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Characterization of the interaction between *Oidium heveae* and *Arabidopsis thaliana*

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SUMMARY

Oidium heveae, an obligate biotrophic pathogen of rubber trees (*Hevea brasiliensis*), causes significant yield losses of rubber worldwide. However, the molecular mechanisms underlying the interplay between *O. heveae* and rubber trees remain largely unknown. In this study, we isolated an *O. heveae* strain, named HN1106, from cultivated *H. brasiliensis* in Hainan, China. We found that *O. heveae* HN1106 triggers the hypersensitive response in a manner that depends on the effector-triggered immunity proteins EDS1 (Enhanced Disease Susceptibility 1) and PAD4 (Phytoalexin Deficient 4) and on salicylic acid (SA) in the model plant *Arabidopsis thaliana*. However, SA-independent resistance also appears to limit *O. heveae* infection of *Arabidopsis*, because the pathogen does not produce conidiospores on *npr1* (*nonexpressor of pr1*), *sid2* (*SA induction deficient 2*) and *NahG* plants, which show disruptions in SA signalling. Furthermore, we found that the callose synthase PMR4 (Powdery Mildew Resistant 4) prevents *O. heveae* HN1106 penetration into leaves in the early stages of infection. To elucidate the potential mechanism of resistance of *Arabidopsis* to *O. heveae* HN1106, we inoculated 47 different *Arabidopsis* accessions with the pathogen, and analysed the plant disease symptoms and *O. heveae* HN1106 hyphal growth and conidiospore formation on the leaves. We found that the accession Lag2-2 showed significant susceptibility to *O. heveae* HN1106. Overall, this study provides a basis for future research aimed at combatting powdery mildew caused by *O. heveae* in rubber trees.

Keywords: Arabidopsis thaliana, enhanced disease susceptibility 1, Hevea brasiliensis, incompatible interaction, Oidium heveae, powdery mildew resistant 4, salicylic acid.

INTRODUCTION

Powdery mildews are a group of parasitic ascomycete fungi that infect many plant species and result in significant crop yield losses worldwide. Outbreaks of powdery mildew disease of rubber trees (*Hevea brasiliensis)* in Malaysia, India, Brazil and Papua New Guinea (Beeley, 1933; Mitra and Mehta, 1938; Saranya *et al.*, 2005) have significantly reduced rubber yields. *Oidium heveae*, the powdery mildew that infects rubber trees, usually grows in living host cells of young leaves or buds, resulting in defoliation of young leaves and discoloration and curling of the margins of old leaves. When an *O. heveae* conidiospore lands on a leaf, a primary germ tube is generated which produces an appressorium at the site at which the fungal pathogen attempts to breach the plant cell wall. Successful penetration leads to invagination of the host plasma membrane to form a lobe-shaped structure, the haustorium. Subsequently, secondary hyphae form from the spore and new appressoria penetrate neighbouring cells and develop secondary haustoria (Saranya *et al.*, 2005). Mycelia on the leaves are amphigenous and conidiospores develop from the vegetative hyphae, forming straight, cylindrical foot cells, followed by one to three additional cells. The first conidium that matures on a conidiospore is ellipsoidal with a rounded tip; secondary conidia are ellipsoidal-cylindrical in shape (Saranya *et al.*, 2005). At present, brimstone powder is often used to prevent outbreaks of powdery mildew on rubber trees. However, this substance is toxic to the environment. Therefore, there is an urgent need to elucidate the molecular profiles of *O. heveae*–host interactions, and subsequently to develop an effective strategy to cope with the disease.

To protect themselves against pathogen invasion, plants possess two layers of immune recognition. The first line of disease resistance, triggered by pathogen/microbe-associated molecular patterns (PAMPs/MAMPs) (Boller and He, 2009; Zhou and Chai, 2008), is termed PAMP-triggered immunity (PTI). For example, chitin present in the growing hyphae and conidia of the powdery mildew *Golovinomyces cichoracearum* functions as a PAMP that elicits PTI responses during infection of *Arabidopsis thaliana* (Ramonell *et al.*, 2005). The second, effector-triggered immunity (ETI), is activated by plant resistance (R) proteins on recognition of race-specific effectors, and is usually associated with a hypersensitive response (HR), which involves rapid programmed host cell death at the infection site (Jones and Dangl, 2006). Two

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avirulence (AVR) proteins, AVR_{a10} and AVR_{k1}, encoded by the powdery mildew *Blumeria graminis* f. sp. *hordei*, elicit host defences in resistant barley (*Hordeum vulgare*) varieties (Catanzariti *et al*., 2007; Ridout *et al*., 2006).

