Received: 11 June 2022 | Revised: 17 October 2022 | Accepted: 1 December 2022

DOI: 10.1111/mpp.13290

Molecular Plant Pathology WILEY

REVIEW

Genetic approaches to dissect plant nonhost resistance mechanisms

Ying Wu[1] | William Sexton[1] | Bing Yang[2,3] | Shunyuan Xiao[1,4]

[1]Institute for Bioscience and Biotechnology Research, University of Maryland College Park, Rockville, Maryland, USA

[2]Division of Plant Science and Technology, Bond Life Sciences Center, University of Missouri, Columbia, Missouri, USA

[3]Donald Danforth Plant Science Center, St. Louis, Missouri, USA

[4]Department of Plant Science and Landscape Architecture, University of Maryland, College Park, Maryland, USA

Correspondence
Shunyuan Xiao, Institute for Bioscience and Biotechnology Research, University of Maryland College Park, Rockville, MD 20850, USA.
Email: xiao@umd.edu

Funding information
Directorate for Biological Sciences, Grant/Award Number: IOS-1901566

Abstract

Nonhost resistance (NHR) refers to the immunity of most tested genotypes of a plant species to most tested variants of a pathogen species. Thus, NHR is broad spectrum and durable in nature and constitutes a major safety barrier against invasion of a myriad of potentially pathogenic microbes in any plants including domesticated crops. Genetic study of NHR is generally more difficult compared to host resistance mainly because NHR is genetically more complicated and often lacks intraspecific polymorphisms. Nevertheless, substantial progress has been made towards the understanding of the molecular basis of NHR in the past two decades using various approaches. Not surprisingly, molecular mechanisms of NHR revealed so far encompasses pathogen-associated molecular pattern-triggered immunity and effector-triggered immunity. In this review, we briefly discuss the inherent difficulty in genetic studies of NHR and summarize the main approaches that have been taken to identify genes contributing to NHR. We also discuss new enabling strategies for dissecting multilayered NHR in model plants with a focus on NHR against filamentous pathogens, especially biotrophic pathogens such as powdery mildew and rust fungi.

KEYWORDS
Arabidopsis, filamentous pathogen, immunity, nonhost resistance, penetration resistance, post-penetration resistance, rice

1 | INTRODUCTION

In nature, plants are resistant to most potential phytopathogens. Conversely, a majority of phytopathogens are capable of only infecting certain plant species, which defines their host ranges. The effective resistance or immunity exhibited by an entire plant species to all genetic variants of a phytopathogen species is described as nonhost resistance (NHR) (Heath, 1977, 2000; Nurnberger & Lipka, 2005; Thordal-Christensen, 2003). NHR is a phenomenological term that also reflects the inability of a nonadapted but potentially pathogenic microbe to complete its asexual or sexual life cycle on a particular plant species (Heath, 2000; Nurnberger & Lipka, 2005; Panstruga & Moscou, 2020). Because NHR means robust and complete resistance, there is strong motivation to understand the molecular basis of NHR with the hope of using the NHR mechanisms for improvement of disease resistance against adapted and aggressive pathogens (Borlaug, 2000; Fan & Doerner, 2012; Gill et al., 2015). However, there are two major constraints that limit genetic dissection of NHR. First, there is no or rarely polymorphism in NHR as a genetic trait within a plant species, hence conventional forward genetics (which is often based on segregation of the target trait in an intraspecific population for identifying genes controlling the trait)

is not easily applicable to genetic studies of NHR. Interspecific segregating populations can be useful for studying NHR, but sexual hybridization between plant species is often prohibited due to various barriers. When sexual hybridization is feasible in some cases, there may not be a suitable pathogen that has adapted to one plant species but not the other, although exceptions do exist and have been used for studying NHR (Bartaula et al., 2018, 2019; Newcombe, 2005). Second, the inability of a potential pathogen to conquer resistance of an entire plant species implies that NHR consists of multiple layers of effective immunity that collectively can block even accidental successful invasion from the most capable individuals of the pathogen population. This ability of NHR may explain why only incremental erosion of NHR rather than complete breakdown of NHR has been achieved in most cases through single rounds of random mutagenesis (Collins et al., 2003; Hematy et al., 2020; Lipka et al., 2005; Stein et al., 2006) or virus-induced gene silencing (VIGS) (Ramu et al., 2020; Rojas et al., 2012). This is especially the case for filamentous pathogens, whose reproductive success requires brutal penetration of plant cells to derive adequate nutrients from the attacked plants. Hence, the high (theoretical) potential of NHR contrasted with its poor mechanistic characterization makes the question "what is the molecular basis of NHR" among the top 10 unanswered questions in the field of molecular plant–microbe interactions (Harris et al., 2020).

Despite the aforementioned inherent difficulty, there have been considerable efforts in NHR studies in the past two decades and around 30 genes involved in NHR have been identified in *Arabidopsis* and other plants using various forward and reverse genetics approaches (Fonseca & Mysore, 2019; Lee et al., 2017; Panstruga & Moscou, 2020). Diverse and probably layered defence mechanisms have been shown to contribute to NHR (Figure 1). Preformed physical barriers such as leaf cuticular layers (Yu et al., 2019) and preformed chemical barriers such as constitutively synthesized secondary metabolites of antimicrobial activity (Bowyer et al., 1995; Papadopoulou et al., 1999) help certain plants prevent infection from nonadapted pathogens. Apart from the preformed barriers, NHR is built on the same molecular basis of host resistance against adapted pathogens (Panstruga & Moscou, 2020). In other words, pathogen-associated molecular pattern (PAMP)-triggered immunity (PTI) and/or effector-triggered immunity (ETI), the two well-characterized immune mechanisms responsible for host resistance (Jones & Dangl, 2006; Ngou et al., 2022), probably also underlie NHR in most cases (Lee et al., 2017; Panstruga & Moscou, 2020; Senthil-Kumar & Mysore, 2013). A complete genetic dissection of most robust NHR such as rice's immunity to rust and powdery mildew, if possible, may also lead to discovery of unexpected novel mechanisms that either may not exactly fit the PTI–ETI framework or may reveal how PTI or ETI is regulated at an unknown level(s), or host factors that are strictly required for pathogenesis (Figure 1). This prospect has motivated and will continue to motivate researchers to take novel and enabling approaches to dissect multilayered NHR. Given that there are many comprehensive reviews on the concept and possible mechanisms of NHR (Ayliffe & Sorensen, 2019; Bettgenhaeuser et al., 2014; Fonseca & Mysore, 2019; Lee et al., 2017; Panstruga & Moscou, 2020; Schulze-Lefert & Panstruga, 2011; Senthil-Kumar & Mysore, 2013), we will focus our review on summarizing the approaches employed for NHR studies and propose improved strategies for dissecting multilayered NHR against biotrophic filamentous phytopathogens in model dicot (*Arabidopsis*) and monocot (rice) plants.

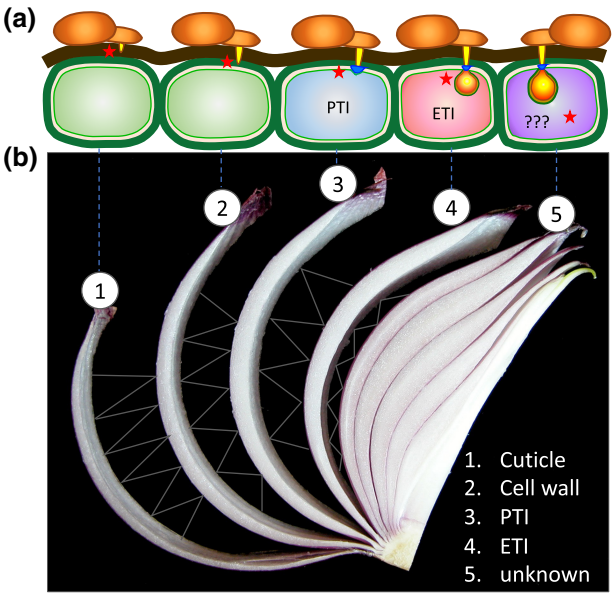

FIGURE 1 Multilayered defence mechanisms underlying nonhost resistance (NHR) may be dissectible. For any filamentous pathogens to achieve reproductive success in a particular plant species, they must overcome several spatiotemporally distinct or connected defence barriers of the plant (a). Likely defence layers of NHR include (1) the epicuticular wax, (2) the cell wall, (3) antimicrobials by pathogen-associated molecular pattern (PAMP)-triggered immunity (PTI), (4) antimicrobials and hypersensitive response (HR) by effector-triggered immunity (ETI), and/or (5) possible novel but currently unknown mechanisms. Like an onion, multilayered NHR against a nonadapted filamentous pathogen is dissectible using appropriate strategies and tools (b). Red stars in (a) indicate the defence layers where the microbial invasion is halted; grey lines in (b) between layers imply possible mechanistic connections

2 | APPROACHES USED TO ELUCIDATE NHR MECHANISMS

To understand the molecular genetic basis of a biological trait, one needs to identify the gene(s) that controls the target trait. This entails genetic association (linkage) studies with a population(s) that possesses phenotypic polymorphism (i.e., trait segregation) for the identification of a candidate gene(s). Indeed, over the past decades, researchers have utilized various strategies to identify natural or create polymorphisms in NHR against a particular phytopathogen for the identification of genes and specific cellular mechanisms contributing to NHR. Because NHR is typically exhibited by an entire plant species, natural polymorphism in NHR is rare and only found in special circumstances. Major efforts have been focused on creating

plant (or pathogen in some cases) mutants and/or conditions in which NHR is partially or significantly eroded or completely abolished by chemical inhibitors, random genome-wide mutagenesis, or targeted mutagenesis. Below, we summarize the main approaches that have been used for genetic studies of NHR with a few of the most relevant examples and propose a few new improved strategies.

