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Good Riddance? Breaking Disease Susceptibility in the Era of New Breeding Technologies

Stefan Engelhardt, Remco Stam and Ralph Hückelhoven *

Chair of Phytopathology, TUM School of Life Sciences Weihenstephan, Technical University of Munich, 85354 Freising, Germany; stefan1.engelhardt@tum.de (S.E.); stam@wzw.tum.de (R.S.)

* Correspondence: hueckelhoven@wzw.tum.de; Tel.: +49-8161-713-682

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Abstract: Despite a high abundance and diversity of natural plant pathogens, plant disease susceptibility is rare. In agriculture however, disease epidemics often occur when virulent pathogens successfully overcome immunity of a single genotype grown in monoculture. Disease epidemics are partially controlled by chemical and genetic plant protection, but pathogen populations show a high potential to adapt to new cultivars or chemical control agents. Therefore, new strategies in breeding and biotechnology are required to obtain durable disease resistance. Generating and exploiting a genetic loss of susceptibility is one of the recent strategies. Better understanding of host susceptibility genes (*S*) and new breeding technologies now enable the targeted mutation of *S* genes for genetic plant protection. Here we summarize biological functions of susceptibility factors and both conventional and DNA nuclease-based technologies for the exploitation of *S* genes. We further discuss the potential trade-offs and whether the genetic loss of susceptibility can provide durable disease resistance.

Keywords: plant immunity; effector-triggered susceptibility; necrotrophic effector; biotroph; susceptibility gene; host reprogramming; pathogen nutrition; plant cell development; natural diversity; CRISPR

1. Introduction

In crop production systems, plant diseases are controlled by standard field management practices (e.g., crop rotation, ploughing), usage of disease-resistant cultivars and pesticide applications. However, disease resistance and pesticide efficacy are often not durable because pathogen populations rapidly adapt to the selection pressure that is exerted by these disease control mechanisms. This and potentially harmful effects of pesticides on off-target organisms can render plant protection unsustainable, necessitating novel approaches to combat plant pathogens. In recent years, fundamental research on molecular plant-microbe interactions has revealed new insights on how plants defend themselves against pathogens and how pathogens subvert plant immunity. This knowledge and the development of new breeding technologies holds the potential for innovative approaches in genetic plant protection, which could complement the limitations of conventional technologies to provide greater resistance durability [1].

In plants, invading pathogens are challenged at several levels of plant-pathogen interactions [2]. Preformed defensive barriers together with pathogen-induced plant defense responses successfully restrict parasitic growth on resistant plants. Induced plant defenses have, however, led to the adaptation of pathogens to certain host species and the evolution of host-specific virulence strategies. This includes the secretion of proteinaceous and non-proteinaceous pathogenicity factors that support pathogen virulence. Since these so-called effector molecules are required to actively overcome host immune barriers, the term "effector-triggered susceptibility" (ETS) was coined [1]. Most reports on

effector activities show that effectors function as suppressors of plant immune receptor functions, signal transduction and defense reactions [3]. Hence, the suppression of plant defense appears to be pivotal for virulence. Effectors can either specifically modulate host immune processes or more broadly influence host physiology. The latter often contributes to the development of disease symptoms. Such effectors may even provoke strong symptoms when applied as pure substances to plants. In this case, they are considered as toxins that can act either host-specifically or host-nonspecifically. Some effectors influence plant development and some pathogens produce plant hormones or hormone analogs to manipulate host development or physiology.

Pathogen detection either takes place at the plant cell surface, where surface receptor complexes function, or in the host plant cytoplasm or nucleoplasm by intracellular receptor proteins or receptor complexes [1,4]. Plant immune receptors (so-called resistance proteins encoded by major disease resistance genes [*R*]) detect the presence of effector proteins in a race-cultivar specific manner as determined by monogenic inheritance in both the host and parasite. At the molecular level, this classical gene-for-gene model is described by the term "effector-triggered immunity" (ETI) [1]. A more basal, race-nonspecific type of immunity operates within the broader context of ETS and ETI and it is mediated by the detection of a broad spectrum of non-self or altered-self molecules. This is collectively summarized as pattern-triggered immunity (PTI) [4,5].

2. The Principle of Susceptibility Genes and How to Find Them

Host immune components are encoded by dominantly inherited genes, which show either major (qualitative) or minor (quantitative) effects on disease resistance. However, the observation that disease resistance can also be recessively inherited indicates that pathogens can also profit from dominantly inherited host functions or susceptibility factors [1,6,7]. The corresponding dominantly inherited genes are called susceptibility genes (*S*). Recessive *s* genes have been successfully used in conventional and marker assisted plant breeding for the improvement of disease resistance. Recessive *mlo* (*mildew locus o*), several virus resistance genes and ToxA-insensitive *tsn1* genes are prominent examples for this [8,9]. Here we discuss the mechanisms of disease susceptibility and how provoking and exploiting genetic loss of susceptibility can aid durable disease resistance.