Several proteins play crucial roles in the activation of R proteins in *Arabidopsis* (Day *et al*., 2006; Hammond-Kosack and Parker, 2003). For example, EDS1 (Enhanced Disease Susceptibility 1) is generally required for the activation of the Toll-Interleukin1 Receptor (TIR)-nucleotide binding (NB)-leucine-rich repeat (LRR) subclass of R proteins, such as resistance to *Peronospora parasitica* RPP(s) and resistance to *Pseudomonas syringae* (RPS) 4 (Aarts *et al*., 1998; Moreau *et al*., 2012; Parker *et al*., 1996; Rietz *et al*., 2011). NDR1 (Non-Race-Specific Disease Resistance 1) is largely required for ETI conditioned by coiled-coil (CC)-NB-LRR R proteins, such as resistance to *Pseudomonas syringae* pv. *maculicola* (RPM) 1, RPS2 and RPS5 (Aarts *et al*., 1998; Century *et al*., 1997; Day *et al*., 2006; Moreau *et al*., 2012). In *Arabidopsis*, EDS1 interacts with two signalling partners, PAD4 (Phytoalexin Deficient 4) and SAG (Senescence-Associated Gene) 101, to form soluble cytoplasmic and nuclear signalling complexes (Cui *et al*., 2015; Feys *et al*., 2001, 2005; Wagner *et al*., 2013). The Heat Shock Protein 90 (HSP90) co-chaperones RAR1 (Required for *Mla12* Resistance) and SGT1 (Suppressor of G-Two Allele of Skp1) are required to stabilize some R proteins, and *rar1* and *sgt1* mutants often fail to induce R protein-mediated resistance (Hubert *et al*., 2009).

R protein-mediated resistance responses are usually accompanied by increased levels of salicylic acid (SA) in local and distant parts of the plant, as well as the up-regulation of a large set of defence genes, including pathogenesis-related (*PR*) genes (Gaffney *et al*., 1993; McDowell and Dangl, 2000; Ward *et al*., 1991). *Arabidopsis* mutants that are impaired in pathogen-induced SA accumulation include *eds1* (Falk *et al*., 1999), *eds5* (Nawrath *et al*., 2002), *sid2* (*SA induction deficient 2*) (Wildermuth *et al*., 2001) and *pad4* (Jirage *et al*., 1999). In addition, mutants exist that are defective in SA responsiveness, for example *npr1* (*nonexpressor of pr1*) (Cao *et al*., 1997). Transgenic *Arabidopsis* plants expressing *NahG*, a bacterial SA hydroxylase gene, are also unable to accumulate SA and show enhanced susceptibility to incompatible pathogens (Delaney *et al*., 1994). In *Arabidopsis*, SA and EDS1 are thought to function in a positive feedback loop, because SA up-regulates *EDS1* expression and EDS1 enhances SA production (Falk *et al*., 1999; Venugopal *et al*., 2009). EDS1 mediates resistance via both SA-dependent and SA-independent pathways (Bartsch *et al*., 2006).

In this study, we isolated the *O. heveae* strain HN1106 from the rubber species *H. brasiliensis GT-1*. When infected by *O. heveae* HN1106, *H. brasiliensis GT-1* displays severe disease symptoms, such as irregular patches on the upper and lower surfaces of the leaves. Interestingly, we found that *Arabidopsis* displays a typical resistance response to *O. heveae*, characterized by cell death activation, hydrogen peroxide (H_2O_2) production, callose deposition and *PR1* (*Pathogenesis-Related 1*) gene expression. In addition, we found that the *eds1* and *pad4* mutants, but not the *ndr1* mutant, are significantly compromised in resistance to *O. heveae* HN1106. These results collectively suggest that EDS1-mediated ETI signalling is involved in the resistance to the pathogen *O. heveae*.

RESULTS

Arabidopsis and *O. heveae* HN1106 exhibit an incompatible interaction

In this study, we isolated a powdery mildew strain from *H. brasiliensis GT-1* leaves displaying powdery mildew symptoms, and named it *O. heveae* HN1106. The ribosomal DNA internal transcribed spacer (ITS) sequence of *O. heveae* HN1106 was analysed and found to be identical to the Thailand *O. heveae* MUMH2602 isolate (Fig. S1, see Supporting Information). Furthermore, we generated a phylogenetic tree based on 11 powdery mildew ITS sequences, and showed that *O. heveae* is more closely related to *Erysiphe cruciferarum* (Fig. S2, see Supporting Information), a powdery mildew that infects *Arabidopsis* (Gollner *et al*., 2008), than it is to *B. graminis* f. sp. *hordei* DH14, a non-adapted pathogen in *Arabidopsis* (Stein *et al*., 2006).

