Susceptibility Genes 101: How to Be a Good Host

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Abstract

To confer resistance against pathogens and pests in plants, typically dominant resistance genes are deployed. However, because resistance is based on recognition of a single pathogen-derived molecular pattern, these narrow-spectrum genes are usually readily overcome. Disease arises from a compatible interaction between plant and pathogen. Hence, altering a plant gene that critically facilitates compatibility could provide a more broad-spectrum and durable type of resistance. Here, such susceptibility (*S*) genes are reviewed with a focus on the mechanisms underlying loss of compatibility. We distinguish three groups of *S* genes acting during different stages of infection: early pathogen establishment, modulation of host defenses, and pathogen sustenance. The many examples reviewed here show that *S* genes have the potential to be used in resistance breeding. However, because *S* genes have a function other than being a compatibility factor for the pathogen, the side effects caused by their mutation demands a one-by-one assessment of their usefulness for application.

INTRODUCTION

Pattern recognition receptors (PRRs): recognizing either pathogen-derived molecules, called PAMPs, or plant-derived damage-associated molecular patterns (DAMPs)

PAMP-triggered immunity (PTI): defenses induced after PAMP/DAMP perception by PRRs

Callose: a plant polysaccharide deposited in the cell wall as a response to wounding and pathogen-infection; the main constituent of papillae

Effector: secreted pathogen-derived molecule that changes host responses to support compatibility, often by suppressing the immune response

Resistance (*R*) gene: immune receptor conferring recognition of a pathogen-derived avirulence product (often an effector), resulting in the activation of host defense

Effector-triggered immunity (ETI): defenses induced after effector perception by a resistance gene

Hypersensitive response (HR): a programmed cell death response that often accompanies ETI

Plants have evolved sophisticated defense mechanisms to ward off pathogens. In fact, a high degree of adaptation by a pathogen is required to allow it to colonize a suitable host and to overcome its preformed and pathogen-inducible defenses. The stringent requirements imposed by the host force the pathogen to coevolve, resulting in a high degree of host specialization. The phenomenon of host specificity is especially widespread among biotrophic filamentous (fungal and oomycete) pathogens that have long-lasting interactions with living host cells and frequently form specialized feeding structures inside these cells (217). A perturbation in this delicate balance between host and pathogen can result in incompatibility and thus loss of susceptibility.

After recognition of a suitable host, a pathogen breaches the host's constitutive defense barriers, penetrates its tissues and cell walls, and is then confronted with pathogen-induced defenses. These postpenetration defenses are launched either upon direct recognition of the pathogen or upon indirect recognition mediated by its actions or the damage it inflicts to the host during infection. At their cell surface, plants carry pattern recognition receptors (PRRs) that mediate recognition of pathogen-derived molecules, called PAMPs (pathogen-associated molecular patterns; e.g., flagellin), or plant-derived DAMPs (damage-associated molecular patterns; e.g., plant cell wall fragments). DAMP/PAMP recognition by PRRs activates a defense response, designated PAMP-triggered immunity (PTI). PTI includes the production of reactive oxygen species (ROS), secretion of antimicrobial compounds and hydrolytic enzymes that target pathogen cell walls (chitinases and glucanases), and the trigger of local cell wall fortifications, such as callose (23). To suppress PTI, pathogens produce effector molecules that change host responses to support compatibility (57). To counteract the activity of effectors, plants evolved the ability to recognize either the effector itself or its modification inflicted on a host protein using resistance (*R*) genes. Most *R* genes encode intracellular nucleotide-binding leucine-rich-repeat (NB-LRR) proteins, and *R*-gene-mediated immunity is referred to as ETI (effector-triggered immunity), which is often accompanied by local cell death [hypersensitive response (HR)] (55, 180). Whereas PTI confers broad recognition, as it is activated by evolutionarily conserved molecules, ETI is mostly race specific, as effectors are highly polymorphic (55). Although activated differently, the output of PTI and ETI shows merely quantitative differences and there is no apparent clear-cut separation between both signaling pathways (184). Pathway overlap at multiple levels has been suggested (155, 189), and both immune signaling pathways are tightly regulated, with major roles for the defense hormones salicylic acid (SA), jasmonic acid (JA), and ethylene (Eth) (152). Effectors triggering ETI are under strong negative selection pressure and, consequently, the encoding genes often rapidly evolve. The resulting arms race forces a pathogen to continuously evolve new strategies to evade or suppress PTI and ETI and drives selection for an expanded recognition repertoire by the plant (96).

Along with suppressing or evading plant immunity, most pathogens, and especially biotrophs, require cooperation of the host for establishment of a compatible interaction. Accommodating the pathogen involves enabling it to establish feeding structures, such as haustoria, inside the host cell to obtain nutrients. All plant genes that facilitate infection and support compatibility can be considered susceptibility (*S*) genes. Mutation or loss of an *S* gene can therefore limit the ability of the pathogen to cause disease. Whereas *R* genes are typically dominant, resistance conferred by loss or alteration of *S* genes is generally recessive.

When mutated, *S* genes can cause pathogen-specific resistance when they no longer support a compatible interaction because of impaired prepenetration requirements (e.g., host recognition, penetration) or impaired support of specific postpenetration requirements (e.g., nutrients). Alternatively, *S* genes can provide relatively broad-spectrum resistance when they cause prolonged or constitutive defenses, although the latter may cause autoimmune-like fitness penalties.

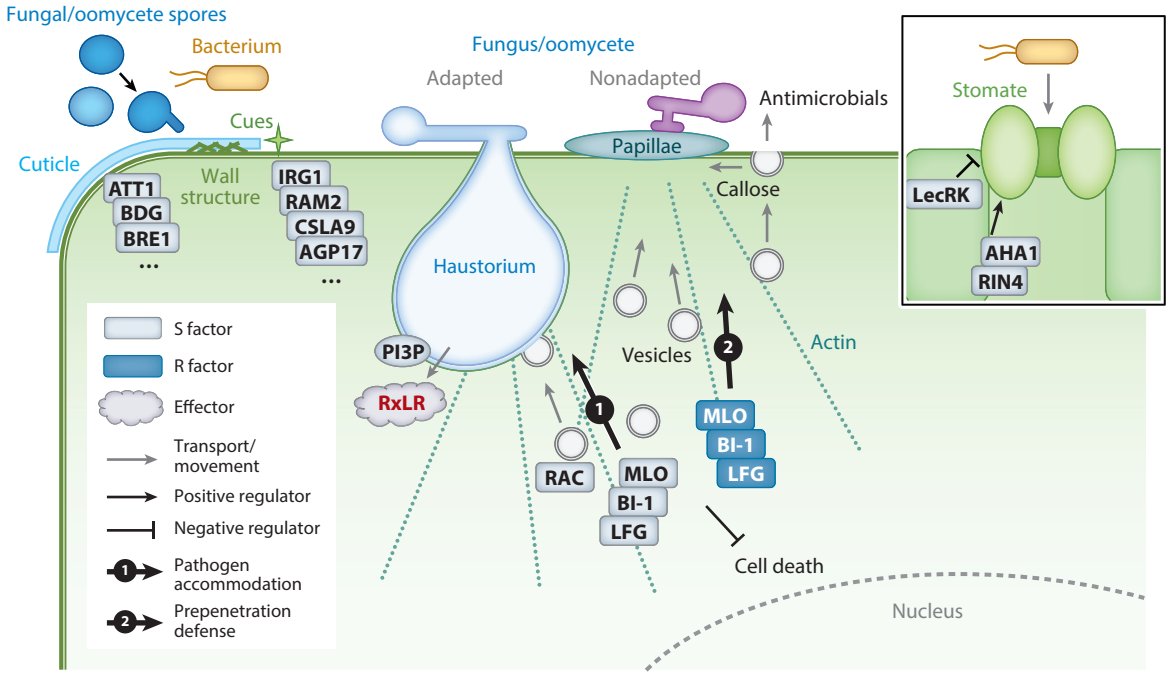

Figure 1

Susceptibility genes involved in host recognition and (pre)penetration. Examples are shown of proteins involved in early infection processes, such as (*top left*) synthesis of pathogen cues and cuticle and cell wall components, (*middle*) formation of the extrahaustorial membrane, which requires vesicular trafficking and actin polymerization, and penetration defense. (*top right*, *inset*) Genes involved in stomatal (re)opening required for bacterial entry. Mutations in these genes confer loss of susceptibility to adapted pathogens. For a more detailed description of gene names and function, please refer to **Supplemental Table 1** (follow the **Supplemental Material link** from the Annual Reviews home page at **http://www.annualreviews.org**).

Supplemental Material

The potential robustness of an *S* gene is exemplified by the *Mlo* gene, of which a recessive mutant was shown to confer powdery mildew (PM) resistance in barley seven decades ago and this mutant is still used and confers resistance to all PM races in the field (67, 97). These characteristics, conferring non-race-specific and potential durable resistance, make *S* genes an interesting alternative to *R* genes in breeding programs. Although recessive resistances have been used for decades, the concept of a susceptibility gene was first explored in 2002 after the identification of *pmr6* (PM resistance) in *Arabidopsis*. *PMR6* was described as "...a novel form of disease resistance based on the loss of a gene required during a compatible interaction..." (198), after which the term susceptibility gene was proposed (59).

On the basis of the distinct phases of the host-pathogen interaction processes, we distinguish three major mechanisms by which *S* genes facilitate susceptibility and contribute to infection (**Figures 1–3**):

1. Genes allowing basic compatibility (prepenetration), facilitating host recognition and penetration.
2. Genes encoding negative regulators of immune signaling.
3. Genes allowing sustained compatibility (postpenetration), fulfilling metabolic or structural needs, and allowing pathogen proliferation.