Farmers and breeders have selected naturally occurring mutations of *s* genes for the improvement of crop health. Additionally, breeders and researchers searching for mutagenesis-induced resistance in crop and model plants have identified a broad variety of recessive disease resistances. High research effort over the last three decades enabled the identification of several of the corresponding mutations in *S* genes and first insights into the mechanisms of disease susceptibility.

The *mlo*-mediated powdery mildew resistance is perhaps the most prominent example of recessive plant disease resistance. It is of particular interest, because it is race-nonspecific and durable in the field. The *MLO*-gene was originally characterized in spring barley but it seems to function in all interactions in which *MLO S*-gene functions have been studied in detail [8]. Ethiopian highland farmers may have originally selected the barley *mlo-11* allele in old land races, collected during expeditions in the 1930s, and used later in European plant breeding in the 1970s [10]. However, the first description of *mlo* goes back to an X-ray induced powdery mildew mutant generated in the 1940s [11]. Across multiple plant species, many mutagenesis-derived loss-of-function *mlo* alleles exist and *MLO* null-mutants are generally resistant to powdery mildew assuming no genetic redundancy with other *MLO* family members exists. The exact biochemical function of the f protein is not understood, but it may act as a negative regulator of pathogen-triggered and spontaneous defense reactions, putting *mlo* mutants in a primed defensive status [8,12].

The model plant *Arabidopsis thaliana* has been instrumental for the identification of many more susceptibility factors. For instance, forward genetic screens for powdery mildew resistance (*pmr*) or gene expression studies of compatible interactions with diverse biotrophic pathogens, followed by reverse genetic approaches have identified several candidate *S* genes [6,13–15]. Additionally, educated guesses and translational approaches have proved similarly successful in discovering

S genes in crop plants [16,17]. In vivo protein-protein interaction screens are yet another suitable approach to identify susceptibility factors using effector proteins as bait [18,19]. Candidate susceptibility genes were also identified via host gene expression profiling, as it was shown with *Hyaloperonospora arabidopsidis*-infected *Arabidopsis thaliana* and *Phytophthora cinnamoni*-infected *Castanea* [20,21]. Once an *S* gene is identified, studying the physiological function of the susceptibility factor and genetic or physical interactions can identify susceptibility mechanisms or associated pathways and thereby new susceptibility factors [22,23].

3. Biological Functions of Susceptibility Genes

Considering the role of susceptibility genes in compatible plant-microbe interactions, the question arises as to what physiological function host susceptibility factors (or compatibility factors in terms of microbial symbiosis) may exert in healthy and microbe-attacked plants (Figure 1). Some plant susceptibility factors are regulators of host defense responses or cell death. Depending on whether the pathogen is a biotroph, hemibiotroph, or a necrotroph, it can be more or less sensitive to individual plant defense reactions or even profit from host cell-death. Biotrophs often profit from negative regulators of host defense reactions or cell death whereas necrotrophs can profit from host programmed cell-death. This might explain why certain host susceptibility factors show an ambivalent character and can turn into a resistance factor in interaction with another pathogen (see also chapter 5 for trade-offs below). Similarly, individual plant hormone pathways can positively or negatively influence plant-pathogen interactions, depending on the pathogen's lifestyle [24]. In other cases, susceptibility factors do not have reported functions in regulating plant defense. They could be involved in physiological reprogramming of the susceptible host to establish and maintain a compatible interaction. This is particularly well described for the interaction with biotrophic pathogens that show a tight parasitic symbiosis with their host plants and appear to depend on many host functions for disease development. An increasing amount of publications support that successful obligate biotrophs not only successfully inhibit plant immunity, but also heavily rely on and reorganize host cell physiology and development. In the next paragraphs we discuss some prominent examples of *S* gene functions. For more comprehensive overviews, we refer to other review articles [25–27].