To test whether *O. heveae* HN1106 is pathogenic to *Arabidopsis*, we inoculated *A. thaliana* Col-0 leaves and *H. brasiliensis GT-1* leaves (as a control) with the pathogen. We collected whole leaves every 3 h post-inoculation (hpi) during the early stages of infection, and examined the leaves by light microscopy. Germ tubes arose from the ends of conidia on both Col-0 and *GT-1* leaves by 3 hpi (Fig. 1A). The primary germ tubes produced appressoria on *GT-1* leaves by 3 hpi and on *Arabidopsis* leaves by 6 hpi (Fig. 1A). Secondary germ tubes often emerged from conidia on *GT-1* leaves by 6 hpi and on *Arabidopsis* by 24 hpi (Fig. 1A). By 24 hpi, the majority of the germ tubes had penetrated the epidermal cells on *GT-1* leaves, but a large proportion of the germ tubes had not completely penetrated the *Arabidopsis* cells (Fig. 2A). By 2 days post-inoculation (dpi), the hyphae had elongated over 300 μm on *H. brasiliensis*, but only about 50 μm on *Arabidopsis* (Fig. 2B). By 4 dpi, fungal growth was apparent to the naked eye on *H. brasiliensis* leaves (Fig. 1C), and conidiospores had begun to develop perpendicular to the leaf surface (Fig. 1B). In the following days, fungal mycelia spread rapidly, adjacent patches of mycelia coalesced and mature conidia appeared at the ends of the conidiospores by 10 dpi on *H. brasiliensis* leaves (Fig. 1B, 2C). By contrast, chlorotic/necrotic flecks at the inoculation sites were apparent on *Arabidopsis* leaves by 10 dpi (Fig. 1C). Microscopy analysis showed that *O. heveae* HN1106 had a significantly lower penetration ratio on *Arabidopsis* than on *H. brasiliensis*, and had limited growth and failed to form a hyphal network