2.1 | Chemical genetics to understand major requirement of NHR

Chemical genetics is the investigation of the function of genes or their engaged signalling pathways by using small molecules to perturb a particular cellular function (Specht & Shokat, 2002). Pharmacological inhibitors were used to infer an important role of the actin cytoskeleton for NHR of plants against nonadapted powdery mildew (Kobayashi et al., 1997a, 1997b), rust (Song et al., 2012; Wang et al., 2015a), and *Colletotrichum* species (Shimada et al., 2006). For example, treatment of *eds1-2* mutant leaves with cytochalasin E, an actin polymerization inhibitor, significantly compromised *Arabidopsis* NHR against barley powdery mildew (*Blumeria graminis* f. sp. *hordei*; Bgh) (Yun et al., 2003). Likewise, inactivation of myosins by pharmacological inhibitors in *Arabidopsis* leaves compromised penetration resistance against Bgh, which was further substantiated by genetic mutations of genes encoding myosin XI proteins (Yang et al., 2014). Similarly, chemical inhibition of phosphatidic acid production by phospholipase D (PLD) increased Bgh penetration success on wild-type *Arabidopsis* leaves, which led to the identification of *PLDδ* to be important for NHR against nonadapted powdery mildew. Miklis et al. (2007) found that functional actin cytoskeleton is required for both NHR and *mlo*-mediated resistance against powdery mildew in barley (Miklis et al., 2007). In a recent screen for chemical suppressors of *mlo*-mediated resistance to powdery mildew, Wu and colleagues found that alloxan (5,5-dihydroxyl pyrimidine-2,4,6-trione) and some of its structural analogues can partially suppress *mlo*-mediated resistance as well as NHR to powdery mildew, probably through destabilization of the cytoskeletal architecture (Wu et al., 2017, 2020). These results further suggest that *mlo*-mediated resistance and NHR share certain common cellular mechanisms (Humphry et al., 2006). Notably, Qin et al. (2021) recently provided genetic evidence that corroborates the observations made with chemical inhibitors. These authors found that genetic disruption of the ARP2/3 complex and formins, two actin-nucleating systems, compromised *Arabidopsis* penetration resistance to nonadapted fungal pathogens (Qin et al., 2021). Besides the findings concerning the roles of actin cytoskeleton and phospholipases in NHR, chemical inhibitors of protein kinases and protein phosphatases were also found to impair pea NHR to a bean pathogen, *Fusarium solani* f. sp. *phaseoli*, although the specific cellular mechanism(s) impacted is not known (Hartney et al., 2007).

With the availability of relevant chemicals, screening for chemicals impacting NHR via leaf infiltration is simple to perform in model plants such as *Arabidopsis thaliana* and *Nicotiana benthamiana*. However, due to potential chemical toxicity and/or the lack of precise mechanistic information about the cellular targets of most chemical inhibitors, the utility of chemical genetics in NHR studies is rather limited.

2.2 | Exploring natural variations to identify genes underpinning NHR in near-hosts

In nature, host adaptation of most pathogens is thought to be gradual and continuous, although host jumps do happen (Morris & Moury, 2019; Panstruga & Moscou, 2020; Thines, 2019). Hence, in many cases, there may be no strict demarcation of host and nonhost. Accordingly, plant species that are evolutionarily close to a host plant species may exhibit less robust NHR to a corresponding pathogen, therefore they can be described as "near-hosts" (Figure 2a). Conversely, such a pathogen may be in a "near-pathogen" state with respect to its poor or limited adaptation to the near-host. Plants as near-hosts have been used for genetic studies of NHR against rust and powdery mildew fungi (Atienza et al., 2004; Delventhal et al., 2017; Dracatos et al., 2016; Jafary et al., 2008). One common strategy for using near-hosts to understand NHR is to find intraspecific or interspecific variation in NHR and identify genes underlying the differences through genetic mapping. For example, in a recent report, Haghdoust et al. (2021) tested 492 barley accessions and two mapping populations with pathogenically diverse cereal rust (*Puccinia*) isolates representing distinct formae speciales adapted to different cereal species. The authors found that about 80% of the barley accessions were either immune or near immune to all pathogens tested and identified major quantitative trait loci (QTLs) in barley that may contribute to NHR (Haghdoust et al., 2021). These observations support the notion that there is a continuum between NHR and host resistance, and in some cases the resistance phenotypes are quantitative traits (Bettgenhaeuser et al., 2014). Through a similar strategy, Wang et al. found that the lectin receptor-like kinases encoded by orthologous genes at the *Rphq2* locus from cultivated barley and wild barley confer resistance to rust pathogens. Interestingly, the resistance in either background is much stronger to their respective nonadapted rust compared to their respective adapted rust, indicating that the host status of a particular barley genotype to different leaf rust fungi is quantitatively affected by the same orthologous receptor kinase (Wang et al., 2019). In rare cases where natural or deliberately constructed interspecific hybrids between a near-host and a host are fertile, NHR in the near-host plant may be amenable to genetic studies for mapping genes controlling the NHR. For example, barberry (*Berberis* spp.) is known to be the alternate host of grass rust fungi (*Puccinia* spp.). A natural population of *Berberis xottawensis*, derived as an interspecific hybrid of *B. vulgaris* (host of *Puccinia graminis*) and *B. thunbergia* (nonhost of *Puccinia graminis*), was used for the identification of several QTLs that are associated with the NHR against *P. graminis* in *B. thunbergia* (Bartaula et al., 2018, 2019). Another example concerns triticale, a man-made interspecific hybrid between wheat (*Triticum*) and rye (*Secale*) made