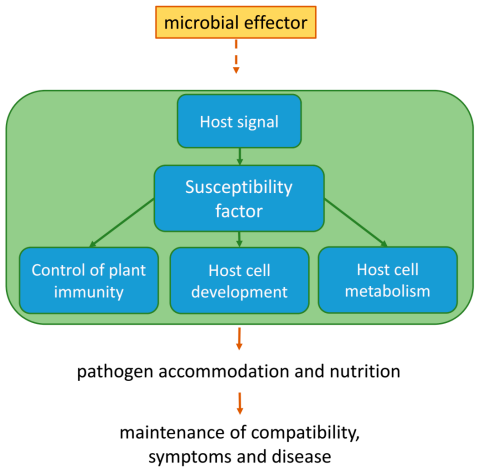

Figure 1. Plant physiological functions and microbial utilization of host susceptibility factors (S). Host S factors can have diverse physiological functions (highlighted by the green background box), which would also operate in pathogen-free plants. This can include control of immune responses or normal host cell development and metabolism. In healthy plants, S factors are regulated by host endogenous signals (e.g., hormones, peptides, second messengers, protein-protein interaction, and protein modification). Microbial pathogens can simply profit from S factor functions or they actively take advantage of S factors via virulence effectors. Effectors might directly act on S factors or on their physiological environment.

3.1. Host Cues for Recognition by the Pathogen

Upon first contact of a pathogen with the host aerial surface or rhizosphere, silent pathogen genes need to be activated e.g., for germination, directed growth, the development of infection structures, and for secretion of virulence factors. Gene activation requires recognition of host cues that trigger pathogen development. For example, epicuticular waxes and cutins of plants provide such cues for germination and formation of appressoria (the pathogen organ for surface adherence and cell wall penetration) by oomycetes, powdery mildew, anthracnose, and rust fungi. Correspondingly, plant mutants that show alterations in leaf wax composition can be less susceptible to fungal invasion [28–33]. Wild type forms of mutated genes that function in or interfere with biosynthesis of wax or cutin components can be considered as *S* genes. There are also reports about other types of chemical cues from the host (e.g., volatiles, flavonoids, acetosyringone, etc.), which are considered to be responsible for metabolic compatibility with adapted pathogens [34–36], and corresponding biosynthetic pathways may contain S factors. Additionally, plant surface hydrophobicity and topology trigger early pathogen development on susceptible plants [37,38].

3.2. Support of Pathogen Demands

Host-adapted necrotrophs can usually tolerate preformed and induced plant defensive barriers, but biotrophs, in particular, require additional support from the host because they lack saprophytic potential [39]. This host support means that, for example, the plant is involved in establishing pathogen feeding structures (haustoria or analogous feeding hyphae) in living host cells. Additionally, the host actively provides nutrients, e.g., by changes in carbohydrate metabolism, sugar transport, or carbohydrate source-sink transitions. Some obligate biotrophs have lost certain biosynthetic pathways, and hence, they might depend on host metabolite supply for primary or secondary metabolite biosynthesis. Thus, the pathogen requires plant components, whilst simultaneously suppressing the same plant's defense. In plant-virus interactions, host primary metabolism and basic cell functions are involved in susceptibility because they provide the building blocks and biochemical machinery for synthesis of the virus itself. For instance, several components of the plant translation machinery contribute to virus replication and they are S factors in virus diseases [40]. An example for a host protein that supports fungal infection is the ROP GTPase RACB of barley, which is required for the susceptibility to fungal growth into epidermal cells and the expansion of haustoria of the powdery mildew pathogen *Blumeria graminis* f.sp. *hordei*. The physiological function of RACB in a healthy plant lies in polar cell development of leaf and root epidermal cells. RACB supports the outgrowth of root hairs from trichoblasts and the ingrowth of haustoria in leaf epidermal cells [41,42]. Another example is SWEET proteins; SWEET sugar transporters transport sucrose out of plant cells for reallocation of sugars. SWEETS are S factors, because they can be overexpressed in pathogen interactions and function in providing nutrients for the pathogen [43]. In summary, host cellular processes support certain demands of pathogens that feed from live tissue and the components of these processes can be S factors.

3.3. Control of Plant Defense Responses

Many S genes encode negative regulators of plant defense responses. Consequently, corresponding homozygous loss-of-function-mutants show either instantaneous defense responses or stronger defense responses after pathogen contact, which can be considered as a genetic priming mechanism. Mutant screens provided several lesion mimic or constitutive defense gene expression mutants, and in many of these mutants, stress hormone signaling is imbalanced. Prominent examples are lesion-simulating disease 1 (*lsd1*) or constitutive expressor of PR genes (e.g., *cpr1* or *cpr5*). These mutants are usually less susceptible to biotrophic pathogens. In fewer cases, such mutants show a resistance to necrotrophs or broad-spectrum resistance [44]. Powdery mildew resistant *mlo* mutants show primed defense in young tissues and spontaneous defense in older tissues. However, in this

particular case, it remains unclear as to whether deregulated defense is decisive for disease resistance because double mutants in *mlo* and stress hormone pathways lose spontaneous defense yet they retain pathogen resistance [45]. Genetic studies have shown that secondary indole metabolism appears crucial for *mlo*-mediated resistance in Arabidopsis [46], and vesicle fusion involved in protein secretion appears to be generally crucial for *mlo*-mediated resistance [45,47].