In the first part of this review, typical representatives of *S* genes and potential *S* genes of the three different categories are described. These examples are selected from a comprehensive list of

Biotroph: microbial pathogen that requires living host cells for its growth and proliferation

Haustoria: infection structures of specialized biotrophic filamentous pathogens that are used for nutrient absorption from host cells and secretion of effectors

Susceptibility (*S*) gene: any plant gene that facilitates the infection process or supports compatibility with a pathogen

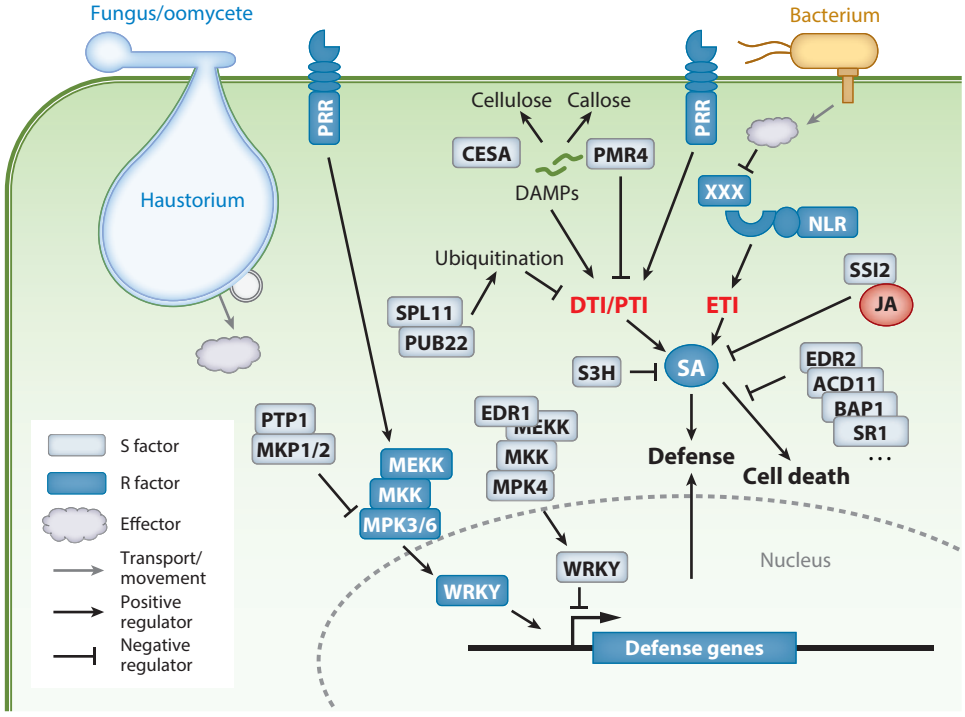

Figure 2

Susceptibility genes involved in suppression of host defense. Examples of genes are shown that suppress erroneous DAMP (damage-associated molecular pattern)-triggered immunity (DTI) (CESA/PMR4) or pattern recognition receptor (PRR)-triggered PAMP (pathogen-associated molecular pattern) (PTI) by affecting MAP kinase (MPK) and WRKY transcription factor pathways with positive or negative regulatory roles, control degradation of PTI components by ubiquitination, suppress salicylic acid (SA) signaling by SA catabolism (S3H), mediate SA antagonism by oxylipins and jasmonic acid (JA), and suppress effector-triggered immunity (ETI) and programmed cell death. For a more detailed description of gene names and function, please refer to **Supplemental Table 1** (follow the **Supplemental Material link** from the Annual Reviews home page at **http://www.annualreviews.org**). Abbreviations: CESA, cellulose synthase; XXX, effector target.

Supplemental Material

S genes provided in **Supplemental Table 1** (follow the **Supplemental Material link** from the Annual Reviews home page at **http://www.annualreviews.org**). We also briefly highlight how pathogens control and use *S* genes. Finally, we discuss the possibilities and potential drawbacks of using *S* genes to increase crop resistance.

TYPES OF *S* GENES

S Genes Allowing Basic Compatibility: A Warm Welcome

Bacterial pathogens enter the apoplast through stomates or wounds and often form type III and type IV secretion systems for injection of effectors. Fungi and oomycetes form spores that germinate and form runner hyphae that either enter the host via natural openings or force entry using appressoria that can penetrate cell walls. A haustorium can then be established for feeding and effector translocation (217). Below and in **Figure 1**, typical examples are given of plant genes involved in the

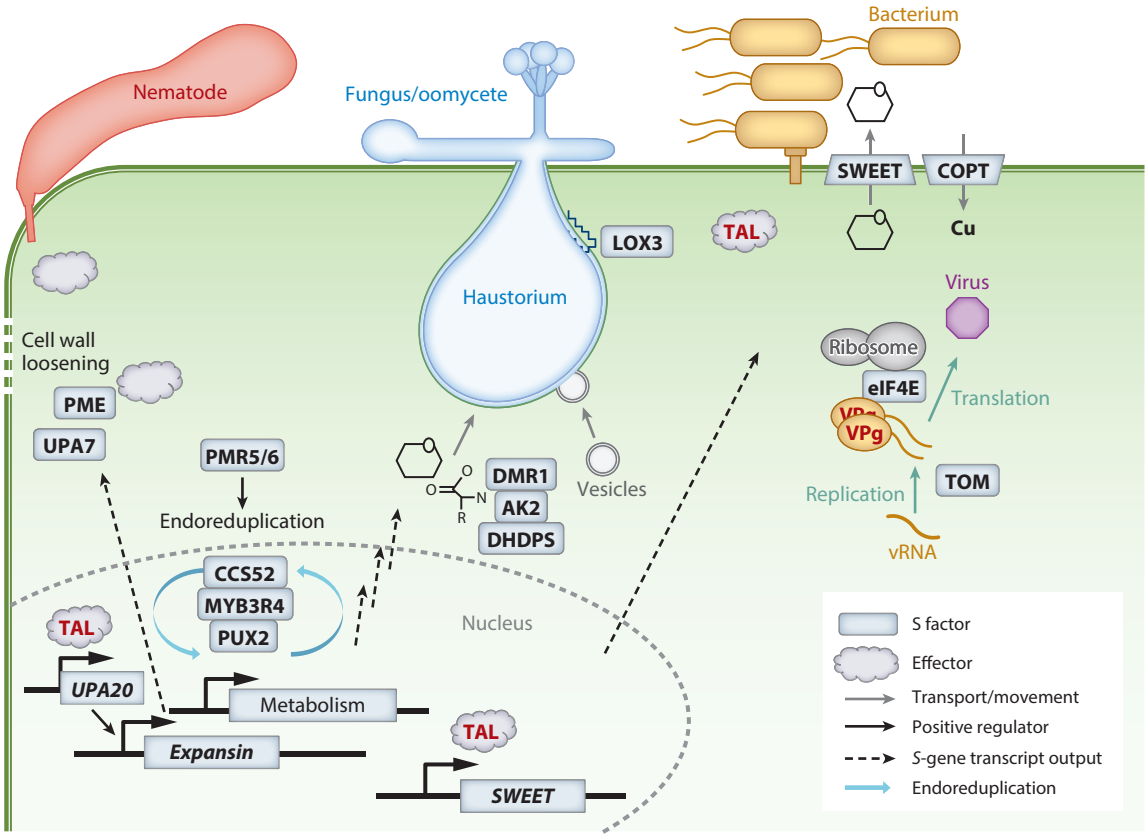

Figure 3

Susceptibility genes involved in pathogen sustenance. Examples are shown of plant proteins involved in cell expansion and endoreduplication, which allow increased metabolism (*left*), production of metabolites such as amino acids, sugars, or lipids (*center*), metabolite transport (*top right*), and virus replication (*right*). For a more detailed description of gene names and function, please refer to **Supplemental Table 1** (follow the **Supplemental Material link** from the Annual Reviews home page at **http://www.annualreviews.org**). Abbreviations: Cu, copper; DHDPS, dihydrodipicolinate synthase; PME, pectin methylesterase; TAL, transcription activator–like.

Supplemental Material

early infection steps, from the production of attractants to the formation of membrane structures to establish a feeding site, determining whether a compatible interaction can be established.

Cuticle and cell wall structure. The leaf surface is covered with a waxy layer, the cuticle, which is composed of cutin, wax, polysaccharides, and minor compounds such as flavonoids (17). The corn mutant *glossy11* has decreased very-long-chain aldehyde levels in leaf cuticles, resulting in poor germination of PM spores (75). A *Medicago* mutant, *irg1*, with reduced primary alcohols in surface wax caused reduced differentiation of fungal rust and anthracnose pathogens (*Phakopsora pachyrhizi*, *Puccinia emaculata*, and *Colletotrichum trifolii*) (191). Another *Medicago* mutant, *ram2*, has altered cutin composition because of compromised glycerol-3-phosphate acyltransferase activity, which results in reduced susceptibility to *Phytophthora palmivora* because of perturbed appressoria formation (202). These examples indicate that the leaf cuticle contains components that are used by filamentous pathogens as essential developmental cues for pathogenicity. The plant genes/enzymes involved in synthesis of such compounds contribute to susceptibility and can be regarded as *S* genes.