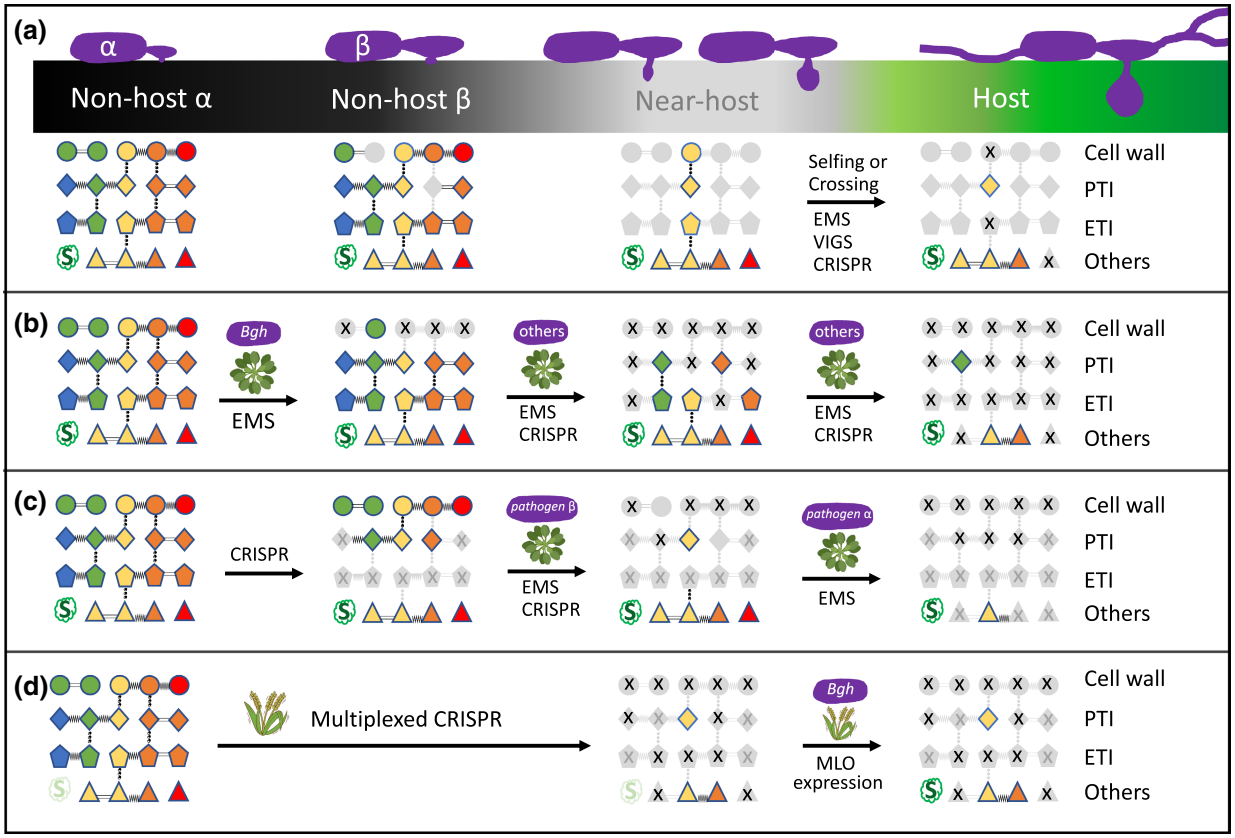

FIGURE 2 Major strategies for dissecting genetic components contributing to nonhost resistance (NHR). Distinct defence layers are depicted by different shapes; different components in the same layer are indicated by different colours, with grey being indicative of functional suppression by the invading pathogen; functional impairment of unknown immunity genes by mutagenesis is indicated by a dark "x", while targeted ablation of known genes is indicated by grey "x". Waved lines depict linear mechanistic relationships, dotted lines depict interconnection between different mechanisms, "=" indicates functional redundancy, and circled "S" indicates a host susceptibility factor. Pathogen-associated molecular pattern (PAMP)-triggered immunity (PTI); effector-triggered immunity (ETI); virus-induced gene silencing (VIGS). (a) Using natural near-hosts. NHR in nonhost α and nonhost β is completely or largely intact and thus is not easily amenable to genetic studies. In contrast, NHR in a near-host is conferred by only one or a few genes and thus is genetically tractable using various approaches as shown. (b) Employing "top-down" forward and/or reverse genetics. Ethyl methanesulfonate (EMS) mutagenesis was used to identify *pen* mutants compromised in penetration resistance; the *pen* mutant could be used to identify genes involved in postpenetration resistance by further EMS or CRISPR mutagenesis. *Arabidopsis*–powdery mildew interaction is used as an example. Bgh, barley powdery mildew *Blumeria graminis* f. sp. *hordei*. (c) Employing "bottom up"- and/or "nullify knowns"-based forward genetics. Removal of postpenetration resistance can allow more sensitized and saturated forward genetic screens for novel *pen* mutants, while next-round forward and/or reverse genetics screens in the background of a higher-order, immunocompromised mutant can reveal unknown residual defence mechanisms contributing to NHR. (d) Breaking rice's NHR to powdery mildew and rust fungi. One or more rounds of multiplexed CRISPR mutagenesis of all known or candidate immunity genes may create rice mutants susceptible to the nonadapted fungi. If not, a susceptible factor(s) may be inadequate or missing in rice. In this case, the known barley S factor Mildew locus O may be expressed in the rice mutant to enable pathogenesis of Bgh or other cereal powdery mildew

in the 19th century (Longin et al., 2012). Triticale used to be resistant to powdery mildew (*Blumeria graminis*) formae speciales either adapted to wheat (*B. graminis* f. sp. *tritici*) or rye (*B. graminis* f. sp. *secalis*) (Walker et al., 2011), suggesting that genes contributing to NHR from wheat and rye are dominant and complementary. Interestingly, Menardo and colleagues discovered that the powdery mildew forma specialis that has overcome the resistance in triticale (and wheat but not rye) is a sexual hybrid of the wheat powdery mildew and rye powdery mildew (hence named *B. graminis* f. sp. *triticale*) (Menardo et al., 2016). This finding not only sheds insight onto evolutionary mechanisms of new powdery mildew speciation through hybridization but also implies an important role of powdery mildew effectors in host adaptation via suppressing immunity genes contributing to NHR.

Definitive determination of genes underlying NHR in a near-host was provided by Cevik and colleagues in 2019. The authors screened 593 inbred lines from an *Arabidopsis* multiparent advanced generation intercross (MAGIC) mapping population derived from 19 accessions and identified two transgressive segregants that are susceptible to *Albugo candida*, indicating an absence of gene(s) critical for NHR (Cevik et al., 2019). Through gene mapping using F_2 progenies derived from crossing these two individuals back to the 19 MAGIC parental accessions, the authors found that resistance to *A. candida* race 2 (Ac2V) can be explained in each accession by

at least one of the four *NLR* (nucleotide-binding site leucine-rich repeat) genes identified. These findings demonstrated the utility of *A. thaliana* as a near-host of *A. candida* for revealing a critical role of ETI in NHR (Cevik et al., 2019). Using wheat as a near-host to powdery mildew adapted to related cereals, Bourras and colleagues analysed the allelic polymorphisms at the wheat resistance gene locus *Pm3* (encoding an *NLR*) and those of the corresponding *Avr* genes in the adapted and the nonadapted powdery mildew formae speciales and concluded that *Pm3*-enabled ETI also explains NHR against the nonadapted powdery mildew (Bourras et al., 2019).

In addition, the molecular basis of NHR in a particular near-host species can also be inferred from the genetic polymorphisms and pathogenesis patterns among related pathogen species (including nonadapted and adapted) or their sexual hybrids. By comparing the effector repertoires of *Pseudomonas syringae* DC3000 (adapted to *Arabidopsis*) and *P. syringae* T1 (adapted to tomato but not *Arabidopsis*), Sohn et al. (2012) identified two effector genes, *avrRpt2* and *hopAS1*, responsible for ETI in nonhost *A. thaliana* (Sohn et al., 2012). Interestingly, while *hopAS1* is broadly present in *P. syringae* strains contributing to virulence in tomato, all tested *P. syringae* strains that are pathogenic in *Arabidopsis* carry truncated *hopAS1* variants (presumably to avoid ETI). These observations suggest that ETI plays an important role in *Arabidopsis* NHR against a broad range of *P. syringae* strains (Sohn et al., 2012). More recently, Laflamme et al. (2020) constructed the pan-effectorome from 494 *P. syringae* strains based on the pan-genomic analyses, screened for core effectors that can elicit ETI in *A. thaliana*, and identified 59 ETI-eliciting effectors with orthologues distributed among 96.8% of *P. syringae* strains. Interestingly, ETI in *Arabidopsis* was found to be activated by only a small number of NLRs, with CAR1 and ZAR1 being able to recognize orthologous effectors present in about 95% of *P. syringae* strains, many of which are not adapted to *Arabidopsis* (Laflamme et al., 2020). The new findings further provide genome-scale evidence that ETI underpins NHR of *Arabidopsis* as a near-host to many nonadapted *P. syringae* biotypes that are close relatives of the adapted *P. syringae* strains.

The above examples demonstrate that NHR in a near-host against bacterial or fungal pathogens may be genetically tractable and that typical ETI may underpin NHR under such pathocontexts. These findings also imply that PTI in near-hosts has been partially or largely overcome by the invading pathogens, hence the delivery of effectors and ETI. Obviously, the availability of near-host/near-pathogen accessions/biotypes is a prerequisite for successful employment of the above-mentioned strategies to study the genetic basis of NHR. Infection tests of large segregating populations and/or whole-genome sequence analyses are required to map and identify genes contributing to NHR.

2.3 | Applying mutagenesis-aided forward genetics to identify components of NHR

In cases where NHR consists of more than one layer with each being effective in halting the invasion of pathogens, the resistance is unlikely breakable by the nonadapted pathogens. For example, NHR of dicot plants such as *Arabidopsis* against powdery mildew fungi adapted to monocot plants is probably multilayered and insurmountable by the pathogens (Figure 2a, nonhost α). The inducible defences of the NHR against filamentous pathogens can be divided into two major layers, penetration resistance and postpenetration resistance. Logically, removal of the first layer (i.e., cell wall-based penetration resistance) of NHR via mutagenesis is considered the very first step towards dissecting the genetic mechanisms of NHR.

Indeed, ethyl methanesulfonate (EMS) mutagenesis of the *A. thaliana* Col-0 accession followed by screening mutagenized M_2 seedlings with barley powdery mildew (Bgh) has led to the identification of a series of penetration (*pen*) mutants with increased penetration success of the nonadapted powdery mildew (Figure 2b). The cloning and functional characterization of *PEN1* to *PEN4* genes made a significant contribution to our understanding of plant cell wall-based penetration resistance (Collins et al., 2003; Hematy et al., 2020; Lipka et al., 2005; Stein et al., 2006). Using the same strategy, Fukunaga and colleagues identified two allelic *n*ecrotic spotted *l*esion (*nsl*) mutants on inoculation of nonadapted fungi and identified the causal mutations in *NSL1*, which is predicted to encode a protein containing a putative membrane-attack complex/perforin (MACPF) domain without known function (Fukunaga et al., 2017). Interestingly, the *nsl1* mutants also developed necrotic spotted lesions on flg22 treatment and the cell death phenotype was abrogated in the absence of PEN2 or when the biosynthesis of salicylic acid (SA) is defective. This suggests that PAMPs from bacteria or fungi can enhance production of a PEN2-dependent, tryptophan metabolism-derived secondary metabolite that can induce programmed cell death via the SA-dependent pathway in the absence of NSL1 (Fukunaga et al., 2017). A similar approach was also used for identifying genes contributing to NHR to nonadapted bacterial pathogens. Zhou and colleagues identified *NHO1*, a gene encoding glycerol kinase, to be important for NHR and host resistance against nonadapted *P. syringae* pv. *phaseolicola* (Kang et al., 2003; Lu et al., 2001). For inducing random mutations in DNA, besides chemical mutagen treatments, other mutagenesis methods, including radiation mutagenesis, T-DNA insertion, and transposon tagging, can also be used to introduce mutations into genes associated with NHR. For example, using *Medicago truncatula Tnt1* retrotransposon insertion lines, Uppalapati and colleagues revealed a role of abaxial leaf epicuticular wax of *M. truncatula* in NHR against nonadapted rust fungi (Uppalapati et al., 2012).