4. Effector Targets

Plant pathogenic microbes benefit from a certain repertoire of secreted effector proteins that interact with host molecules aiming to create a more favorable environment for the microbe (Figure 1). Hence, any host protein that directly or indirectly supports the susceptibility to any given phytopathogen represents an attractive potential effector target. Our knowledge of microbial effector proteins and susceptibility factors as genuine targets of these effectors has increased significantly over the last years [3,26], and some examples are described below.

4.1. Hub Proteins

Proteins at the center of signaling networks constitute hubs that are by definition key players during plant development and hence represent potential effector targets. In a recent study, a protein-protein interaction network was generated based on *Arabidopsis thaliana* host proteins and virulence effector proteins of biotrophic (*Golovinomyces orontii* and *Hyaloperonospora arabidopsidis*) and hemibiotrophic (*Pseudomonas syringae*) phytopathogens. The authors showed that certain plant proteins are targeted simultaneously by effector proteins from bacterial, fungal, and oomycete pathogens, thereby demonstrating exemplary effector convergence on key targets [19]. Several of these concurrently targeted plant proteins have also been shown to be susceptibility factors. Mutants of the COP9 signalosome complex subunit 5A (CSN5A), for example, showed enhanced disease resistance against both biotrophic and hemibiotrophic pathogens, suggesting a role for CSN5A in facilitating pathogen sustenance in the host. In contrast, transcription factor TCP14, being the most effector-targeted protein, seems to act as a susceptibility factor only in hemibiotrophic interactions, similar to other TCPs. Likewise, APC8, a protein involved in cell-cycle phase transitions, is one of the five most targeted hub proteins and acts as susceptibility factor in hemibiotrophic interactions [19]. The C3HC4 RING finger protein HUB1 was found to be targeted by only one pathogen effector protein. However, its function as susceptibility factor was demonstrated in *hub1* mutant plants that showed enhanced disease resistance against biotrophic pathogens [19].

RIN4 (RPM1-INTERACTING PROTEIN 4) is another excellent and well-studied example of an effector-targeted susceptibility factor. This negative regulator of plant immune responses is also guarded by R proteins RPM1 (RESISTANCE TO PSEUDOMONAS SYRINGAE PV. MACULICOLA 1) and RPS2 (RESISTANT TO PSEUDOMONAS SYRINGAE 2), which are activated upon perception of the effectors AvrB-, AvrRpm1- or AvrRpt2-induced state modifications of RIN4 to subsequently trigger ETI. RIN4 nicely demonstrates that guarding of susceptibility factors by R-proteins sometimes is the plant's only efficient way for protection, as opposed to *S*-gene mutation or the entire elimination from the genetic background, which regarding RIN4 would have detrimental pleiotropic consequences [48,49].

4.2. Bacterial Effector Targets

Certain race-specific susceptibility genes are targeted by transcription activator-like effectors (TALEs) of phytopathogenic bacteria from the genus *Xanthomonas*. They are known to drive host gene expression in a sequence-specific manner, leading to enhanced disease symptoms [50]. For example, AvrXa7 and PthXo3 activate the expression of sugar transporter OsSWEET14 in rice cultivars by directly binding to the effector binding element (EBE), which is located in the promoter region of *OsSWEET14*, *OsSWEET11*, and *OsSWEET13*, like *OsSWEET14*, are likewise major susceptibility genes and targets of TAL effectors [51,52]. Intriguingly, host transcription factors seem to be an attractive target for TALEs,

as several other studies have now shown [53–55]. With the increasing amount of EBEs found in nature, the next years will continue to enrich the arsenal of TALE-targeted susceptibility genes.

4.3. Effector Targets of (Hemi)Biotrophic Pathogens

In contrast to bacteria, filamentous pathogens like fungi and oomycetes possess a plethora of effector proteins, indicating probable effector function redundancy. Biotrophs might, furthermore, require multiple effectors for manipulating host S factors, as suggested recently [56]. Barley MLO has not been described yet as a powdery mildew effector target, in contrast to its functional ortholog MLO2 from *Arabidopsis thaliana*, which is targeted by bacterial *Pseudomonas syringae* effector HopZ2 [8,57]. Likewise, the barley S factor ROP GTPase RACB was recently shown to interact with the ROP-interactive peptide 1 (ROPIP1) from *Blumeria graminis* f.sp. *hordei*. ROPIP1 is encoded on repetitive DNA, supports fungal penetration, and can provoke host cell microtubule disorganization. It may therefore represent an unconventional powdery mildew effector [58].