Cuticle: waxy protective film covering the nonwoody aerial plant parts, such as leaves and shoots

Necrotroph: microbial pathogen that kills host cells before colonizing them

Adapted pathogen: a pathogen specialized on a specific host species, able to overcome or suppress PTI

Supplemental Material

Interestingly, several *Arabidopsis* cuticle mutants are resistant to infection by *Botrytis* and *Sclerotinia*. This is surprising because these pathogens are necrotrophs with a very broad host range and are quite indiscriminate when choosing a surface on which to grow. Could, in this case, the mechanism of resistance and susceptibility be based on something other than host recognition and attachment? Cuticle mutants *Att1*, *Bdg*, *Lcr*, *Rwa2*, *Bre1/Lacs2/Sma4*, and *Fdh* all have altered cuticle composition due to loss of enzymes such as fatty acid oxidase, fatty acid hydroxylase, and long-chain acyl CoA synthetase (see **Supplemental Table 1** and Reference 34). These changes have structural consequences, and cuticles of these mutants have increased permeability. Conceivably, a more permeable cuticle could facilitate perception of *Botrytis* elicitors by the plant, resulting in more effective defense activation. Alternatively, increased levels of cutin monomers might activate a DAMP-induced immune response (see section Suppressors of PTI/DTI). In addition, antimicrobial compounds such as camalexin and ROS are more easily released through a permeable cuticle (34, 112). Thus, components of the cuticle and the genes that encode their biosynthetic enzymes are susceptibility factors for many pathogens, but the mechanisms mediating compatibility vary.

The cell wall and its composition, as well as its density and stretching, are important factors in determining compatibility. Plant cells use expansins to facilitate cell wall stretching and growth. The expansin EXLA2 is required for susceptibility to *Botrytis* and *Alternaria*, and although the underlying mechanism is not clear, it is plausible that expansins facilitate pathogen entry (2) (see also section Endoreduplication and Cell Expansion to Boost Metabolic Output Potential). Cellulose is a major structural component of cell walls, and the cellulose synthase-like gene *CSLA9* (*rat4*) is required for susceptibility to *Agrobacterium* infection/transformation. *Agrobacterium* attachment to the root surface is strongly reduced in *csla9/rat4* mutants, indicating that the CSLA9 product could be an essential cue for host recognition (227, 228). In the same screen, *rat1* was identified, which encodes a cell wall–localized arabinogalactan protein (AGP17) that is also required for *Agrobacterium* attachment (73).

Stomates are entry portals. Bacterial pathogens are not able to breach the cell wall or cuticle and instead use wounds or natural openings, such as stomata or hydathodes, to get access to the apoplast or vasculature. Pathogen-induced stomatal closure is an important basal defense response and pathogens actively counteract this process (165). After the pathogen threat diminishes, plants need to (re)open their stomata to establish gas exchange. LecRK (a receptor kinase) is a negative regulator of pathogen-induced stomatal closing (52), and RIN4, together with H^+ ATPase AHA1, is required for stomatal reopening (119). Consequently, loss-of-function mutants of their encoding genes are less susceptible to pathogen entry, qualifying them as *S* genes.

Membrane dynamics support establishment of haustoria. Many biotrophic filamentous pathogens penetrate the cell wall, but not the host plasma membrane, to form specialized feeding structures. One of the best-known susceptibility genes, required for PM penetration of epidermal cells, is *MLO* (mildew resistance locus O). *MLO* was discovered in barley (67) and later mapped and characterized as a membrane-anchored protein (27). The role of *MLO* in PM susceptibility has been confirmed in *Arabidopsis*, pea, tomato, pepper, wheat, and strawberry (12, 47, 86, 95, 149, 194, 225) (**Supplemental Table 1**). *MLO* seems to be required for susceptibility to adapted pathogens, and *mlo* mutants display loss of susceptibility resembling that described for nonhost resistance (85). Pepper *MLO2* is also required for susceptibility to the adapted bacterial pathogen *Xanthomonas campestris* but not for avirulent bacteria (105). *mlo*-based resistance is independent of JA, Eth, and SA but requires vesicular trafficking and, to some extent, actin polarization (47, 129). Independent of its role in (pre)penetration, MLO also seems to suppress programmed cell death (see section Side Effects of Mutating Prepenetration Factors).

The highly conserved BAX inhibitor-1 (BI-1) proteins fulfill a function similar to MLO. BI-1 proteins are known cell death suppressors in plants as well as animals and are membrane proteins with six to seven transmembrane domains. In addition, a role of BI-1 in susceptibility to penetration of PM (*Blumeria graminis*) has been established in barley (60, 61). *BI-1* silencing resulted in decreased PM penetration, whereas overexpression increased PM penetration. Overexpression of *BI-1* also fully restored PM penetration efficiency in *mlo* mutants and *MLO* overexpression restored PM penetration in *BI-I* mutants, suggesting that MLO and BI-1 cause susceptibility through similar and perhaps redundant mechanisms (60, 83). Interestingly, perturbing BI-1 increases susceptibility to necrotrophic fungi, such as *Botrytis cinerea* and *Chalara elegans* in barley and carrot, respectively, and (hemi)biotrophs, such as *Fusarium graminearum* and stripe rust (*Puccinia striiformis*) in barley and wheat, respectively (11, 87, 205). This indicates that BI-1 plays a very different role in accommodating infection structures of PM. BI-1 proteins belong to the larger family of Lifeguard (LFG) proteins that have also been shown to negatively regulate programmed cell death (81). Recently, five other LFG proteins outside the BI-1 clade have been identified in *Arabidopsis* as well as in barley. Also, these LFG proteins have a function in susceptibility to PM penetration (208). These five proteins may function redundantly to some extent, but individual knockdowns and knockouts already showed significantly reduced susceptibility to *B. graminis* and *Erysiphe cruciferarum*. The exact mechanism of PM accommodation by BI-1 and LFGs remains unclear, but colocalization of LFG with extrahaustorial membranes suggests that these proteins have a prolonged role at the site of interaction, perhaps to suppress local defense responses or to facilitate nutrient transfer through vesicular traffic (208).

Hemibiotroph: microbial pathogen that initially grows as a biotroph and later switches to a necrotrophic lifestyle

Cytoskeleton dynamics and vesicle traffic require small G proteins (Rho-GTPases, or RAC/ROP) and GTPase activating proteins (GAP) (134). A RAC/ROP from barley, HvRACB, was originally identified as a susceptibility factor for PM penetration (167). Three other gene family members, *HvRAC1*, *HvRAC3*, and *HvROP6*, also increase PM susceptibility when overexpressed (148, 168). Interestingly, *HvRAC1* acts as a resistance factor against the nonadapted fungus *Magnaporthe oryzae*, indicating that the phenotype (resistance versus susceptibility) conferred by these genes is pathogen specific (148). The rice genes *OsRAC4*, *OsRAC5*, and *OsRACB* act as susceptibility factors for *M. oryzae*, which is adapted to rice (39, 98). A negative regulatory role for HvRACB, as well as MLO, in actin reorganization and focusing (cell polarity) was proposed as a possible mechanism for susceptibility to (adapted) PM in barley, and RAC/ROPs seem to depend on MLO in this process (129, 144). Also, in *Arabidopsis*, ROPs are required for susceptibility to (adapted) PM. Likely, multiple *ROP* genes function redundantly, as a single knockout of *ROP6* did not show a phenotype, but plants expressing a dominant negative *ROP6* displayed a lower susceptibility to PM (154). Lastly, an *Arabidopsis* ARF-GAP protein, AGD5, is a susceptibility factor for *Hyaloperonospora arabidopsidis*, the causal agent of adapted downy mildew (DM), but is involved in resistance to the nonadapted PM *Erysiphe pisi* (166).

An emerging concept is that (pre)penetration compatibility and susceptibility to biotrophic fungi are defined by factors involved in membrane-associated cytoskeleton rearrangements and vesicular trafficking, such as MLO, BI-1, LFG, RAC/ROP, and GAP, as outlined above. Whereas these factors ensure susceptibility to adapted fungi, they mediate resistance to nonadapted biotrophs and necrotrophic fungi. To explain this paradox, one could hypothesize that there are two distinct phases during infection that utilize similar tools: (*a*) Phase 1, in which prepenetration defenses involving actin focusing and vesicle delivery induce papillae formation and secretion of other antimicrobial deposits/secretions, and (*b*) Phase 2, which includes membrane invagination, vesicle delivery to form the extrahaustorial membrane and support formation of haustoria, and possibly vesicle-mediated nutrient exchange. Nonadapted fungi are susceptible to the focal prepenetration defenses (Phase 1), whereas adapted fungi bypass or tolerate Phase 1 and

require events in Phase 2 for proper establishment. On the above-mentioned *S*-gene mutants, penetration and development of adapted fungi may be halted because the host membrane is not able to invaginate and interact with the cytoskeleton/vesicle/membrane-dynamics machinery to support haustorium formation. In conclusion, many *S* genes involved in (pre)penetration define whether a plant is a suitable host and, consequently, resistance based on mutation of such *S* genes may confer a nonhost type of resistance (85).

S Genes Encoding Immune Suppressors: Keeping Security at Bay

Although pathogens should not prematurely activate their infection machinery, plants need to suppress immune responses in the absence of a pathogen threat. Negative regulators of immunity can be considered *S* genes because their activity increases susceptibility (**Figure 2**).

Controlling salicylic acid levels. Typically, mutations in SA defense suppressors increase resistance to biotrophic pathogens because of constitutive defense signaling, characterized by high SA levels and pathogenesis-related (PR) gene expression. However, these mutants often exhibit growth retardation and, in some cases, HR-like symptoms known as lesion mimics (123, 130). One way of controlling SA signaling is to catabolize SA, and genes involved in SA conversion may contribute to susceptibility. SA catabolism is an important control mechanism, illustrated by the diversity of enzymes that convert SA. SA can be glucosylated, methylated, hydroxylated, and conjugated to amino acids (197). *Arabidopsis* SA 3-hydroxylase (S3H), which converts SA to 2,3-DHBA, was recently characterized (224). An *s3h* mutant was less susceptible to *Pseudomonas syringae*, indicating that SA hydroxylation contributes to susceptibility. However, mutants also display increased senescence. Whether S3H merely serves to reduce SA levels or whether 2,3-DHBA itself may have specific functions in aging and defense remains to be elucidated. Most of the other enzymes responsible for SA conversions do not contribute significantly to susceptibility (51, 170, 171). Interestingly, a mutant in a glucosyltransferase, *UGT76B1*, had elevated SA levels and reduced susceptibility to biotrophic pathogens. However, its substrate appeared to be isoleucic acid rather than SA. Isoleucic acid may act as a suppressor of the SA pathway (200). So, whereas genes involved in SA conjugation/conversion may conceptually be *S* genes, their actual contribution to susceptibility seems limited, and specific functions of SA conjugates in other processes will likely be discovered.