Random, genome-wide mutagenesis allows nonbiased forward genetic screens to identify key genes that control a specific phenotype. The efficiency of this approach depends on the efficacy of DNA mutagenesis and identification of phenotypic alteration. If the defect in NHR is minor and requires microscopy or other laborious assays for phenotyping, mutant screening and subsequent identification of causal mutations by positional cloning can be time-consuming, hence mutant screening and gene identification may be difficult to reach saturation. Also, because a single round of forward genetic screen cannot circumvent genetic redundancy, this approach is ineffective in identifying genes that are functionally redundant.

2.4 | Using reverse genetics to identify components of NHR

This approach has been used in several laboratories to search for genetic components contributing to NHR against nonadapted bacterial pathogens. The success of this strategy depends on the establishment of a relatively efficient gene-manipulation method such as virus-induced gene silencing (VIGS) and/or targeted mutagenesis method such as CRISPR in a near-host plant (Figure 2a). Using VIGS, a role of SGT1 in both NHR and host resistance was identified (Peart et al., 2002; Wang et al., 2010). Notably, by using VIGS in *N. benthamiana* for initial gene identification (Senthil-Kumar et al., 2013) and functional studies of orthologous/homologous genes in *Arabidopsis*, the Mysore laboratory identified a dozen novel genes involved in NHR of *N. benthamiana* and *Arabidopsis* against the nonadapted bacterial pathogen *P. syringae* pv. *tomato* T1 (Fonseca et al., 2020; Fonseca & Mysore, 2019; Pant et al., 2020; Ramu et al., 2020), demonstrating the power of reverse genetics. These studies have collectively revealed how the leaf apoplast environment is modified during NHR to restrict the survival and proliferation of nonadapted bacteria.

With the development of the next-generation sequencing technologies, candidate genes involved in NHR can be more efficiently identified through (pan)genomics and comparative transcriptomics studies (Gangurde et al., 2021; Iven et al., 2012; Tufan et al., 2009). When such gene expression information is coupled with VIGS and CRISPR, the reverse genetics approach for identification of genes involved in NHR can become more efficient. An obvious advantage of this approach is that when multiple members of a gene family are targeted simultaneously, this can reveal relevant mechanisms of NHR where genetic redundancy exists.

More and more mutant lines of model plants have been generated, characterized, and made available to the research community. One may collect a panel of mutants with mutations in genes belonging to a gene family or involved in a particular signalling or metabolic pathway that may serve a role in immunity and test them with one or a group of nonadapted pathogens. This more targeted reverse genetics approach may identify causal mutations that compromise NHR. For example, infection tests with a panel of phosphoinositide signalling-related *Arabidopsis* mutants with T-DNA insertions led to the identification of *PLDδ* in both penetration and postpenetration resistance to nonadapted powdery mildew (Pinosa et al., 2013; Zhang et al., 2018).

2.5 | Combining stepwise forward and reverse genetics to identify novel components of NHR

To dissect multilayered NHR once a major component of NHR is discovered, the corresponding mutant can be used to identify genes that function in the next layer via another round of forward or reverse genetic screen using the same or other nonadapted pathogens (Figure 2b). Because of the essential role of PEN2-dependent glucosinolate metabolic pathway in penetration resistance against a broad range of pathogens (Bednarek et al., 2009; Clay et al., 2009; Lipka et al., 2005; Sanchez-Vallet et al., 2010), most stepwise genetic studies described in published literature were conducted in *pen2* mutant backgrounds. For example, Kopischke et al. (2013) further mutagenized the *Arabidopsis pen2-1* mutant and screened for new mutants with an altered response to infection by the nonadapted pathogen *Phytophthora infestans*. Characterization of the first new mutant led to the identification of a phospholipid: sterol acyltransferase (PSAT1) in negative regulation of mesophyll cell death associated with excessive callose deposits (Kopischke et al., 2013). Characterization of an additional mutant from the same screen implicated EDR1 in negative regulation of PTI response induced by flg22 and elfl8 (Geissler et al., 2015). Using a stepwise reverse genetics strategy, Langenbach et al. (2013) found that *BRT1*, encoding a UDP-glucosyltransferase in the phenylpropanoid pathway, was highly induced in *Arabidopsis* plants lacking *pen2* on inoculation with soybean rust *Phakopsora pachyrhizi*. They constructed the *pen2 brt1* double mutant and found that it could support more mesophyll haustorium formation, despite the fact that the haustoria are not functional enough to support further fungal development (Langenbach et al., 2013). Interestingly, the *brt1* mutation in the wild-type (Col-0) background exhibited normal NHR, suggesting that BRT1 plays a role in postpenetration resistance activated only when PEN2-dependent penetration resistance is breached (Langenbach et al., 2013).

By introducing the *pad3-1* mutation, which impairs camalexin production (Zhou et al., 1999), into the *pen2-1* mutant background, Schlaeppi and colleagues found that the *pen2-1 pad3-1* displayed susceptibility to nonadapted *Phytophthora brassicae*, indicating that NHR of *Arabidopsis* against *P. brassicae* relies on a combined action of PEN2-dependent production of 4-methoxyindol-3-yl methylglucosinolate and PAD3-dependent production of camalexin, both of which are derived from the tryptophan metabolic pathway (Schlaeppi et al., 2010). Since then, more specific studies have shown that PTI signalling components, including transcription factors and MPK3/6, play important roles in regulating the production of camalexin during NHR (Frerigmann et al., 2016; Pastorczyk et al., 2020; Saga et al., 2012; Xu et al., 2016; Yang et al., 2020). Using a similar approach, SOBIR1, a co-receptor for PAMP recognition, was found to be required for NHR against *Pyricularia oryzae* in *Arabidopsis* (Takahashi et al., 2016, 2018), and BAK1, another PAMP co-receptor, has recently been shown to play a role in *Arabidopsis* postpenetration resistance against *Alternaria brassicicola* independent of tryptophan-derived metabolites (Kosaka et al., 2021).

Notably, the *pen2* mutant was also tested with different nonadapted pathogens for exploring new mechanisms of NHR. For example, it was recently found that in epidermal cells of the *pen2* mutant, atypical small chloroplasts act as defence-related motile organelles by specifically positioning immune components in the plant epidermis. Blocking the distinct steps of such responses decreases NHR against nonadapted fungi (Irieda & Takano, 2021).

2.6 | Going forward: Improved/new strategies for identifying novel genes contributing to NHR

The efficiency of any forward genetics approach depends on how readily the desirable mutants can be identified. A typical "top-down" forward genetic screen (Figure 2b) was employed to identify *pen1* to *pen4* mutants based on the detection of impaired penetration resistance as a top layer of NHR by microscopy (Collins et al., 2003; Hematy et al., 2020; Lipka et al., 2005; Stein et al., 2006). Although extremely successful in setting the early milestones for understanding penetration resistance as part of NHR, this screen by design was laborious and thus difficult to reach saturation. Moreover, the identification of the causal mutations using microscopy-based phenotyping for positional cloning was also time-consuming, which probably in part explains why the characterization of *PEN4* was only recently published (Hematy et al., 2020).

Inspired by an early observation that the *Arabidopsis pen2 pad4 sag101* triple mutant supported profuse sporulation visible to the naked eye from the nonadapted pea powdery mildew (Lipka et al., 2005), we can envisage a sensitized "bottom-up" forward genetics strategy to more efficiently identify genes contributing to NHR in penetration resistance (Figure 2c). Specifically, we made an *Arabidopsis* mutant in which key components of postpenetration resistance, *EDS1*, *PAD4*, and *SID2*, were mutated and found the *eds1 pad4 sid2* triple mutant to be supersusceptible to the adapted *Arabidopsis* powdery mildew isolate *Golovinomyces cichoracereaum* UCSC1 (Zhang et al., 2018). Interestingly, the triple mutant still possesses largely intact penetration resistance to a strawberry powdery mildew pathogen *Podosphaera aphanis* (pathogen β in Figures 2c and 3a). Knocking out *PEN2* in the *eds1 pad4 sid2 pen2* background (Figure 3c) by CRISPR resulted in profuse sporulation of *P. aphanis* on the quadruple mutant plant visible to the naked eye (Figure 3b). Therefore, one may anticipate that screening for genes involved in penetration resistance as part of NHR against nonadapted cell wall-penetrating filamentous pathogens using an EMS-mutagenized *eds1 pad4 sid2* population would be efficient and possible to reach saturation because potential *pen* mutants can be recognized by the naked eye instead of having to use microscopy (Figure 2c).

To efficiently identify novel genes involved in postpenetration resistance, we can also envisage a "nullify-knowns"-based forward genetics strategy that requires the construction of an *Arabidopsis* mutant in which key components of both penetration resistance (e.g., *PEN1*, *PEN2*, and *PEN3*) and postpenetration resistance (e.g., *EDS1*, *PAD4* and *SID2*) are mutated. If a higher-order *Arabidopsis* mutant such as *eds1 pad4 sid2 pen1 pen2 pen3* is still resistant to cereal powdery mildew fungi or any other filamentous pathogens (pathogen α in Figure 2c), a genetic screen with the EMS mutagenized M_2 population of such a mutant line may enable identification of mutants that permit growth of nonadapted pathogen α visible to the naked eye (Figure 2c).