Hemibiotrophic pathogens require living host tissue during the initial stage of infection, suggesting that any host proteins that are involved in suppressing early resistance-associated cell death reactions might constitute susceptibility factors. For example, the effector Avr3a from the late blight pathogen *Phytophthora infestans* targets and stabilizes the E3 ubiquitin ligase CMPG1. CMPG1 degradation is required for INF1 elicitor-mediated cell death, which would, in turn, obstruct further pathogen spreading during the biotrophic phase of the infection [59]. A similar stabilization, followed by an enhanced infection, was observed with the putative potato K-homology (KH) RNA-binding protein KRBP1 that is targeted by *P. infestans* effector Pi04089 [60]. Targeting S factors is a well-exploited strategy that oomycetes like *P. infestans* follow, as additional studies have shown [61,62].

4.4. Effector Targets of Necrotrophic Pathogens

The pathogenicity of necrotrophs can also rely on secreted effectors that interact with host susceptibility factors to establish foliar necrosis and/or chlorosis in a cultivar-specific manner. For example, it was recently reported that some subunits of the membrane tethering exocyst complex from solanaceous plants seem to act as susceptibility factors for the grey mold pathogen *Botrytis cinerea* [63]. Profound knowledge of necrotrophic effector proteins was gained from studying effector proteins ToxA and ToxB from the tan spot pathogen *Pyrenophora tritici-repentis*, with ToxA being the best studied to date. The *ToxA* gene was acquired by horizontal gene transfer from the leaf blotch pathogen *Parastagonospora nodorum* [64]. ToxA itself interacts directly with a chloroplast-localised protein, ToxABP1. The presence of both, ToxA and ToxABP1, or the silencing of *ToxABP1* in the absence of ToxA leads to similar necrotic phenotypes, indicating ToxA altering ToxABP1 function [65,66]. ToxA genetically interacts with the product of the toxin sensitivity gene *Tsn1*, which encodes a protein that resembles canonical Resistance proteins [9,67]. Functional TSN1 is involved in triggering ToxA-dependent cell death, which favors necrotrophic pathogen growth, and is thus a major S factor with agronomic relevance for both tan spot and leaf blotch in wheat [68].

5. Trade-offs and Prediction of Durability

S gene-based resistance has been suggested to be more durable than the widely applied *R* gene-based resistance [25]. Indeed, there are many reasons why *R* gene resistances, even when combined, might not be durable [69]. Thus, loss-of-susceptibility could potentially be a way to create more durable resistance. However, little is known about general *S* gene durability with only a few examples of long term durability being reported: *Mlo* resistance in barley has been in use for over 35 years and eIF4E-mediated virus resistance in pepper for almost 60 [11,70]. It is often thought that a combination of (functional) redundancy and pleiotropic effects contribute to the durability of *S* genes. Yet, which factors specifically contribute to this durability remains unclear. However, the non-race-specific nature of *s* gene-mediated resistance (e.g., *mlo*-mediated powdery

mildew resistance) makes it less likely that population dynamics rapidly select for single races that are capable of circumventing the loss of susceptibility.

5.1. Broad Spectrum Resistance & Functionality

It has been hypothesized that *S* genes are more durable because of the central role they play in facilitating the initial infection stages. This could be tightly linked to biological functions and the physical environment of S factors. As described above, S factors can generally be considered as (indirect) negative regulators of immunity [71]. In *s* mutant genotypes, it is therefore possible that the whole plant is in a primed state or that certain defense-associated processes are more active, preventing the pathogen from establishing infection [13].

S factors are often intra-cellular and well-embedded in physiological pathways. It has been shown that certain *S* genes can be classified as protein hubs [19]. They are therefore unlikely to steer a single physiological process, which, when missing, can be easily circumvented by the pathogen. In the case of host *S* gene null mutants, pathogens would have to evolve new functions to overcome loss of susceptibility. This would be particularly relevant if an S factor serves an essential requirement for the pathogen. Such evolution of a new biological function can be considered to be slow or even impossible, according to complexity. In addition, the subcellular location and the presence of many possible guard and decoy proteins will impede even rapidly evolving effectors to reach and modify their intracellular target, as opposed to cell-surface receptors or apoplastic R proteins [72]. Thus, it is easier for a pathogen to lose or modify a single R-protein-recognized effector to tackle ETI than to overcome the loss of a host susceptibility gene.