Suppressors of PTI/DTI. Cellulose synthases (CESAs) are essential enzymes for plant cell wall formation (see section Cuticle and Cell Wall Structure), but their role may well reach beyond the structural aspect of providing a physical barrier. *CESA3* (*cev1*) mutants have increased resistance to various PMs and show constitutively activated JA and Eth defenses and reduced stature (63, 64). *CESA4*, *CESA7*, and *CESA8* are also involved in pathogen susceptibility; the respective mutants display increased resistance to fungal and bacterial pathogens (77) (**Supplemental Table 1**). Although it cannot be ruled out that decreased cellulose content may hamper pathogen attachment, a more likely hypothesis is that in *cesa* mutants, cellulose precursors (oligogalacturonides) accumulate and induce DTI. Indeed, expression of several defense-related genes is induced in *cesa4*, *cesa7*, and *cesa8* mutants, yet their phenotypes are not dependent on JA, Eth, or SA (77).

Supplemental Material

The role of callose in plant defense is controversial. Callose deposition at the site of pathogen perception/penetration is primarily an induced defense response that limits pathogen entry. Overexpression of a callose synthase, *GSL5/PMR4*, leads to complete resistance against PM in *Arabidopsis* through formation of enlarged callose deposits, preventing pathogen entry (62). However, *pmr4* loss-of-function also reduces susceptibility to PMs as well as to DMs (*Erysiphe*

cichoracearum, *Erysiphe oriontii*, and *Phytophthora parasitica*) (89, 141). It seems counterintuitive that plants with less callose are also more resistant to pathogens. Possibly pathogen-induced callose, or *PMR4* itself, suppresses SA defenses as a negative feedback loop. Alternatively, callose precursors (oligosaccharides) that accumulate in *pmr4* mutants may induce DTI. loss of susceptibility in *pmr4* mutants is indeed dependent on SA and associated with moderately increased expression of defense genes (141). The function of *PMR4* as a susceptibility factor is thus independent of the role of callose as a physical barrier but probably lies in PTI suppression. Silencing the tomato *PMR4* abolished susceptibility to the PM *Oidium neolycopersici*, indicating that the role of *PMR4* in PM susceptibility is conserved (84). However, this appears to apply only to adapted PM fungi and DM oomycetes given that *Arabidopsis pmr4* mutants have shown greater penetration of a nonadapted PM, *Blumeria graminis* (89).

Genes that encode negative regulators of PTI or DTI can also be considered *S* genes; e.g., phosphorylation-mediated MAP kinase (MAPK) signaling cascades are known to relay PTI activation. These pathways are repressed by MAPK phosphatases (MKPs). MKP1, MKP2 and, to a lesser extent, protein tyrosine phosphatase PTP1 target MPK3 and MPK6 to suppress PTI in *Arabidopsis*. Consequently, *mkp* mutants are less susceptible to virulent *Pseudomonas* and *Ralstonia* bacteria (5, 15, 124). Not all MAPK cascades positively regulate PTI, as the MPK4 pathway in *Arabidopsis* suppresses PTI (18, 70, 151). The soybean MPK4 and rice MAPK5 have similar PTI or SA defense suppressor activities, and the mutants show reduced susceptibility to oomycete and viral or fungal and bacterial pathogens, respectively (120, 212) (**Supplemental Table 1**). *EDR1* (enhanced disease resistance) was identified in a mutant screen for enhanced resistance to PM in *Arabidopsis* and turned out to encode a MAPK kinase kinase. *EDR1* is required for susceptibility to biotrophic fungal and bacterial pathogens, and the rice homolog *OsEDR1* has a similar function in susceptibility (42, 68, 169). Therefore, depending on the pathway, MAP kinases as well as MAPK phosphatases can act as *S* factors.

Supplemental Material

Transcription factors are common substrates of MAP kinases, and WRKY transcription factors play key roles in transcriptional reprogramming upon pathogen perception. WRKYs can function as positive or negative regulators of defense and thus contribute to either resistance or susceptibility. A fairly recent overview of dozens of defense-suppressing WRKYs from *Arabidopsis*, rice, and other plants can be found elsewhere (146). Later, several more WRKY defense suppressors were identified in rice, pepper, and *Arabidopsis* (41, 44, 183, 206, 218). Interestingly, *Arabidopsis WRKY8* has, besides a role in susceptibility to *Pseudomonas*, a role in resistance against *Tobacco mosaic virus* (40, 41). In addition, the rice gene *WRKY45-1* is a susceptibility factor for the bacterial pathogen *Xanthomonas oryzae*, whereas the homologous gene *WRKY45-2*, differing in only ten amino acids, is a resistance factor for the same pathogen (183). Most WRKYs have been characterized in relation to microbial pathogens, but one report suggests that *AtWRKY23* is a susceptibility factor for root cyst nematodes. It is not clear yet whether this WRKY is a defense suppressor or perhaps required for tissue remodeling and cyst formation (74). Clearly, enzymes suppressing DTI and signaling components suppressing PTI are involved in susceptibility to diverse pathogens but their role can be very specific.

Protein degradation is another important theme in defense signaling (187). In *Arabidopsis*, ubiquitin ligases PUB22/23/24 function redundantly in suppressing PTI. Double and triple mutants were less susceptible to oomycete and bacterial pathogens (186). Intriguingly, *PUB22/23/24* mutants did not show any constitutive defense phenotypes, but the oxidative burst, MPK activation, and PR gene expression were enhanced and prolonged once induced by infection. This ubiquitin ligase triplet was shown to target the PTI regulator Exo70B2, whose degradation results in dampening of PTI (175). In addition to protein degradation, protein stabilization also appears crucial for defense. Deubiquitinating enzymes AtUBP12, AtUBP13, and NtUBP12 suppress defense, as

silenced plants were less susceptible to virulent *Pseudomonas* bacteria and showed increased *Cf9*-mediated HR (65). A ubiquitin ligase from rice, SPL11, is a negative regulator of defense and HR. Mutants are less susceptible to fungal and bacterial pathogens and show constitutive defense and a lesion mimic phenotype (223).

Jasmonic acid and salicylic acid antagonism. Jasmonates (JAs) are lipid-derived defense hormones required for defense against necrotrophs and chewing insects (13). Suppressors of the JA pathway may contribute to susceptibility to these pests, although not many *S* genes in this category have been described. The transcription factors bHLH3/13/14/17 collectively suppress the JA response; a quadruple *bHLH* mutant has a strongly reduced susceptibility to *Botrytis cinerea* and *Spodoptera exigua* larvae (172). Owing to the antagonistic interaction between JA and SA, the JA pathway can suppress SA-mediated defense, and the SA pathway can suppress JA-mediated defense (160). Consequently, the *bHLH* quadruple mutant has decreased SA defenses and is more susceptible to the biotroph *P. syringae* (172). This antagonistic interaction can explain why many *S*-gene mutants with reduced susceptibility to biotrophic pathogens and increased SA defense have reduced JA defense and become more susceptible to necrotrophic pathogens (see section Side Effects of Mutating Defense Suppressors and **Supplemental Table 1**).

Supplemental Material

Calcium and (sphingo)lipids mediate defense and ETI suppression. Calcium plays an essential role in signaling ETI and HR (125). BON1/CPN1, BAP1/2, and SR1 are calcium- and calmodulin-binding proteins that suppress ETI. SR1 directly binds to and suppresses the promoter of the important defense mediators *EDS1*, *NDR1*, and *EIN3* (58, 137, 215, 216). In addition, cyclic nucleotide–gated calcium channels CNGC2/4/11/12 (*dnd1/2* and *cpr22*) seem to specifically suppress the HR, and mutants are more resistant to virulent oomycetes and bacteria (4, 45, 192).

Lipids (other than JA) also mediate ETI suppression, and a significant number of lesion mimic mutants with constitutive defense represent genes encoding lipid-binding proteins (EDR2, VAD1, ACD11, BON1/CPN1, BAP1/2) (**Supplemental Table 1**). Lipid signals, such as phospholipids, are known to be required for ETI and HR (6). In addition, sphingolipid metabolism affects ETI, HR, and susceptibility (16). Lesion mimic mutant *acd11* (sphingosine transfer protein) and mutants of sphingolipid fatty acid hydroxylases *AtFAH1/2* have increased SA levels and reduced susceptibility to biotrophic pathogens (25, 107). Notably, calcium and sphingolipid signals were recently suggested to be interconnected parts of the ETI and HR pathways (185). Lastly, fatty acid desaturase SSI2 is required for susceptibility to biotrophic fungi, bacteria, oomycetes, and viruses in rice, soybean, and *Arabidopsis* (93, 99, 100). *Ssi2* mutants have decreased 18:1 to 18:0 lipid ratios, and it was suggested that 18:1 lipids negatively regulate the SA pathway. Similarly, *Arabidopsis* and tomato fatty acid desaturase FAD7 suppresses SA defense and *fad7* mutants show decreased susceptibility to aphids. Although basal levels of SA are unchanged, SA levels and defense are enhanced in *fad7* upon aphid infestation (9). It has become clear that pathogen defense pathways are filled with genes that suppress defense at almost any level. These genes control defenses to prevent inappropriate activation (see section Side Effects of Mutating Defense Suppressors), and as such they also contribute to susceptibility.