CRISPR/Cas9-targeted mutagenesis has been widely used for knocking out genes in several model plant species (Gaillochet et al., 2021). The ability to simultaneously knock out up to 15 genes by multiplexed CRISPR system in *Arabidopsis*, *N. benthamiana*, and rice (Stuttmann et al., 2021; Zhang et al., 2021) has created unprecedented opportunities to perform highly efficient reverse genetic screens targeting genes and gene families in single or multiple signalling networks involved in control of complex biological traits such as NHR. This can not only circumvent the functional redundancy constraint inherent to conventional forward genetic screens but also should greatly facilitate the "nullify knowns"-based forward genetics strategy described above. By taking advantage of rice's high efficiency in both transformation and CRISPR-mutagenesis, Meng and colleagues constructed the first large-scale CRISPR/Cas9 mutant library in rice that is of high quality, with good coverage and uniform distribution (Meng et al., 2017). This rice library consists of 14,000 independent transgenic rice lines expressing *Cas9* and one or more small guide (sg) RNAs targeting one or more of 12,802 rice genes, which can be used for screening mutants with biological phenotypes of interest, including compromised NHR. Such genetic screens and subsequent gene identification seem to blur the boundary of forward genetics and reverse genetics, and should make gene discovery much easier using the sgRNA sequence information. We further envision generation of a rice mutant population expressing Cas9 and a library of multiplexed (e.g., 16×) sgRNA targeting all possible known or predicted rice immunity genes (>500). Such a rice mutant library may be very useful for identification of high-order rice mutants with compromised NHR to cereal rust and powdery mildew fungi (Figure 2d), and if so, we can address perhaps the most challenging and symbolic question regarding NHR (i.e., why is rice immune to these cereal fungi?).

However, it is also possible that a host factor(s) required for pathogenesis of either one of the two biotrophic pathogens may be missing or insufficient in rice and, as such, immunocompromised higher-order rice mutants may be unable to support infection of the nonadapted fungi. Given that one or more host Mildew locus O (MLO) proteins are strictly required for host-entry of powdery mildew (Buschges et al., 1997; Consonni et al., 2006) and that overexpression of barley *MLO* can increase host penetration by a nonadapted powdery mildew in barley (Elliott et al., 2002), it is possible that expression of a cognate rice MLO is inadequate in rice epidermal cells, hence contributing to NHR. Therefore, expressing barley *MLO* from its native promoter or the rice ubiquitin promoter in an immunocompromised rice mutant may further increase the chances for breaking down rice's NHR to barley or other cereal powdery mildew (Figure 2d).

Furthermore, nonadapted pathogens may lack a mechanism for nutrient acquisition from nonhost plants, (partly) accounting for their failure in reproduction or proliferation. For example, phytobacteria *Xanthomonas* spp. use transcription activator-like (TAL) effectors to target and manipulate the transcription of plant *SWEET* sucrose transporter genes for increasing sugar availability in the apoplast (Bezrutczyk et al., 2018). If there are no binding sites of TAL effectors from a *Xanthomonas* species in the promoter region of any *SWEET* genes of a nonhost plant under attack, the bacteria may remain nonpathogenic even if the

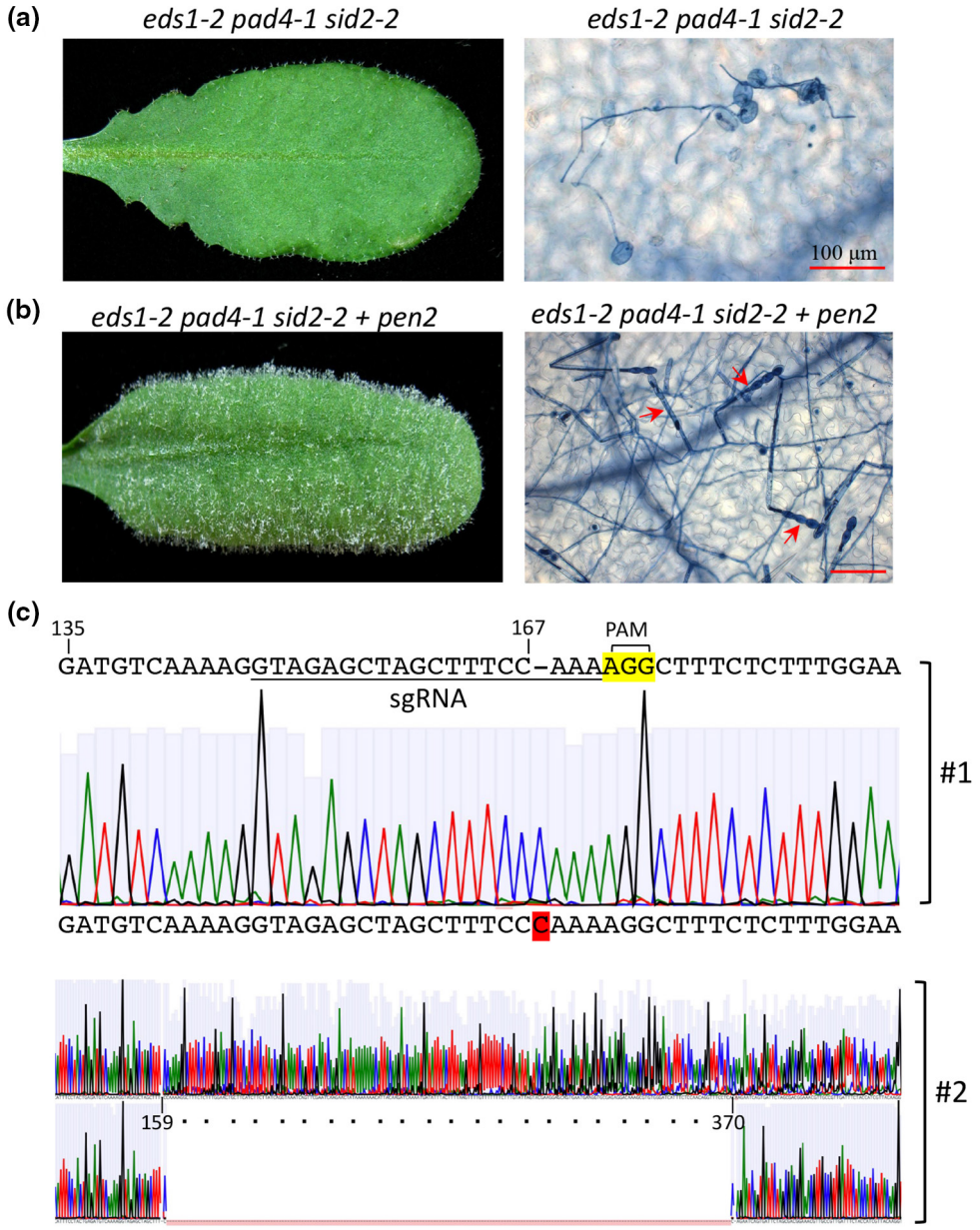

FIGURE 3 Making it visible: a sensitized "bottom-up" forward genetics strategy for identifying genes involved in penetration resistance against nonadapted powdery mildew fungi. The immunocompromised *eds1-2 pad4-1 sid2-2* (*eps*) triple *Arabidopsis* mutant is supersusceptible to the adapted powdery mildew isolate *Golovinomyces cichoracearum* UCSC1 (Zhang et al., 2018). However, the *eps* mutant remains resistant to the nonadapted strawberry powdery mildew pathogen *Podosphaera aphanis*, as evidenced by the lack of fungal mass visible to the naked eye at 8 days postinoculation (dpi) (a, left) and restricted fungal growth revealed by trypan blue staining followed by microscopy (a, right). CRISPR-targeted mutagenesis using the pHEE401E vector containing *Cas9* under control of an egg cell-specific promoter (Wang et al., 2015b) was deployed to mutate *PEN2*. Inoculation of 6-week-old 20 T_1 plants with *P. aphanis* identified two plants to be susceptible to this nonadapted pathogen as shown by profuse fungal sporulation visible to the naked eye (b, left), which was confirmed by trypan blue staining and microscopy at 8 dpi (b, right; red arrows indicate conidiophores). Sequencing the target site of *PEN2* revealed a single nucleotide insertion in the second exon of *PEN2* in plant #1 and a 211 nucleotide deletion in plant #2 (c), both of which result in an early stop codon (TGA). All plants were grown under 22°C, 65% relative humidity, short-day (8 h light at 125 µmol/m^2/s, 16 h dark) for 6 weeks before fungal infection. The above results demonstrate that an immunocompromised mutant with defective postpenetration resistance can be used to effectively identify genes involved in penetration resistance against nonadapted cell wall-penetrating filamentous pathogens through saturated mutagenesis followed by efficient mutant screening with the naked eye

plant is immunocompromised. Ectopic expression of a heterologous *SWEET* gene known to be targeted by a TAL effector of the pathogen (thus such a *SWEET* gene is a susceptibility factor) in an immunocompromised nonhost plant can help ascertain if lack of adequate nutrient acquisition could also attribute to NHR against some phytobacteria.

