilities for generating desired mutations in S genes. The use of CRISPR/Cas systems allows the induction of targeted changes in S genes. Indels in target genes can easily be generated through CRISPR/Cas9. Furthermore, coupling of CRISPR/Cas9 with base editors allows the generation of targeted SNPs in genes. Recently, allele replacement through homology-directed repair (HDR) in plants using a CRISPR/LbCas12 system was reported [77]. Replacement of functional S alleles with natural or synthetically generated loss-of-function alleles through HDR can help bypass time-consuming procedures by direct replacement of alleles. This figure was created using BioRender (https://biorender.com/).

broad-spectrum resistance that exhibits all characteristics of NHR against powdery mildew and potyviruses, respectively [12,69]. This highlights the potential of using mutant S genes to achieve NHR-like resistance.

The genetic diversity of S genes is understudied. However, a number of natural mutant alleles in crops have been identified [21,28,72]. Introgression of natural mutant alleles into elite cultivars or the generation of EMS populations are options for the breeding of resistant genotypes. However, both approaches are time-consuming and may introduce unwanted changes into the elite background. Here we propose options for the breeding of resistant cultivars based on precise genome editing techniques (Figure 3).

Uncoupling of adverse pleiotropic effects and resistance is the main challenge in the application of mutant S genes in breeding [6,12]. Nevertheless, the development of new genetic engineering tools is offering new possibilities to breeders. The discovery of the CRISPR/Cas9 genome editing technology sparked a revolution in biology [73]. The CRISPR/Cas9 system has already been used in the study of multiple S genes [48,53,54,74].

Recently, base editors combined with the CRISPR/Cas9 system were used for the generation of single-base changes in plants [75,76]. In our opinion, the use of base editors for the generation of SNPs in S genes is an attractive option. Identification of interacting sites between bacterial and plant proteins (or genes) can localise the target region for the introduction of a SNP [75]. In this way, fitness costs may be avoided by introducing SNPs without altering the catalytic domains of proteins.

Changes in the promoters of S genes targeted by TALEs has been shown to be a fitness cost–free strategy. Induced susceptibility by TALEs is highly modifiable due to the predictable nature of **effector binding elements (EBEs)**. Prediction of TALEs and manipulation of their cognate EBEs can lead to fitness cost–free resistance. Additionally, simultaneous mutations of EBEs through multiplex CRISPR can lead to broad-spectrum resistance [48].

Where natural mutants are available in the germplasm, they can be used to directly replace functional S genes in cultivars. Where the germplasm is limited, synthetically generated alleles containing crucial mutations can be used instead. Introduction of loss-of-function alleles in the susceptible background can be achieved through **homology-directed repair (HDR)**, as recently demonstrated by the efficient replacement of the salinity tolerance *HKT1;2* (*High-affinity* K^+ *Transporter 1;2*) allele through HDR in tomato via the use of a CRISPR/LbCas12a complex [77].

Concluding remarks

The very nature of bacteria makes the management of the diseases they cause a challenge. Their ability to reach population sizes that favour epidemics in a short period of time, the rapid evolution of their effectors, and the development of resistance to antibiotics require the development of novel breeding strategies that can lead to durable resistance. In this review, we highlight the potential of using mutated S genes in breeding for plant resistance to bacteria. Furthermore, we propose that transporter proteins represent a new important category of S genes. As more S genes are identified, our knowledge of plant susceptibility and how S genes can be edited will further expand (see Outstanding questions). Although the use of mutated S genes in breeding remains a challenge, because of adverse pleiotropic effects, we are confident that the development of new genetic engineering tools will soon solve some of these problems. In different parts of the world, the use of such technologies is now becoming a reality. Whether these technical solutions will be freely used in Europe without genetic modification–associated regulations remains to be seen.

Outstanding questions

Are there protein domains encoded by S genes that specifically interact with bacterial proteins? Can modification of such domains lead to fitness cost–free resistance?

To what extent are S genes identified in model species functionally conserved across crops? Does impairment of conserved S genes lead to the same pleiotropic defects in different crops?

Is the molecular mechanism of broad-range resistance conferred by mutant S genes the same for different pathogens?

Are there resistant natural loss-of-function variants in the germplasm that do not exhibit pleiotropic defects? Can we introduce the same variants in susceptible genotypes using precise gene editing?

What is the link between nonhost resistance and susceptibility genes?

Is there crosstalk between biotic and abiotic stresses governed by S genes? Could additional abiotic stresses complement the resistant phenotype back to a susceptible one? Multiple mutant S genes have been screened for their ability to provide broad-range resistance against biotic factors, but knowledge on the combination of stresses is lacking.

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Declaration of interests

The authors have no interests to declare.

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