S Genes Allowing Sustained Compatibility: Sustenance for the Guests

Once a pathogen-host interaction has been established, pathogens continue to utilize the host cell machinery to meet their metabolic and structural requirement for replication and proliferation (**Figure 3** and **Supplemental Table 1**). The interaction between rice and bacterial blight (*Xanthomonas oryzae*) revealed that approximately 10 out of 30 *R* genes are inherited in a recessive

manner (88). In addition, virus resistance is recessively inherited in almost half of the cases (201). Apparently, in these systems, pathogens heavily rely on host susceptibility factors for a successful interaction. Interestingly, the majority of known susceptibility genes in these two types of interactions fall in the category discussed in this section: host genes important for sustained compatibility.

Sugar transport. Most pathogens require nutrient uptake from the host apoplastic space or vasculature and, thus, nutrient secretion from plant cells. In rice, two recessive genes that confer resistance against *X. oryzae* (*Xoo*), *Xa13* and *Xa25*, encode SWEET proteins (SWEET11 and 13, respectively) (37). These are plasma membrane–localized sugar (efflux) transporters required for *Xoo* susceptibility through loading the apoplastic space with sugars, thereby providing nutrients to the pathogen. A similar role is predicted for SWEET12, 14, and 15 (37, 176). Expression of *Arabidopsis SWEET* genes is upregulated by several pathogens, and these genes may contribute to susceptibility as well (37). Interestingly, SWEET11 (*Xa13*) interacts with copper transporters COPT1 and COPT5, and these proteins were also found to be required for full *Xoo* susceptibility. *COPT* and *SWEET11* silencing in rice resulted in increased copper levels in xylem sap and reduced pathogen growth (221). Apparently, sugar export is linked to copper import, and both processes contribute to susceptibility.

Metabolite biosynthesis. In *Arabidopsis*, several mutants with loss of DM (*H. arabidopsidis*) susceptibility because of altered amino acid metabolism have been identified, particularly in the Asp-derived amino acid pathways. *DMR1* encodes a homoserine kinase (HSK) that catalyzes phosphorylation of homoserine (HS), a derivative of the amino acid Asp and a precursor for Met, Thr, and Ile. *Hsk* knockouts are lethal, implying that a basal level of HS conversion is required for synthesis of essential metabolites. The resistant *HSK* mutants overaccumulate HS, but this metabolite does not seem directly toxic to the pathogen. Also, the abundance of HS derivatives, Met, Thr, and Ile, was not significantly reduced (193). *RSP1* encodes an aspartate kinase (AK2) that converts Asp to aspartyl-4-P, used for Met, Thr, and Ile but also for Lys biosynthesis. *Rsp2* encodes a dihydrodipicolinate synthase (DHDPS), catalyzing the first committed step to Lys synthesis, two steps downstream from AK2. Both *AK2* and *DHDPS* mutants lost susceptibility to DM (177). Interestingly, these mutants also do not appear to be deficient in downstream metabolites, probably due to redundancy, as there are five AKs and two DHDPSs. The mechanism of reduced DM susceptibility in *HSK*, *AK2*, and *DHDPS* mutants is not clear; unknown metabolites upstream or downstream of the enzymes may be toxic to, or required by, DM. Alternatively, an amino acid imbalance may affect biosynthesis pathways in the pathogen because of feedback regulation.

Attempts to pinpoint a key metabolite responsible for susceptibility have been inconsistent. One report observed a prominent effect of exogenous Thr, but not HS, on DM susceptibility (177), whereas previous studies found that exogenous HS, but not Thr, decreases DM susceptibility (193). Further work is needed to uncover how Asp-derived amino acid biosynthesis pathways affect DM susceptibility.

Along with a role in host defense signaling (see sections Jasmonic Acid and Salicylic Acid Antagonism and Calcium and (Sphingo)Lipids Mediate Defense and ETI Suppression), lipids are used by pathogens directly. *LOX3* of maize is required for full susceptibility to three fungal pathogens from distinct genera (*Fusarium*, *Colletotrichum*, *Cochliobolus*) (72). Lipoxygenases convert 18:2 and 18:3 lipid substrates toward synthesis of, among others, JA. Intuitively, one might therefore assume that susceptibility is due to LOX3-mediated JA synthesis that can suppress SA defenses directed to the fungi. Indeed, *Lox3* mutants become more susceptible to necrotrophic *Aspergillus* fungi, most likely due to decreased JA synthesis (71). However, for the biotrophic fungi mentioned above, it appears that the fungal biomass in leaves was similar to wild type, but mainly

the disease symptoms were reduced in *lox3* mutants. Moreover, production of conidia/spores was strongly reduced and production of the fungal toxin fumonisin B1 from *Fusarium* was eliminated (72). Hence, LOX3 may produce lipid compounds that are essential for fungal reproduction and toxin biosynthesis.

Endoreduplication: replication of the nuclear genome without cell division, resulting in elevated gene content (polyploidy) and enhancing metabolic capacity

Endoreduplication and cell expansion to boost metabolic output potential. Besides transport, metabolite production can also be increased to benefit pathogens. In barley, ADH (alcohol dehydrogenase) is induced upon PM infection and supports pathogen growth by increasing (anaerobic) glycolytic metabolism. *ADH*-silenced plants were less susceptible to PM (147). Host cells in overall metabolic overdrive provide an even better environment for pathogens. PM infection is accompanied by a marked increase in cell ploidy by endoreduplication at the site of infection (31). The transcription factor MYB3R4 and the ubiquitin X domain protein PUX2 are both required for PM susceptibility through endoreduplication regulation and cell cycle progression (30, 32). Surprisingly, the PM susceptibility genes *PMR5* and *PMR6*, which encode pectate lyases, also contribute to mesophyll ploidy level increase (31). The mechanism for pectate lyase–mediated susceptibility has been obscure, as the *PMR5* and *PMR6* mutant phenotypes are independent of known defense signaling pathways and although their cell walls accumulate more pectin, penetration efficiency is unaltered. Yet, the fungus produces less hyphae, conidiophores, and conidia at later stages (198, 199). A plausible hypothesis is that a more rigid pectin-rich cell wall imposes physical constraints to cell expansion, which feedback-inhibits the endoreduplication machinery (31). Endoreduplication also promotes susceptibility to giant cell– or syncytia-inducing nematodes and symbiotic, nitrogen-fixing *Rhizobium* bacteria. In both cases, the endocycle activating CCS52 proteins was shown to be required for establishing an efficient interaction (50, 195).

Hypertrophy, the enlargement of cells, is induced by *Xanthomonas* infection in pepper, and was found to be mediated by the bHLH transcription factor Upa20 and its target, expansin Upa7 (103). Although expansin-mediated facilitation of cell wall penetration by pathogens was suggested elsewhere (see section Cuticle and Cell Wall Structure), Upa20 and Upa7 likely facilitate susceptibility to *Xanthomonas* through increased nutrient production by enlarged host cells (103). We can conclude that pathogens and symbionts utilize the plant's capacity to increase metabolic (and therefore nutrient) output. The facilitation of this process by several host genes, acting as susceptibility genes/factors, seems to be a conserved theme in diverse microbe-host interactions.

Virus replication. Viruses have very compact genomes and heavily rely on host factors for completing their life cycle, most notably the host's replication and translation machinery. Different virus genera seem to require different plant proteins. TOM1, TOM2, and TOM3 are tonoplast transmembrane proteins required for formation of the RNA replication complex of tobamoviruses like *Tomato mosaic virus* (ToMV) and *Tobacco mosaic virus* (TMV). TOM proteins were first characterized in *Arabidopsis* (190, 213, 214). The genes are partly redundant, and mutating two genes, *TOM1* and *TOM3*, led to complete inhibition of virus replication (213). Silencing the *TOM* homologs in various tobacco species resulted in efficient inhibition of TMV multiplication but not of *Cucumber mosaic virus* (CMV), a bromovirus (8, 35, 110). This indicates that the function of TOM proteins as tobamovirus susceptibility factors is highly conserved across plant species. The ARL8 proteins are small GTP-binding proteins encoded by a small gene family with three and four homologs in *Arabidopsis* and tobacco, respectively. ARL8 is also part of the RNA replication complex, and mutating two *ARL8* genes in *Arabidopsis* also resulted in complete inhibition of tobamovirus replication (140). Interestingly, both *TOM* and *ARL8* mutants do not show any other obvious phenotypes, so the function of these genes for the plant remains unknown.

Translation of potyvirus RNA requires the host translation initiation complex, consisting of cap-binding protein eIF4E, scaffold protein eIF4G, and seven other complex members, including the ribosomal subunits (102). The 5′ end of potyvirus RNA is occupied by viral protein VPg, which interacts with the plant eIF4E protein (209). This interaction is essential for viral RNA translation and eIF4E is the most important susceptibility factor for potyviruses. eIF4E (as well as eIF4G) has two isoforms, and an impressive number of *eIF4E* and *eIF(iso)4E* mutations have been identified in many plant species that abolish susceptibility to potyviruses (201) (**Supplemental Table 1**). There are a few examples of eIF4E being identified as a susceptibility factor for families other than potyviruses. These include eIF4E for a tombusvirus [*Melon necrotic spot virus* (MNSV)] in melon and eIF4E as well as eIF4G for a bromovirus (CMV) in *Arabidopsis* (138, 219). eIF4G has also been found essential for several viruses but to a lesser extent than eIF4E. As mentioned above, this was shown for a bromovirus (CMV) in *Arabidopsis* but also for a tombusvirus [*Turnip crinkle virus* (TCV)] in *Arabidopsis*, a sobemovirus [*Rice yellow mottle virus* (RYMV)] in rice, a sequivirus (*Rice tungro spherical virus* (RTSV)] in rice, and four potyviruses [Plum pox virus (PPV), *Turnip mosaic virus* (TuMV), *Lettuce mosaic virus* (LMV), and *Clover yellow vein virus* (ClYVV)] in *Arabidopsis* (3, 22, 115, 136, 219) (**Supplemental Table 1**). It is interesting to note that for the plant itself, two possible initiation factor complex isoforms, containing either eIF4E and eIF4G or eIF(iso)4E and eIF(iso)4G, exist and seem to function redundantly; mutating one of them does not cause any observable developmental phenotype (46). In contrast, most potyviruses are completely dependent on one of the two isoforms; the VPg interaction with eIF4E (or eIF4G) is isoform specific. Few other host factors important for virus multiplication have been reported so far. Those that have include RNA helicases RH8 and PpDDXL for potyviruses in *Arabidopsis* and peach (82), a NAC domain transcription factor, *rim1*, for a reovirus [*Rice dwarf virus* (RDV)] in rice (220), and a DNA binding protein phosphatase, AtDBP1, for potyviruses in *Arabidopsis* (28).