3 | CONCLUSIONS AND PERSPECTIVES

While genetic control of NHR in near-hosts is relatively simple and thus genetically amenable, NHR in most cases is often multilayered and thus dissection of its genetic basis may require multiple rounds of forward and/or reverse genetic studies. The increased understanding of plant immune mechanisms using various host–pathogen systems, together with the development of advanced enabling technologies, including various OMICS and CRISPR technologies, should greatly facilitate future studies towards a complete dissection of the most canonical NHR such as rice's immunity to powdery mildew and rust fungi. As discussed in a recent review (Panstruga & Moscou, 2020), NHR is just a phenomenological term, and genetic/molecular mechanisms underlying NHR may vary depending on the context of the plant–pathogen interaction under study. However, the mechanisms of defence programmes induced by nonadapted pathogens during NHR most likely are the same as those occurring in host resistance that encompasses PTI and ETI. Therefore, while NHR studies may not lead to discoveries of entirely new immune mechanisms independent of PTI and ETI, elucidation of multilayered NHR may help reveal hidden components or regulatory nodes of PTI and ETI, thereby advancing our understanding of the plant immunity architecture in general and realizing the great potential of NHR for crop improvement by engineering effective immunity in susceptible crop plants.

ACKNOWLEDGEMENTS

This work was supported by the National Science Foundation (IOS-1901566; IOS-2224203) for S.X. and B.Y. (IOS-2224204). The authors would like to thank other members of the Xiao laboratory for useful discussion.

CONFLICT OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as potential conflicts of interest.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analysed.

ORCID

Bing Yang https://orcid.org/0000-0002-2293-3384
Shunyuan Xiao https://orcid.org/0000-0003-1348-4879

REFERENCES

Atienza, S.G., Jafary, H. & Niks, R.E. (2004) Accumulation of genes for susceptibility to rust fungi for which barley is nearly a nonhost results in two barley lines with extreme multiple susceptibility. *Planta*, 220, 71–79.

Ayliffe, M. & Sorensen, C.K. (2019) Plant nonhost resistance: paradigms and new environments. *Current Opinion in Plant Biology*, 50, 104–113.

Bartaula, R., Melo, A.T.O., Connolly, B.A., Jin, Y. & Hale, I. (2018) An interspecific barberry hybrid enables genetic dissection of nonhost resistance to the stem rust pathogen *Puccinia graminis*. *Journal of Experimental Botany*, 69, 2483–2493.

Bartaula, R., Melo, A.T.O., Kingan, S., Jin, Y. & Hale, I. (2019) Mapping nonhost resistance to the stem rust pathogen in an interspecific barberry hybrid. *BMC Plant Biology*, 19, 319.

Bednarek, P., Pislewska-Bednarek, M., Svatos, A., Schneider, B., Doubsky, J., Mansurova, M. et al. (2009) A glucosinolate metabolism pathway in living plant cells mediates broad-spectrum antifungal defense. *Science*, 323, 101–106.

Bettgenhaeuser, J., Gilbert, B., Ayliffe, M. & Moscou, M.J. (2014) Nonhost resistance to rust pathogens – a continuation of continua. *Frontiers in Plant Science*, 5, 664.

Bezrutczyk, M., Yang, J., Eom, J.S., Prior, M., Sosso, D., Hartwig, T. et al. (2018) Sugar flux and signaling in plant–microbe interactions. *The Plant Journal*, 93, 675–685.

Borlaug, N.E. (2000) Ending world hunger. The promise of biotechnology and the threat of antiscience zealotry. *Plant Physiology*, 124, 487–490.

Bourras, S., Kunz, L., Xue, M., Praz, C.R., Muller, M.C., Kalin, C. et al. (2019) The AvrPm3–Pm3 effector–NLR interactions control both race-specific resistance and host-specificity of cereal mildews on wheat. *Nature Communications*, 10, 2292.

Bowyer, P., Clarke, B.R., Lunness, P., Daniels, M.J. & Osbourn, A.E. (1995) Host-range of a plant-pathogenic fungus determined by a saponin detoxifying enzyme. *Science*, 267, 371–374.

Buschges, R., Hollricher, K., Panstruga, R., Simons, G., Wolter, M., Frijters, A. et al. (1997) The barley *Mlo* gene: a novel control element of plant pathogen resistance. *Cell*, 88, 695–705.

Cevik, V., Boutrot, F., Apel, W., Robert-Seilaniantz, A., Furzer, O.J., Redkar, A. et al. (2019) Transgressive segregation reveals mechanisms of *Arabidopsis* immunity to brassica-infecting races of white rust (*Albugo candida*). *Proceedings of the National Academy of Sciences of the United States of America*, 116, 2767–2773.

Clay, N.K., Adio, A.M., Denoux, C., Jander, G. & Ausubel, F.M. (2009) Glucosinolate metabolites required for an *Arabidopsis* innate immune response. *Science*, 323, 95–101.

Collins, N.C., Thordal-Christensen, H., Lipka, V., Bau, S., Kombrink, E., Qiu, J.L. et al. (2003) SNARE-protein-mediated disease resistance at the plant cell wall. *Nature*, 425, 973–977.

Consonni, C., Humphry, M.E., Hartmann, H.A., Livaja, M., Durner, J., Westphal, L. et al. (2006) Conserved requirement for a plant host cell protein in powdery mildew pathogenesis. *Nature Genetics*, 38, 716–720.

Delventhal, R., Rajaraman, J., Stefanato, F.L., Rehman, S., Aghnoum, R., McGrann, G.R.D. et al. (2017) A comparative analysis of nonhost resistance across the two Triticeae crop species wheat and barley. *BMC Plant Biology*, 17, 232.

Dracatos, P.M., Nansamba, M., Berlin, A., Park, R.F. & Niks, R.E. (2016) Isolate specificity and polygenic inheritance of resistance in barley to the heterologous rust pathogen *Puccinia graminis* f. sp. *avenae*. *Phytopathology*, 106, 1029–1037.

Elliott, C., Zhou, F., Spielmeyer, W., Panstruga, R. & Schulze-Lefert, P. (2002) Functional conservation of wheat and rice *Mlo* orthologs in defense modulation to the powdery mildew fungus. *Molecular Plant-Microbe Interactions*, 15, 1069–1077.

Fan, J. & Doerner, P. (2012) Genetic and molecular basis of nonhost disease resistance: complex, yes; silver bullet, no. *Current Opinion in Plant Biology*, 15, 400–406.

Fonseca, J.P. & Mysore, K.S. (2019) Genes involved in nonhost disease resistance as a key to engineer durable resistance in crops. *Plant Science*, 279, 108–116.

Fonseca, J.P., Lee, H.K., Boschiero, C., Griffiths, M., Lee, S., Zhao, P. et al. (2020) Iron-sulfur cluster protein nitrogen fixation S-like1 and its interactor frataxin function in plant immunity. *Plant Physiology*, 184, 1532–1548.

Frerigmann, H., Pislewska-Bednarek, M., Sanchez-Vallet, A., Molina, A., Glawischnig, E., Gigolashvili, T. et al. (2016) Regulation of pathogen-triggered tryptophan metabolism in *Arabidopsis thaliana* by MYB transcription factors and indole glucosinolate conversion products. *Molecular Plant*, 9, 682–695.

Fukunaga, S., Sogame, M., Hata, M., Singkaravanit-Ogawa, S., Pislewska-Bednarek, M., Onozawa-Komori, M. et al. (2017) Dysfunction of *Arabidopsis* MACPF domain protein activates programmed cell death via tryptophan metabolism in MAMP-triggered immunity. *The Plant Journal*, 89, 381–393.

Gaillochet, C., Develtere, W. & Jacobs, T.B. (2021) CRISPR screens in plants: approaches, guidelines, and future prospects. *The Plant Cell*, 33, 794–813.

Gangurde, S.S., Nayak, S.N., Joshi, P., Purohit, S., Sudini, H.K., Chitikineni, A. et al. (2021) Comparative transcriptome analysis identified candidate genes for late leaf spot resistance and cause of defoliation in groundnut. *International Journal of Molecular Sciences*, 22, 23.

Geissler, K., Eschen-Lippold, L., Naumann, K., Schneeberger, K., Weigel, D., Scheel, D. et al. (2015) Mutations in the *EDR1* gene alter the response of *Arabidopsis thaliana* to *Phytophthora infestans* and the bacterial PAMPs flg22 and elf18. *Molecular Plant-Microbe Interactions*, 28, 122–133.

Gill, U.S., Lee, S. & Mysore, K.S. (2015) Host versus nonhost resistance: distinct wars with similar arsenals. *Phytopathology*, 105, 580–587.

Haghdoust, R., Singh, D., Park, R.F. & Dracatos, P.M. (2021) Characterizing the genetic architecture of nonhost resistance in barley using pathogenically diverse *Puccinia* isolates. *Phytopathology*, 111, 684–694.

Harris, J.M., Balint-Kurti, P., Bede, J.C., Day, B., Gold, S., Goss, E.M. et al. (2020) What are the top 10 unanswered questions in molecular plant–microbe interactions? *Molecular Plant-Microbe Interactions*, 33, 1354–1365.