5.2. Pleiotropy and Trade offs

S genes often have key physiological functions within the host. This implies a high chance of pleiotropic effects of *S* gene mutation. On the one hand, if the recessive *s* gene of interest displays deleterious phenotypes, even in a different genetic background, it would not be easily exploitable [26]. On the other hand, mutants that do not show pleiotropic effects are extremely valuable and they could provide sustainable solutions in resistance breeding. Interestingly, pleiotropic effects also complicate the assessment of both durability and the broadness of the resistance spectrum. For example, elF4E1 provides virus resistance in cultivated tomato (*S. lycopersicum*) and the wild species *S. habrochaites*, yet the null mutant in *S. habrochaites* show stronger and broader resistance than the *S. lycopersicum* mutant. The addition of an independent mutation in a related gene, *elF4E2*, did increase the strength and breadth of resistance, but lead to detrimental growth [73], although *elF4E2* mutants alone do not confer resistance. Also, *Mlo* genes occur as gene families and in *A. thaliana*, independent mutations of three orthologs are required for *mlo* resistance, each having their own major or minor effect [46]. A trade-off has been shown between *mlo* resistance to powdery mildew and increased susceptibility to *Magnaporthe grisea* [74] or the toxins of the necrotroph *Bipolaris sorokiniana* [75]. On the other side, reduced pleiotropic effects in barley have been reported in moderate *mlo* variants [76]. So far, no trade-offs have been detected in *tsn1* plants, but recent work by See et al. [67] suggested that the tan spot infection could develop in some wheat backgrounds, independent of the *tsn1* allele. Moreover, the strong day/night rhythm of *Tsn1* gene expression might indicate an additional function in wheat.

5.3. Decoys and Protection

Many *S* genes have allelic variants in the genome that may act as a "sponge" decoy [72], attracting effectors and triggering defense responses. In addition, the molecular inclusion of *S* gene domains in *R* genes has been reported, thus combining recessive resistance with active defense signaling. *Pi21* is a gene that encodes an HMA domain containing protein that suppresses the plant defense response in rice [77]. HMA domains are virulence targets for multiple pathogens [78]. Additionally, HMA domains have also been found as integrated domains in *R* genes. Effectors from the rice blast fungus *Magnaporthe oryzae* bind to the HMA domain of the R protein Pik and trigger defense responses [79].

Many more integrated domains have been described to date [80], yet the question remains how many of these are "orthologs" of true *s* genes.

5.4. S Gene Diversity

Durability of *S* genes could possibly contribute to the fact that different homologs and *s* alleles are present in different plant cultivars or ecotypes. Assuming lack of pleiotropic effects, it will be theoretically possible to maintain many different *s* alleles in a population. Yet, evidence seems inconclusive. Additional *elF4E1* alleles have been identified in wild tomato species, albeit with a limited resistance spectrum [81]. Conversely, in chili peppers (*Capsicum annuum*), many variants confer resistance and are thought to have originated from loci under positive selection. Nevertheless, it is striking that *elF4E2* shows no polymorphisms in chili pepper [73]. In barley, the viral S factor HvEIF4E also occurs in many diverse resistance-conferring haplotypes. For another unrelated virus *S* gene, *HvPDIL5-1*, many haplotypes are present in wild barley and landraces, yet none of these are actual virus resistance conferring alleles (*rym*-alleles) [82,83]. The fact that several barley cultivars with different resistance conferring alleles exist, does suggest that the *s* alleles arose by mutation during domestication in an area where the virus was also present [83]. Also, *Tsn1* shows large variety in spelt and other grasses, but in turn, it has hardly any polymorphisms in bread and durum wheat [9].

6. Exploitation of Susceptibility Genes

6.1. Breeding for Reduced Susceptibility/Loss of Susceptibility

In several cases, naturally occurring *s* genes have been identified in breeding material without actually knowing the nature of the corresponding dominant gene. In many other instances, random mutagenesis and selfing have produced disease resistant offspring with mutant *s* genes (Figure 2). Recessive resistance is best identified in inbreeding plant species or artificial double haploid plants. Cloning of *S* genes from crop plants has been successful in several cases and has sometimes facilitated the identification of related genes in other crop or model plant species. *MLO* is an *s* gene with many *mlo* resistance alleles being identified in diverse plant species, making it a "universal weapon" against powdery mildew [8]. Likewise, once *ToxA* and the *Tsn1* tan spot *S*-gene of wheat were identified, it became straightforward to eliminate the susceptibility from breeding material. Even the phenotypic identification of *Tsn1* genotypes became possible because ToxA, which is as a host-genotype specific toxin, could be directly applied to distinguish *Tsn1* from *tsn1* genotypes by differentially ToxA-provoked symptoms [67].