Transcription activator–like (TAL) effectors: effector family from the bacterial pathogen *Xanthomonas* consisting of a DNA binding and transcription activation domain

Supplemental Material

PATHOGENS EXPLOIT *S* GENES: EFFECTOR TARGETS

Pathogens deploy effector proteins to disarm defense networks and to increase nutrient availability. Filamentous pathogens are predicted to secrete up to several hundred effectors, whereas bacteria produce a few dozen (24, 56, 211). Most effectors appear to act inside the host cell; bacteria can inject effectors using a type III secretion system (211), whereas filamentous pathogens secrete effectors and rely on effector uptake by the host cells (24, 56). Identification of effector-host target interactions is essential for our understanding of pathogen virulence and plant susceptibility. To increase virulence, many pathogen effectors suppress host factors involved in resistance (53). However, it makes sense that some effectors can also activate susceptibility factors encoded by *S* genes. In this section, examples are given of *S* genes targeted by pathogen effectors.

The TAL (transcription activator–like) effector family from the bacterial pathogen *Xanthomonas* has received much attention in recent years. TAL effectors bind and activate the promoters of specific host proteins (20). The determinants of binding specificity have been elucidated (21), which opens up great possibilities for genome editing technology. Importantly, this discovery also facilitates prediction and identification of TAL effector targets, which are potential *S* genes (142) (see section Strategies for *S*-Gene Identification and Application). AvrBs3 from *X. campestris* was the first confirmed TAL effector directly regulating an *S*-gene target; the cell size regulator Upa20 (103) (see section Endoreduplication and Cell Expansion to Boost Metabolic Output Potential). The sugar transporter genes *OsSWEET11* and *OsSWEET14* (see section Sugar Transport) are confirmed targets of up to five *Xanthomonas* effectors (37, 162, 176). Lastly, transcription of the rice transcription factor genes *OsTFIIA*γ and *OsTFX1* is induced by two *Xanthomonas* effectors (162, 178).

Although altering *S*-gene expression seems a strategy particularly adopted and expanded by *Xanthomonas* bacteria, there are other pathogen effectors known to target *S* genes. An interesting atypical case is the victorin toxin from the fungus *Cochliobolus* that activates LOV1, an NB-LRR protein in *Arabidopsis*. One role of LOV1 is providing resistance to a biotrophic pathogen by activating ETI and cell death. However, the necrotrophic *Cochliobolus* benefits from necrotizing tissue and thus LOV1 is also a *Cochliobolus* susceptibility factor; victorin hijacks this *R* gene to turn it into an *S* gene (122).

Pseudomonas effector HopZ2 binds AtMLO2, and HopZ2 contributes to virulence in an *AtMLO2* dependent manner, indicating the significance of this *S*-gene interaction (117). In addition, *Pseudomonas* effector AvrB phosphorylates and thereby activates two PTI suppressors, MPK4 and RIN4 (49, 54) (see section Suppressors of PTI/DTI). Lastly, nematodes are notorious for reprogramming host cells to allow formation of enlarged feeding sites (root cysts or root knots) by secreting effectors (78). Two effectors were shown to bind a pectin methylesterase (PME3) and a spermidine synthase (SPDS2), which were both demonstrated to be *S* genes upregulated during infection (79, 80).

In conclusion, although effectors are most known for their suppression of resistance, a significant number of effectors in fact activate *S* genes. Research in this field received a great boost from the uncovering of the TAL effector repertoire and can inspire new strategies for breeding *S* gene–based resistance, which is discussed below.

S-GENE APPLICATION TOWARD BREEDING PATHOGEN RESISTANCE

Supplemental Material

According to our definition of an *S* gene, its activity benefits the pathogen and contributes to disease susceptibility of the host plant. Consequently, disabling an *S* gene enhances host resistance, as demonstrated by many examples of *S*-gene mutants described in this review and listed in **Supplemental Table 1**. The potential of increased resistance, or even nonhost resistance, makes *S* genes highly interesting targets for resistance breeding. However, for an *S*-gene mutant to be usable in crop breeding, several characteristics need to be considered (**Figure 4**). First, will mutating or otherwise impairing *S*-gene function have serious pleiotropic effects, such as sensitivity to other stresses, dwarfing, or other undesirable side effects? Second, will targeting an *S* gene result in sufficiently improved resistance, and is that improvement qualitative or quantitative? Third, if a susceptibility factor is encoded by multiple (redundant) genes of a gene family, is it feasible to target multiple genes and combine multiple alleles? Most known *S* genes have been identified as monogenic recessive gene mutants having a significant resistance phenotype. Some candidate *S* genes, however, have been found using overexpression studies or RNAi, which cause dominant effects/phenotypes, and in these cases the contribution of single gene family members is unclear. If the above criteria are met, the final question is whether *S* gene–based resistance is durable.

S Genes: More Durable than *R* Genes?

Durability of a new resistance-enhancing trait is difficult to predict and depends mostly on the adaptability of the pathogen. However, a limited number of applied examples, and the fundamental difference between *S* gene– and *R* gene–based resistance, provide hints. *S* genes are recessively inherited, and resistance is the result of the loss of function of a host factor required by the pathogen. *R* genes are dominantly inherited and resistance is triggered when a pathogen-derived avirulence determinant (often an effector) is recognized by the R protein. Other dominant resistances can be mediated by PRRs recognizing PAMPS or DAMPs or by genes promoting production of defense compounds and/or structural barriers.

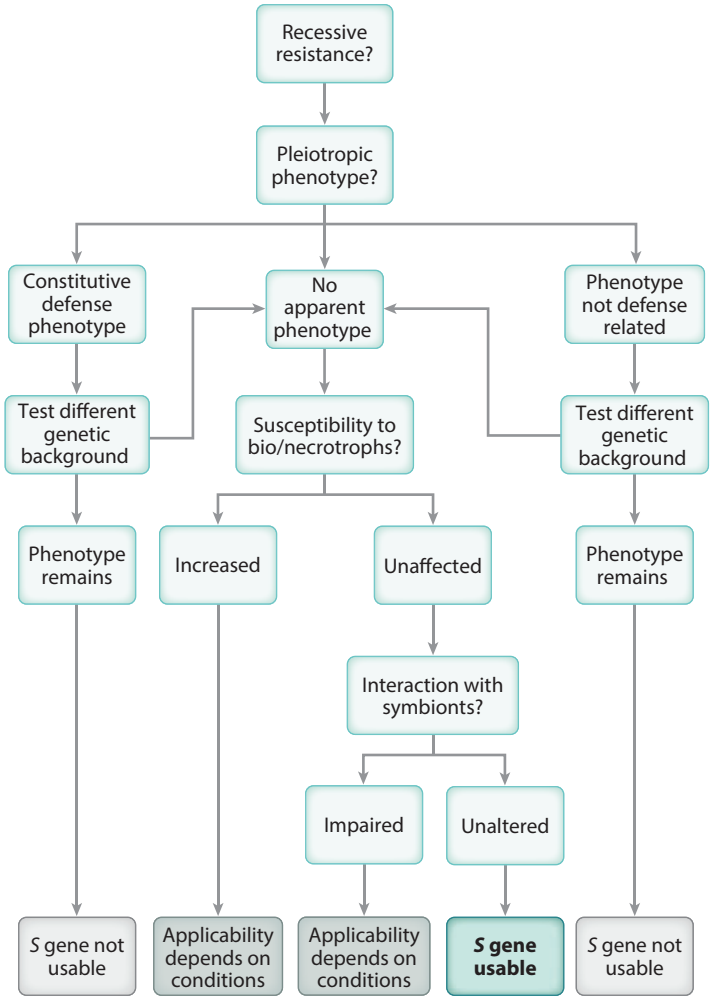

Figure 4

Classification scheme to determine the usability of a susceptibility (*S*) gene. First, recessive resistance is observed (mutant with reduced susceptibility). Second, pleiotropic effects should be monitored (growth, yield, fertility, senescence, and abiotic stress tolerance). If a deleterious phenotype is present, it is important to test whether it can be alleviated in a different genetic background. Third, the plant response to pathogens with a different lifestyle (biotroph versus necrotroph) should be evaluated. Lastly, plant performance should be tested in field conditions, as interactions with beneficial microbes, such as rhizobia and mycorrhiza, may be affected.