Hartney, S., Carson, J. & Hadwiger, L.A. (2007) The use of chemical genomics to detect functional systems affecting the nonhost disease resistance of pea to *Fusarium solani* f. sp *phaseoli*. *Plant Science*, 172, 45–56.

Heath, M.C. (1977) A comparative study of nonhost interactions with rust fungi. *Physiological Plant Pathology*, 10, 73–88.

Heath, M.C. (2000) Nonhost resistance and nonspecific plant defenses. *Current Opinion in Plant Biology*, 3, 315–319.

Hematy, K., Lim, M., Cherk, C., Pislewska-Bednarek, M., Sanchez-Rodriguez, C., Stein, M. et al. (2020) Moonlighting function of Phytochelatin synthase1 in extracellular defense against fungal pathogens. *Plant Physiology*, 182, 1920–1932.

Humphry, M., Consonni, C. & Panstruga, R. (2006) *Mlo* -based powdery mildew immunity: silver bullet or simply nonhost resistance? *Molecular Plant Pathology*, 7, 605–610.

Irieda, H. & Takano, Y. (2021) Epidermal chloroplasts are defense-related motile organelles equipped with plant immune components. *Nature Communications*, 12, 2739.

Iven, T., Konig, S., Singh, S., Braus-Stromeyer, S.A., Bischoff, M., Tietze, L.F. et al. (2012) Transcriptional activation and production of tryptophan-derived secondary metabolites in *Arabidopsis* roots contributes to the defense against the fungal vascular pathogen *Verticillium longisporum*. *Molecular Plant*, 5, 1389–1402.

Jafary, H., Albertazzi, G., Marcel, T.C. & Niks, R.E. (2008) High diversity of genes for nonhost resistance of barley to heterologous rust fungi. *Genetics*, 178, 2327–2339.

Jones, J.D. & Dangl, J.L. (2006) The plant immune system. *Nature*, 444, 323–329.

Kang, L., Li, J., Zhao, T., Xiao, F., Tang, X., Thilmony, R. et al. (2003) Interplay of the *Arabidopsis* nonhost resistance gene *NHO1* with bacterial virulence. *Proceedings of the National Academy of Sciences of the United States of America*, 100, 3519–3524.

Kobayashi, Y., Kobayashi, I., Funaki, Y., Fujimoto, S., Takemoto, T. & Kunoh, H. (1997a) Dynamic reorganization of microfilaments and microtubules is necessary for the expression of nonhost resistance in barley coleoptile cells. *The Plant Journal*, 11, 525–537.

Kobayashi, Y., Yamada, M., Kobayashi, I. & Kunoh, H. (1997b) Actin microfilaments are required for the expression of nonhost resistance in higher plants. *Plant and Cell Physiology*, 38, 725–733.

Kopischke, M., Westphal, L., Schneeberger, K., Clark, R., Ossowski, S., Wewer, V. et al. (2013) Impaired sterol ester synthesis alters the response of *Arabidopsis thaliana* to *Phytophthora infestans*. *The Plant Journal*, 73, 456–468.

Kosaka, A., Pastorczyk, M., Pislewska-Bednarek, M., Nishiuchi, T., Ono, E., Suemoto, H. et al. (2021) Tryptophan-derived metabolites and BAK1 separately contribute to *Arabidopsis* postinvasive immunity against *Alternaria brassicicola*. *Scientific Reports*, 11, 1488.

Laflamme, B., Dillon, M.M., Martel, A., Almeida, R.N.D., Desveaux, D. & Guttman, D.S. (2020) The pan-genome effector-triggered immunity landscape of a host–pathogen interaction. *Science*, 367, 763–768.

Langenbach, C., Campe, R., Schaffrath, U., Goellner, K. & Conrath, U. (2013) UDP-glucosyltransferase UGT84A2/BRT1 is required for *Arabidopsis* nonhost resistance to the Asian soybean rust pathogen *Phakopsora pachyrhizi*. *New Phytologist*, 198, 536–545.

Lee, H.A., Lee, H.Y., Seo, E., Lee, J., Kim, S.B., Oh, S. et al. (2017) Current understandings of plant nonhost resistance. *Molecular Plant-Microbe Interactions*, 30, 5–15.

Lipka, V., Dittgen, J., Bednarek, P., Bhat, R., Wiermer, M., Stein, M. et al. (2005) Pre- and postinvasion defenses both contribute to nonhost resistance in *Arabidopsis*. *Science*, 310, 1180–1183.

Longin, C.F., Muhleisen, J., Maurer, H.P., Zhang, H., Gowda, M. & Reif, J.C. (2012) Hybrid breeding in autogamous cereals. *Theoretical and Applied Genetics*, 125, 1087–1096.

Lu, M., Tang, X. & Zhou, J.M. (2001) *Arabidopsis* NHO1 is required for general resistance against pseudomonas bacteria. *The Plant Cell*, 13, 437–447.

Menardo, F., Praz, C.R., Wyder, S., Ben-David, R., Bourras, S., Matsumae, H. et al. (2016) Hybridization of powdery mildew strains gives rise to pathogens on novel agricultural crop species. *Nature Genetics*, 48, 201–205.

Meng, X., Yu, H., Zhang, Y., Zhuang, F., Song, X., Gao, S. et al. (2017) Construction of a genome-wide mutant library in rice using CRISPR/Cas9. *Molecular Plant*, 10, 1238–1241.

Miklis, M., Consonni, C., Bhat, R.A., Lipka, V., Schulze-Lefert, P. & Panstruga, R. (2007) Barley MLO modulates actin-dependent and actin-independent antifungal defense pathways at the cell periphery. *Plant Physiology*, 144, 1132–1143.

Morris, C.E. & Moury, B. (2019) Revisiting the concept of host range of plant pathogens. *Annual Review of Phytopathology*, 57, 63–90.

Newcombe, G. (2005) Genes for parasite-specific, nonhost resistance in *Populus*. *Phytopathology*, 95, 779–783.

Ngou, B.P.M., Ding, P. & Jones, J.D. (2022) Thirty years of resistance: zig-zag through the plant immune system. *The Plant Cell*, 34, 1447–1478.

Nurnberger, T. & Lipka, V. (2005) Nonhost resistance in plants: new insights into an old phenomenon. *Molecular Plant Pathology*, 6, 335–345.

Panstruga, R. & Moscou, M.J. (2020) What is the molecular basis of nonhost resistance? *Molecular Plant-Microbe Interactions*, 33, 1253–1264.

Pant, B.D., Oh, S., Lee, H.K., Nandety, R.S. & Mysore, K.S. (2020) Antagonistic regulation by CPN60A and CLPC1 of TRXL1 that regulates MDH activity leading to plant disease resistance and thermotolerance. *Cell Reports*, 33, 108512.

Papadopoulou, K., Melton, R.E., Leggett, M., Daniels, M.J. & Osbourn, A.E. (1999) Compromised disease resistance in saponin-deficient plants. *Proceedings of the National Academy of Sciences of the United States of America*, 96, 12923–12928.

Pastorczyk, M., Kosaka, A., Pislewska-Bednarek, M., Lopez, G., Frerigmann, H., Kulak, K. et al. (2020) The role of CYP71A12 monooxygenase in pathogen-triggered tryptophan metabolism and *Arabidopsis* immunity. *New Phytologist*, 225, 400–412.

Peart, J.R., Lu, R., Sadanandom, A., Malcuit, I., Moffett, P., Brice, D.C. et al. (2002) Ubiquitin ligase-associated protein SGT1 is required for host and nonhost disease resistance in plants. *Proceedings of the National Academy of Sciences of the United States of America*, 99, 10865–10869.

Pinosa, F., Buhot, N., Kwaaitaal, M., Fahlberg, P., Thordal-Christensen, H., Ellerstrom, M. et al. (2013) *Arabidopsis* phospholipase Dδ is involved in basal defense and nonhost resistance to powdery mildew fungi. *Plant Physiology*, 163, 896–906.

Qin, L., Liu, L., Tu, J., Yang, G., Wang, S., Quilichini, T.D. et al. (2021) The ARP2/3 complex, acting cooperatively with class I formins, modulates penetration resistance in *Arabidopsis* against powdery mildew invasion. *The Plant Cell*, 33, 3151–3175.

Ramu, V.S., Dawane, A., Lee, S., Oh, S., Lee, H.K., Sun, L. et al. (2020) Ribosomal protein QM/RPL10 positively regulates defence and protein translation mechanisms during nonhost disease resistance. *Molecular Plant Pathology*, 21, 1481–1494.

Rojas, C.M., Senthil-Kumar, M., Wang, K., Ryu, C.M., Kaundal, A. & Mysore, K.S. (2012) Glycolate oxidase modulates reactive oxygen species-mediated signal transduction during nonhost resistance in *Nicotiana benthamiana* and *Arabidopsis*. *The Plant Cell*, 24, 336–352.

Saga, H., Ogawa, T., Kai, K., Suzuki, H., Ogata, Y., Sakurai, N. et al. (2012) Identification and characterization of ANAC042, a transcription factor family gene involved in the regulation of camalexin biosynthesis in *Arabidopsis*. *Molecular Plant-Microbe Interactions*, 25, 684–696.