Another breeding strategy for loss of susceptibility starts from a known *S* gene and looks for natural or induced allelic diversity by TILLING (Targeting Induced Local Lesions In Genomes). TILLING has the advantage that it starts from mutagenesis of any desired genotype, followed by *S* gene re-sequencing, and can identify mutant s alleles even in complex genomes, such as that of hexaploid wheat [84]. The hexaploidy of bread wheat and the presence of three barley *Mlo* orthologues (*TaMlo-A1*, *TaMlo-B1* and *TaMlo-D1*) make the natural occurrence of *mlo* mutants including pathogen resistance quite unlikely for bread wheat. Using TILLING technology to select partial loss-of-function alleles of *TaMlo* however enhanced powdery mildew resistance in some lines without negative pleiotropic effects [84]. Naturally occurring variation of *S* genes can be identified by EcoTILLING: re-sequencing *S* genes in natural populations of crop progenitors or land races. EcoTILLING identified a new allele of *eIF4E* for melon necrotic spot virus resistance [85], and similar to our previous example of *HvPDIL5-1*, a TILLING approach further confirmed the identity of the mutated *S* gene in *rym1* genotypes [83].

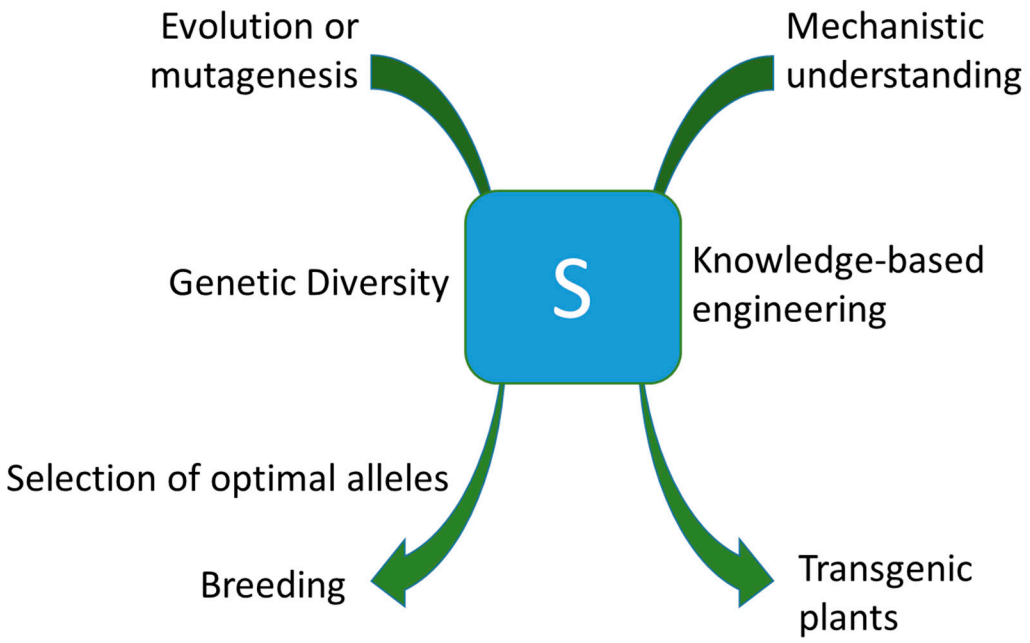

Figure 2. Plant susceptibility genes can be exploited for loss of susceptibility in breeding or biotechnology. Optimal mutant alleles (left side of the panel) that provide a balance between gain of resistance and potential trade-offs can be identified in natural plant populations by ECOTILLING or after random or targeted mutagenesis by Targeting Induced Local Lesions In Genomes (TILLING) or DNA-endonuclease mediated mutagenesis, respectively. Alternatively, knowledge-based (right side of the panel) approaches are enabled if a mechanistic understanding of *S* gene functions exists. This might, amongst others, include artificial effector decoys or silencing *S* genes on demand under control of pathogen-triggered promoters.