For the pathogen to overcome R gene–based resistance, a simple point mutation in a protein/effector recognized by an NB-LRR or a PRR may be sufficient to evade recognition. Many effectors are recognized indirectly by NB-LRRs monitoring a host target. In that case, an effector would need to alter its activity on the host target, or it would need to disappear altogether. Effectors often operate redundantly; dozens of effectors may be injected into a host, and effectors are often on genomic regions prone to rapid mutation and reshuffling (156, 158, 210). Resistance durability can be predicted by assessing the evolutionary potential of pathogens as well as the fitness penalty of losing the effector; recognition of conserved effectors is likely more durable

(114, 128, 196). Durability has also been demonstrated to depend on the host genetic background; *R* genes introduced into plants already having quantitative/partial resistance [quantitative trait loci (QTLs)] may be more stable (14, 26, 174). In addition, *R*-gene stacking can likely be used to increase durability of disease resistance (106, 196, 226).

For a pathogen to overcome *S* gene–based resistance rather than evading *R* gene–based recognition, it must overcome a dependency on a host factor. This may mean the pathogen needs to acquire a new function, which is more difficult to accomplish than loss-of-function. Obligate biotrophs, especially, have a strong dependency on several host factors, such as essential metabolites, they cannot produce themselves (173). Therefore, we predict that *S* gene–based resistance is generally more durable than *R* gene–based resistance. *mlo* is the most frequently mentioned example of an *S* gene providing durable PM resistance. It has been used for many decades, and resistance-breaking pathogen strains have not been found in the field (97). Plants with the recessive *mlo* allele seem to have increased penetration defenses, but more importantly, plants are likely unable to cooperate with membrane and cytoskeleton reorganization to allow formation of haustoria for nutrient exchange (see section Membrane Dynamics Support Establishment of Haustoria). This is a mechanistic/structural requirement that the pathogen cannot easily overcome.

The best-studied type of recessive resistance (*S*-gene mutant) in terms of durability is the *eIF4E*-based resistance against potyviruses. Pepper *pvr1/2* was the first recessive potyvirus (*Potato virus Y*) resistance identified and has been successfully used for more than 50 years (48, 133). Potyvirus isolates breaking *eIF4E*-based resistance have been reported and, in most cases, are explained by mutations in viral protein VPg (10, 33, 126, 132, 188). The virus depends on a physical interaction between its VPg and plant eIF4E to establish successful replication (33, 209). eIF4E (or G) resistance–breaking strains may regain binding capacity to the mutated eIF4, acquire new specificity to a different eIF4 isoform, or even bypass the requirement of eIF4 binding altogether. The latter has been suggested in one study (69). Whereas many studies on resistance-breaking viruses have been conducted in labs by forcing or mimicking virus evolution, resistance-breaking strains have rarely been identified in the field. There have been two instances in which a viral protein other than VPg is responsible for overcoming the requirement for eIF4E (1, 135). For a resistance based on an impaired physical interaction between VPg and a mutated eIF4E, with only a single or a few point mutations, resistance is surprisingly durable. Being encoded by a viral genome, and having very short generation times and notoriously high mutation rates, VPg has the potential to evolve and mutate rapidly. However, there are likely constraints that limit the number of allowed mutations in VPg (131). Interestingly, there seems to be a correlation between the resistance spectrum (number of different potyviruses) of *eIF4E* alleles and their durability (131), which suggests that the resistance spectrum of new *eIF4E* alleles may be used to predict durability. In addition, *eIF4E* durability was found to be much higher when introgressed into a genetic background with quantitative trait loci for partial resistance (145).

The *MLO* and *eIF4E* examples illustrate that pathogens relying on host factors for successful establishment or replication run the risk of reaching a dead end in the evolutionary arms race. They would either have to backtrack and regain a lost function or just abandon that host and find another one. It is this principle that makes *S* genes such interesting targets for resistance breeding. However, as with many promises that seem too good to be true, there is a catch.

Side Effects: Pitfalls of *S*-Gene Mutation

Besides reduced susceptibility, mutating an *S* gene may have other consequences for the plant, as *S* genes have a primary function. The question is, do the benefits of reduced susceptibility outweigh the potential negative effects of losing or changing the function of a plant gene? We here discuss

pleiotropic effects of mutation of an *S* gene in the different phases of pathogen-host interactions (see also **Supplemental Table 1**).

Nonadapted pathogen: a pathogen that triggers a PTI response on attempted colonization, inducing nonhost resistance

Supplemental Material

Side effects of mutating prepenetration factors. *S*-gene mutants with altered cuticle composition may be less susceptible to some pathogens (see section Cuticle and Cell Wall Structure), but several other processes require cuticle integrity. Beneficial mycorrhiza use cuticle cues to initiate their symbiotic interaction. The *Medicago GPA7* mutant illustrates that increased resistance to oomycete pathogens may come at the cost of impaired interaction with mycorrhiza (202). In addition, mutants with increased cuticle permeability are able to ward off necrotrophic fungal pathogens more efficiently, but these mutants turn out to be more sensitive to salt, cold, and drought stress, as well as to biotrophic bacterial pathogens (2, 182, 207).

Fungal penetration and establishment depend on focal membrane and cytoskeleton rearrangement and vesicle traffic mediated by MLO, BI-1, LFG, RAC/ROP, and ARF-GAP, but these likely have additional functions. Indeed, the pleiotropic effects of mutating these genes have been described, most clearly for *mlo*, and they relate to three distinct activities: First, changes in prepenetration defense and secretion adversely affect defense to nonadapted pathogens (e.g., *Arabidopsis agd5* and *rop6*) (154, 166). Secondly, changes in cytoskeleton rearrangement and focal membrane protrusion/penetration adversely affect root hair growth and pollen tube growth and guidance (e.g., *Arabidopsis mlo7* and *rop6*) as well as penetration of beneficial mycorrhizae (e.g., barley *mlo*) (104, 164). Also *RAC/ROP* genes are especially known to be involved in these processes (108, 222). And third, reduced cell death suppression affects growth, senescence, and lesions as well as resistance to necrotrophic pathogens (see also section Side Effects of Mutating Defense Suppressors) (e.g., barley *mlo*, *Arabidopsis mlo2*, *-6*, *-12* and pepper *mlo*) (47, 91, 97, 109, 225).

Despite these issues, barley *mlo* mutants have been successfully used in agriculture. The effects on lesions, senescence, and reduced growth could largely be eliminated by introgression into the right genetic background (19). Issues with increased susceptibility to the blast fungus are mostly avoided by growing *mlo* plants in areas where the rice blast pathogen is absent (northern Europe). Moreover, a mutant (*emr1*) was isolated in the *mlo* background with restored resistance to rice blast (90). The diverse effects of *mlo* mutation in various plants illustrate that the function of *mlo* gene family members has diverged considerably. In tomato, pea, and strawberry, silenced or mutated *mlo* resulted in PM resistance without observable side effects (12, 86, 95, 149, 150). In conclusion, the applicability of *mlo* for reduced PM susceptibility may depend on the crop species, but the range of observed pleiotropic effects provides a glimpse of the trade-offs that need to be considered.

Side effects of mutating defense suppressors. Most *S* genes in this category are involved in direct or indirect suppression of SA-mediated defense pathways. Many mutants with deregulated PTI or ETI have constitutively activated defense and consequently suffer from deleterious effects of high SA levels, such as dwarfing and spontaneous lesions due to ROS and HR cell death (123, 130). In addition, increased SA levels generally cause a decrease in JA-related defense against necrotrophs or chewing insects, a well-known defense hormone antagonism (160). *S*-gene mutants that enhance biotroph resistance but reduce necrotroph resistance are, for example, PTI suppressors BIK1, MKP2, MPK4, EDR1, and AtWRKY4/8/18/33/40/60 (**Supplemental Table 1**). Effects on insect feeding are not usually tested, but an *SR1(/CAMTA3)* mutant that has reduced susceptibility to several pathogens turned out to be more susceptible to insect feeding. If side effects are limited to increased sensitivity to other pests, applicability depends on the local disease pressure.

There are only a few mutants in this class known to provide enhanced defense without major pleiotropic effects. *Arabidopsis cdd1* shows constitutive SA defenses and a decreased susceptibility to bacterial, oomycete, and fungal pathogens, yet no lesions and no effect on growth or development

were reported (179). Callose synthase mutant *Pmr4* also does not exhibit significant growth aberrations despite having heightened SA defenses (141). Lastly, *S* genes required for resetting PTI after activation, such as ubiquitin ligases PUB22/23/24 and MAPK phosphatases MKP1 and MKP2 have no or only mild growth phenotypes. In these mutants, basal SA defenses seem normal, and only after pathogen infection are the responses enhanced and prolonged (5, 124, 186). These examples indicate an important distinction between two types of defense-suppressing *S* genes:

1. Suppressors of defense onset. These are *S* genes that are required for constitutive suppression of defense signaling when no threat is present. Mutations in these genes cause defense signaling to go haywire; examples are suppressors of SA signaling, R-protein signaling, and cell death.
2. Suppressors of defense persistence. These *S* genes reset defense signaling after it was first induced. When mutated, these genes may cause low levels of constitutive defense, but more importantly, they enable enhanced and prolonged defense signaling. Examples are enzymes that inactivate SA, MAPK phosphatases that inactivate MPK-mediated PTI signaling, and ubiquitin ligases that mark PTI components for degradation/inactivation. Type 2 defense suppressors are expected to have fewer pleiotropic effects and are therefore probably more suitable for application.

Side effects of mutating *S* genes that provide pathogen sustenance or replication machinery. The *SWEET* genes encoding sugar transporters are required for sugar efflux, including phloem loading for providing roots and other tissues with energy. The rice *OsSWEET14/11* mutant has reduced stature and suffers from pollen abortion (7, 43), whereas an *Arabidopsis Sweet11/12* double mutant has reduced root length and aboveground growth retardation (38). This indicates that restricting sugar availability for pathogens in the apoplast can affect plant growth as well.