Sanchez-Vallet, A., Ramos, B., Bednarek, P., Lopez, G., Pislewska-Bednarek, M., Schulze-Lefert, P. et al. (2010) Tryptophan-derived secondary metabolites in *Arabidopsis thaliana* confer nonhost resistance to necrotrophic *Plectosphaerella cucumerina* fungi. *The Plant Journal*, 63, 115–127.

Schlaeppi, K., Abou-Mansour, E., Buchala, A. & Mauch, F. (2010) Disease resistance of *Arabidopsis* to *Phytophthora brassicae* is established by the sequential action of indole glucosinolates and camalexin. *The Plant Journal*, 62, 840–851.

Schulze-Lefert, P. & Panstruga, R. (2011) A molecular evolutionary concept connecting nonhost resistance, pathogen host range, and pathogen speciation. *Trends in Plant Science*, 16, 117–125.

Senthil-Kumar, M. & Mysore, K.S. (2013) Nonhost resistance against bacterial pathogens: retrospectives and prospects. *Annual Review of Phytopathology*, 51, 407–427.

Senthil-Kumar, M., Lee, H.K. & Mysore, K.S. (2013) VIGS-mediated forward genetics screening for identification of genes involved in nonhost resistance. *Journal of Visualized Experiments*, 78, e51033.

Shimada, C., Lipka, V., O'Connell, R., Okuno, T., Schulze-Lefert, P. & Takano, Y. (2006) Nonhost resistance in *Arabidopsis–Colletotrichum* interactions acts at the cell periphery and requires actin filament function. *Molecular Plant-Microbe Interactions*, 19, 270–279.

Sohn, K.H., Saucet, S.B., Clarke, C.R., Vinatzer, B.A., O'Brien, H.E., Guttman, D.S. et al. (2012) HopAS1 recognition significantly contributes to *Arabidopsis* nonhost resistance to *Pseudomonas syringae* pathogens. *New Phytologist*, 193, 58–66.

Song, X.H., Ma, Q., Hao, X.Y. & Li, H.L. (2012) Roles of the actin cytoskeleton and an actin-binding protein in wheat resistance against *Puccinia striiformis* f. sp *tritici*. *Protoplasma*, 249, 99–106.

Specht, K.M. & Shokat, K.M. (2002) The emerging power of chemical genetics. *Current Opinion in Cell Biology*, 14, 155–159.

Stein, M., Dittgen, J., Sanchez-Rodriguez, C., Hou, B.H., Molina, A., Schulze-Lefert, P. et al. (2006) *Arabidopsis* PEN3/PDR8, an ATP binding cassette transporter, contributes to nonhost resistance to inappropriate pathogens that enter by direct penetration. *The Plant Cell*, 18, 731–746.

Stuttmann, J., Barthel, K., Martin, P., Ordon, J., Erickson, J.L., Herr, R. et al. (2021) Highly efficient multiplex editing: one-shot generation of 8x *Nicotiana benthamiana* and 12x *Arabidopsis* mutants. *The Plant Journal*, 106, 8–22.

Takahashi, T., Shibuya, H. & Ishikawa, A. (2016) SOBIR1 contributes to nonhost resistance to *Magnaporthe oryzae* in *Arabidopsis*. *Bioscience, Biotechnology, and Biochemistry*, 80, 1577–1579.

Takahashi, T., Murano, T. & Ishikawa, A. (2018) SOBIR1 and AGB1 independently contribute to nonhost resistance to *Pyricularia oryzae* (syn. *Magnaporthe oryzae*) in *Arabidopsis thaliana*. *Bioscience, Biotechnology, and Biochemistry*, 82, 1922–1930.

Thines, M. (2019) An evolutionary framework for host shifts – jumping ships for survival. *New Phytologist*, 224, 605–617.

Thordal-Christensen, H. (2003) Fresh insights into processes of nonhost resistance. *Current Opinion in Plant Biology*, 6, 351–357.

Tufan, H.A., McGrann, G.R.D., Magusin, A., Morel, J.B., Miche, L. & Boyd, L.A. (2009) Wheat blast: histopathology and transcriptome reprogramming in response to adapted and nonadapted *Magnaporthe* isolates. *New Phytologist*, 184, 473–484.

Uppalapati, S.R., Ishiga, Y., Doraiswamy, V., Bedair, M., Mittal, S., Chen, J. et al. (2012) Loss of abaxial leaf epicuticular wax in *Medicago truncatula irg1/palm1* mutants results in reduced spore differentiation of anthracnose and nonhost rust pathogens. *The Plant Cell*, 24, 353–370.

Walker, A.S., Bouguennec, A., Confais, J., Morgant, G. & Leroux, P. (2011) Evidence of host-range expansion from new powdery mildew (*Blumeria graminis*) infections of triticale (*Triticosecale*) in France. *Plant Pathology*, 60, 207–220.

Wang, K., Uppalapati, S.R., Zhu, X., Dinesh-Kumar, S.P. & Mysore, K.S. (2010) SGT1 positively regulates the process of plant cell death during both compatible and incompatible plant–pathogen interactions. *Molecular Plant Pathology*, 11, 597–611.

Wang, J., Zuo, H., Huo, Y., Feng, C.J., Wang, Y. & Ma, Q. (2015a) Evaluation of actin cytoskeleton in nonhost resistance of pepper to *Puccinia striiformis* f. sp *tritici* stress. *Physiological and Molecular Plant Pathology*, 92, 112–118.

Wang, Z.P., Xing, H.L., Dong, L., Zhang, H.Y., Han, C.Y., Wang, X.C. et al. (2015b) Egg cell-specific promoter-controlled CRISPR/Cas9 efficiently generates homozygous mutants for multiple target genes in *Arabidopsis* in a single generation. *Genome Biology*, 16, 144.

Wang, Y., Subedi, S., de Vries, H., Doornenbal, P., Vels, A., Hensel, G. et al. (2019) Orthologous receptor kinases quantitatively affect the host status of barley to leaf rust fungi. *Nature Plants*, 5, 1129–1135.

Wu, H., Kwaaitaal, M., Strugala, R., Schaffrath, U., Bednarek, P. & Panstruga, R. (2017) Chemical suppressors of *mlo*-mediated powdery mildew resistance. *Bioscience Reports*, 37, BSR20171389.

Wu, H.P., Zhang, W.W., Schuster, M., Moch, M., Windoffer, R., Steinberg, G. et al. (2020) Alloxan disintegrates the plant cytoskeleton and suppresses *mlo*-mediated powdery mildew resistance. *Plant and Cell Physiology*, 61, 505–518.

Xu, J., Meng, J., Meng, X., Zhao, Y., Liu, J., Sun, T. et al. (2016) Pathogen-responsive MPK3 and MPK6 reprogram the biosynthesis of indole glucosinolates and their derivatives in *Arabidopsis* immunity. *The Plant Cell*, 28, 1144–1162.

Yang, L., Qin, L., Liu, G., Peremyslov, V.V., Dolja, V.V. & Wei, Y. (2014) Myosins XI modulate host cellular responses and penetration resistance to fungal pathogens. *Proceedings of the National Academy of Sciences of the United States of America*, 111, 13996–14001.

Yang, L., Zhang, Y., Guan, R., Li, S., Xu, X., Zhang, S. et al. (2020) Co-regulation of indole glucosinolates and camalexin biosynthesis by CPK5/CPK6 and MPK3/MPK6 signaling pathways. *Journal of Integrative Plant Biology*, 62, 1780–1796.

Yu, Z.D., Shen, K.C., Newcombe, G., Fan, J.F. & Chen, Q.W. (2019) Leaf cuticle can contribute to nonhost resistance to poplar leaf rust. *Forests*, 10, 12.

Yun, B.W., Atkinson, H.A., Gaborit, C., Greenland, A., Read, N.D., Pallas, J.A. et al. (2003) Loss of actin cytoskeletal function and EDS1 activity, in combination, severely compromises nonhost resistance in *Arabidopsis* against wheat powdery mildew. *The Plant Journal*, 34, 768–777.

Zhang, Q., Berkey, R., Blakeslee, J.J., Lin, J., Ma, X., King, H. et al. (2018) *Arabidopsis* phospholipase Dalpha1 and Ddelta oppositely modulate EDS1- and SA-independent basal resistance against adapted powdery mildew. *Journal of Experimental Botany*, *69*, 3675–3688.

Zhang, Y., Ren, Q., Tang, X., Liu, S., Malzahn, A.A., Zhou, J. et al. (2021) Expanding the scope of plant genome engineering with Cas12a orthologs and highly multiplexable editing systems. *Nature Communications*, 12, 1944.

Zhou, N., Tootle, T.L. & Glazebrook, J. (1999) *Arabidopsis PAD3*, a gene required for camalexin biosynthesis, encodes a putative cytochrome P450 monooxygenase. *The Plant Cell*, 11, 2419–2428.

How to cite this article: Wu, Y., Sexton, W., Yang, B. & Xiao, S. (2023) Genetic approaches to dissect plant nonhost resistance mechanisms. *Molecular Plant Pathology*, 24, 272–283. Available from: https://doi.org/10.1111/mpp.13290