6.2. Gene Editing Possibilities for Targeted Exploitation of Susceptibility Genes

Besides targeted *S* gene-associated traditional breeding strategies, there are several additional opportunities for exploiting *S* gene function (Figure 2). In fundamental research, susceptibility factors can be used as bait to either find antagonists that are involved in resistance or downstream interactors that themselves act as susceptibility factors. If the molecular mode-of-action of an S factor has already been elucidated, one way to inhibit its function can be the application of a biocompatible chemical treatment. Alternatively, the ectopic expression of dominant negative *S* gene variants might outcompete and eventually eliminate the endogenous S factor function. Genome editing using sequence-specific nuclease mutagenesis technologies has gained much attention over the last ten years, as it provides researchers with constantly improving tools like zinc finger nucleases (ZFNs), TALE nucleases (TALENs), and clustered regularly interspaced short palindrome repeats (CRISPR)/CRISPR-associated systems (Cas) to create transgene-free genetically modified crops [86–88]. CRISPR/Cas9-targeted editing has been successfully applied on the citrus susceptibility gene *CsLOB1* and its promoter, resulting in resistance to *Xanthomonas citri* subsp. *citri* (Xcc), and also for creating novel alleles of rice *eIF4G*, which conferred resistance to *Rice tungro spherical virus* [89–91]. A strong disease resistance against powdery mildew has been achieved in tomato by transgene-free genetically CRISPR/Cas-mutated *slmlo1*, one of 16 *Mlo* genes in tomato [92], exemplifying again how powerful and versatile these novel genome editing approaches can be.

Furthermore, the EBE of *OsSWEET14* is targeted by TALEs AvrXa7, PthXo3, TalC and Tal5 from *Xanthomonas oryzae* pv. *oryzae*. TALEN-mediated genome editing of AvrXa7 and Tal5 EBEs conferred resistance to bacterial strains relying on the corresponding TALE [93]. Nature itself teaches us of how to benefit from the EBE-promoter repertoire. To counteract TALE activity, several R proteins

have recently been found in pepper and in rice that contain corresponding EBEs for *Xanthomonas* TALEs AvrBs3, AvrXa10, AvrXa23, and AvrXa27, displaying a promoter-trap strategy in a decoy-like manner to confer disease resistance [94–97]. Using TALENs or genome-based engineering, artificial EBE-promoter traps could be generated using EBEs of *S* gene promoter regions upstream of known *R* genes. Moreover, it is even feasible to combine different EBEs and *R* genes in a consecutive manner aiming for broad-spectrum resistance against a range of microbial pathogens [50]. Additionally, with the help of genome editing technology, broad resistance to different phytopathogenic fungi may be achieved by generating loss-of-function alleles of genes encoding HMA domain-containing proteins, like plant defense suppressor Pi21 [77,98].

6.3. Other Possibilities to Exploit S Factors

In the event that the constitutive knock-out or the silencing of susceptibility genes by genome editing is rendered impossible due to deleterious pleiotropic phenotypes, "silencing on demand" using pathogen-inducible promoters can be an alternative approach. In barley, the pathogen-inducible *Hv-Ger4c* promoter has been successfully used to control the expression of Ta-Lr34res, an ABC transporter that confers resistance against several fungal pathogens in wheat [99].

S genes can also be modified to give rise to artificial decoys that inform R proteins to trigger ETI. This neofunctionalization is of course only applicable for susceptibility factors that are effector targets. Targeting of the artificial decoy by the particular effector protein would consequentially lead to a dead end for this particular effector function. Artificial decoys based on susceptibility genes could eventually be even used to switch plant immunity between pathogen kingdoms, as it was recently shown for artificial R proteins. RPS5, which is an intracellular R protein from *Arabidopsis thaliana*, is normally activated upon the recognition of AVRPPHB SUSCEPTIBLE1 (PBS1) cleavage by *Pseudomonas syringae* effector AvrPphB, with PBS1 serving as a decoy. The AvrPphB cleavage site within PBS1 was substituted with cleavage sites for other pathogen protease effectors, e.g., protease effectors of Turnip mosaic virus, thereby conferring resistance to different pathogens [100].

7. Future Direction

We have discussed several methods and trade-offs for *S* gene exploitation (Figure 2). For the optimal exploitation of *S* genes, future research should focus on further unraveling the molecular mechanisms of *S* gene resistance. This is essential to identify novel susceptibility factors to increase our breeding capacities. Furthermore, intensive research is required to take full advantage of *S* gene exploitation by controlling and, in the best case, diminishing pleiotropic effects. Additionally, whole genome resequencing studies could reveal the diversity and variability of *S* genes in wild crop relatives and heirloom varieties. Combined with large scale protein-protein interaction studies, these findings can be put in a larger *S* gene defense signaling context. Such information will help to understand the durability of *s* gene resistance when compared to *R* gene resistance. One could also try to identify partial *S* gene mutants. Such genes might confer less, but still sufficient field resistance while suffering less from pleiotropic effects. Such genes might be found in natural populations, where they have been selected for, or they might be created by random or knowledge-based approaches.

These new findings can be used for modern breeding and genome editing technologies. In fact, transgenic and marker-assisted breeding have already been utilized for over several decades. More recently, new mutagenesis and gene editing approaches have also been shown to generate strong and functional *s* genes. Thus, the targeted exploitation of susceptibility factors forms a credible and potentially durable route for future resistance breeding.

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