The potyvirus resistance of *eIF4E* mutants is rather unusual among *S* genes. This is one of very few cases in which a subtle mutation does not affect the function of the plant protein but confers loss of susceptibility due to a disturbed interaction between eIF4E and VPg. However, a subtle mutation might allow the pathogen to evolve and regain the ability to interact with the mutant form or with other *eIF4E* isoforms, making this type of loss-of-susceptibility potentially less durable (see section *S* Genes: More Durable than *R* Genes?) (69, 92). Although not commonly observed in the field, avoiding pathogen adaptation may require mutations in multiple *eIF4* genes. However, disabling both isoforms of *eIF4E* or of *eIF4G* results in growth defects or even lethality (116, 127, 136).

In conclusion, one should be aware of possible pleiotropic effects of *S*-gene mutation, and depending on the cellular function of the *S* gene, the nature of these effects can often be anticipated, allowing a proper risk assessment. An illustrative example can be found in Reference 71: "Because *Aspergillus* spp. and *Fusarium* spp. coexist naturally in maize fields, engineered resistance to one pathogen at the expense of susceptibility to the other is useless," which points out not only the importance of the direct costs of introducing the *S* gene for, for example, yield but also its altered sensitivity to other (a)biotic stresses.

Strategies for *S*-Gene Identification and Application

Since the 1940s and 1960s, respectively, the *mlo* alleles in barley and *eIF4E* (*pvr*) alleles in pepper have demonstrated the potential for a more widely adopted application of *S* genes in commercial

crops. The first PM-resistant *mlo* allele in barley was identified from a forward genetic screen after radiation mutagenesis (67), and natural *mlo* alleles were found in tomato and pea (12, 86, 149, 150). The potyvirus resistance based on *eIF4E* mutation was first found as natural alleles in pepper and later also in other crops (48, 163, 201). Besides *mlo* and *eIF4E*, the use of *S* genes in breeding has been rather limited, as their recessive nature hampers identification and complicates breeding. Unbiased functional (forward) screens can be difficult to perform in crops at large scale, and polyploidy of some crops can be another complication in identifying effects of recessive mutations. To identify novel *S* genes, the TAL-effector repertoire from *Xanthomonas* pathogens provides a great resource, as these effectors are known to bind to promoters of *S* genes (see section Pathogens Exploit *S* Genes: Effector Targets). Hence, for any TAL-effector target, an attractive possibility would be to mutate its promoter such that effector binding is abolished but plant gene function stays intact. Although non-TAL-effector families usually suppress the function of resistance factors, a subset interacts with *S*-gene products and thus effectors can generally serve as bait.

Functional screens in the model plant *Arabidopsis* have yielded many *S*-gene candidates (**Supplemental Table 1**), and identification of homologous gene sequences from crops is greatly facilitated by available genome and transcriptome sequencing data. These resources allow a reverse genetic approach in which molecular screening can focus on the gene of interest or mutations can be targeted to specific locations. Whether a crop gene with high homology to a potential *S* gene of *Arabidopsis* also contributes to susceptibility is an important first question. Several crops allow transient silencing with viral vectors to test relatively quickly whether decreased expression of the potential *S* gene indeed decreases susceptibility (113). This was demonstrated for *MLO* homologs in wheat and pepper (194, 225). Alternatively, the candidate crop *S* gene could be tested for its ability to functionally complement susceptibility of the corresponding *Arabidopsis* mutant. Having identified a potential *S* gene in a crop, obtaining a stable mutant can be done by (*a*) screening a (mutant) population with molecular techniques to find mutations in the *S* gene of interest or (*b*) creating *S*-gene mutants in a targeted manner.

Supplemental Material

The first approach involves making a population of mutagenized plants containing typically up to 1,000–20,000 individuals. EMS (ethyl methanesulfonate) is most commonly used for this. Collections of natural accessions are sometimes used as a source of allele variety [ecoTILLING (targeted induced local lesions in genomes)]. Molecular screening techniques have developed from the classic TILLING based on mismatch cleavage with endonucleases and single nucleotide polymorphism (SNP) detection with high-resolution melt-curve (HRM) analysis, Taqman probes, or next-generation sequencing (NGS) (203). EcoTILLING was used to identify allelic variants of *eIF4E* in melon (139). Other TILLING projects yielded multiple new *mlo* alleles in barley (157, 181) and *eIF4E* alleles in tomato (153). In the hexaploid wheat, a large, targeted screen identified mutations in four potential *S* genes, *NFXL1*, *CeSa8*, *PLDb1*, and *PFT1* (**Supplemental Table 1**), using Taqman probes (66). Lastly, an efficient molecular screen using NGS and a multidimensional DNA pooling and (adapter) labeling strategy was employed to identify mutant alleles of *eIF4E* in tomato (159). With these screens, mutation frequencies are variable and depend on the mutagen dose, plant species, and also ploidy; tetra and hexaploids tolerate higher treatment dose (203).

The second approach involves targeted knockdown or knockout of the gene of interest. Silencing with antisense-, hairpin-, or microRNA can yield stable knockdowns. RNAi was successfully used to silence *MLO* in strawberry and tomato (95, 225), and *eIF4E* in melon, plum, and tomato (127, 161, 204). In addition, RNAi of *PMR4* and *DMR1* homologs in tomato demonstrated the applicability of these genes outside *Arabidopsis*, although *DMR1* silencing also caused dwarfing (84).

Interestingly, overexpression of a mutant *eIF4E* allele in tomato and potato demonstrated an alternative approach based on dominant negative effects of the introduced transgenes (29, 101).

An advantage of RNAi (and overexpression) is that expression can be driven by a tissue-specific promoter, thereby minimizing anticipated pleiotropic effects in unrelated tissues. An interesting example here is the rice *Xanthomonas* susceptibility gene *SWEET11*, which was successfully targeted by an artificial microRNA that was expressed only in leaves, so pollen development was unaffected (118). In addition, with RNAi it is possible to silence multiple homologous genes at once. The trait inherits dominantly and is stable across many generations. However, long-term stability has only been evaluated in one rice study and a few shorter studies in *Arabidopsis* (111).

In recent years, targeted genome editing techniques have gained much interest. Engineered zinc finger nucleases or TAL effector nucleases are able to introduce mutations at designated locations (36). Most recently, RNA-guided DNA endonucleases (CRISPR/Cas9) have been added to the genome editing toolkit. Compared with the two earlier techniques, CRISPR/Cas9-mediated genome editing has the advantage that rather than customizing large, repetitive protein domains using complex cloning strategies, only a short oligo of ~20 nucleotides needs to be inserted into the guide RNA (36). Efficiencies of these nuclease-based methods are as much as 1% to 5% and even more than 30% for CRISPR/Cas9 in protoplasts (121). The first demonstrated use of *S*-gene mutation using the novel CRISPR genome editing tool involved *SWEET11* and *SWEET14* in rice (94). As an alternative, oligonucleotide-directed gene targeting or mutagenesis (ODM) had earlier demonstrated its use in plants (143). This involves translocating an oligo with a desired mutation into cells (protoplasts), which anneal to the target sequence with a very low frequency (~0.01%–0.1%), and the mismatch repair machinery copies the mutation into the genome. Despite the lowest mutation frequency, ODM has the advantage of not requiring transgene insertion, as the methods mentioned above do.

Especially for the European market, current GMO (genetically modified organism) regulations are a major hurdle for applying *S* genes using targeted mutagenesis methods. All targeted methods above, except RNAi, can be used to create a crop with only the target site mutated. However, current EU GM legislation states that crops are evaluated based on the technique used (involving alteration of genetic material with non-natural methods and foreign nucleic acids). It could be argued that instead the end product/trait should be evaluated. This principle, as well as regulations on some of the above-mentioned methods specifically, is currently under debate (76). It is ironic to realize that TAL effectors, used by pathogens to induce host *S* genes, can now be modified and used against them by inactivating those same *S* genes and are in fact currently one of the most used genome editing techniques in eukaryotes.

CONCLUSIONS AND PERSPECTIVES

We have outlined the diversity of plant genes that contribute to disease susceptibility and pathogen performance at various stages of pathogen infection. Pathogens may passively benefit from the activity of *S*-gene products or force plants to cooperate by activating or stabilizing *S* genes or their products, using effectors. *S*-gene mutants have increased pathogen resistance and are therefore attractive targets for crop breeding. The underlying mechanisms suggest a higher durability compared with *R* gene–based resistance, especially for obligate biotrophic pathogens, such as viruses and mildews. Indeed, *mlo* in barley and *pvr* (eIF4E) in pepper have been used commercially for decades. However, *S* genes are by no means magic bullets for accomplishing resistance. Given that *S* genes do not merely exist for the pathogen's convenience but have an evolutionarily conserved function in plant processes, one should be aware of possible pleiotropic effects that could outweigh their benefits. Typical effects include reduced growth, yield, and fertility, early senescence, reduced tolerance to abiotic stress, and altered resistance to other pathogens, most notably necrotrophs. We suggest a simple classification scheme of *S*-gene mechanisms. Knowing at which

stage of infection the *S* gene functions allows, to some extent, prediction of pleiotropic effects. Phenotypic analysis of *Arabidopsis* mutants further aids in anticipating the effects of mutating *S* genes in crop species. Optimizing the genetic background or combining with compensatory mutations has been shown to alleviate most pleiotropic effects for *mlo* in barley. In addition, some side effects may be controllable by minimizing exposure to, for example, abiotic stress or necrotrophic pathogens. Identification of new *S* genes by forward genetic screens or identification of effector targets can further expand our understanding of the molecular basis of pathogenicity, helping to uncover processes most crucial to infection. The ever-expanding *S*-gene repertoire, increased access to genome sequencing technology, and novel precise genome editing tools will further support strategies to defeat pathogens using host genes never meant to function as resistance genes.

DISCLOSURE STATEMENT

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