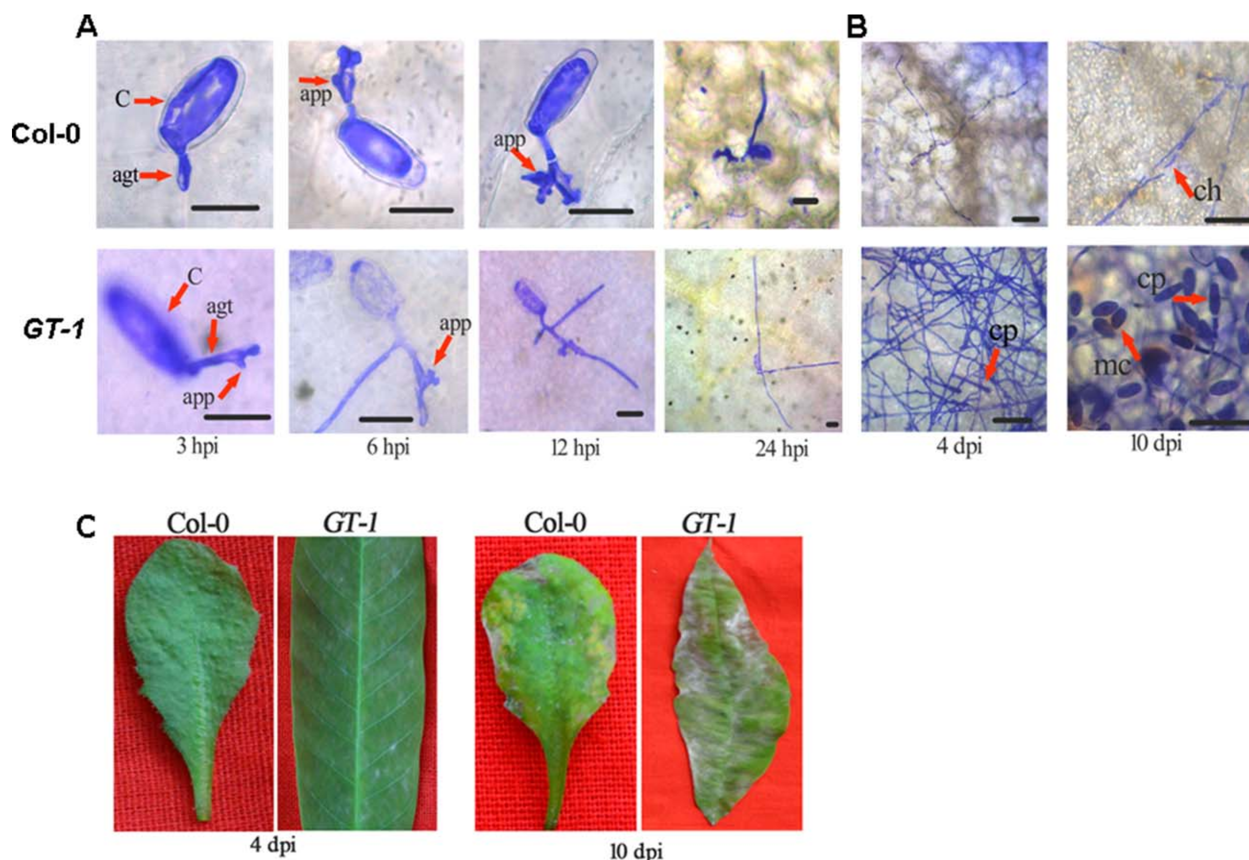

Fig. 1 Light micrographs and symptoms of *Arabidopsis* Col-0 and *Hevea brasiliensis GT-1* plants infected with *Oidium heveae* HN1106. Leaves of 4-week-old Col-0 plants and *H. brasiliensis GT-1* leaves in the light-green phase were inoculated with *O. heveae* HN1106. (A) Infected leaves stained with Coomassie brilliant blue were observed by microscopy at 3, 6, 12 and 24 h post-inoculation (hpi). Bar, 20 μm. (B) Infected leaves stained with Coomassie brilliant blue were observed by microscopy at 4 and 10 dpi. Bar, 50 μm. (C) Symptoms were analysed at 4 and 10 dpi. agt, appressorial germ tube; app, appressorium; C, conidia; ch, collapsed hyphae cp, conidiospores; mc, mature conidia. These experiments were repeated twice with similar results.

and conidiospores on *Arabidopsis* (Fig. 1B). Thus, *O. heveae* HN1106 cannot complete its life cycle on *Arabidopsis* Col-0. Cell death is a second line of defence that is induced when non-host fungi successfully penetrate epidermal cells (Consonni *et al.*, 2006). Here, we found that *O. heveae* HN1106 triggers chlorotic/necrotic symptoms on *Arabidopsis*, suggesting that the interaction between *Arabidopsis* and *O. heveae* HN1106 is incompatible, but not a non-host interaction.

Arabidopsis resistance to *O. heveae* HN1106 requires *EDS1* and *PAD4*, but not *NDR1*

Based on our observation that *O. heveae* HN1106 is an incompatible pathogen on *Arabidopsis*, we next determined whether the disease resistance against *O. heveae* HN1106 depends on some known ETI signalling components. We incubated wild-type *Arabidopsis* (Col-0), *eds1*, *pad4*, *rar1* and *ndr1* mutants with *O. heveae* HN1106, and evaluated the *Arabidopsis* resistance primarily based on conidiospore formation and plant disease symptoms. We observed a higher cell entry ratio (Fig. 3A) and significantly higher mycelium growth (Fig. 3B) on *eds1*, *pad4* and *rar1* mutants compared with Col-0. Typical powdery mildew patches occurred on *eds1* and *pad4* mutants by 10 dpi (Fig. 4A). Whereas *ndr1* mutants and Col-0 plants developed severe chlorosis flecks, *rar1* mutants exhibited relatively mild chlorosis (Fig. 4A). Many fungal conidiospores formed on *eds1* and *pad4* mutants, but none formed on Col-0, *ndr1* and *rar1* plants (Fig. 3D, 4B). Furthermore, the *O. heveae* mature fungal spores produced on *eds1* leaves successfully infected *H. brasiliensis GT-1* leaves (Fig. S3, see Supporting Information). The *rar1* mutant supports the formation of a dense hyphal network (Fig. 4B). These results indicate that *O. heveae* can complete its life cycle on *eds1* and *pad4* plants, and that *O. heveae*-triggered disease resistance partially requires *RAR1*. In addition, we found that cell death (Fig. 4C), H_2O_2 production (Fig. 4D), callose deposition (Fig. 3C) and *PR1* expression (Fig. 3E) were strongly induced in Col-0 and *ndr1*, and mildly in

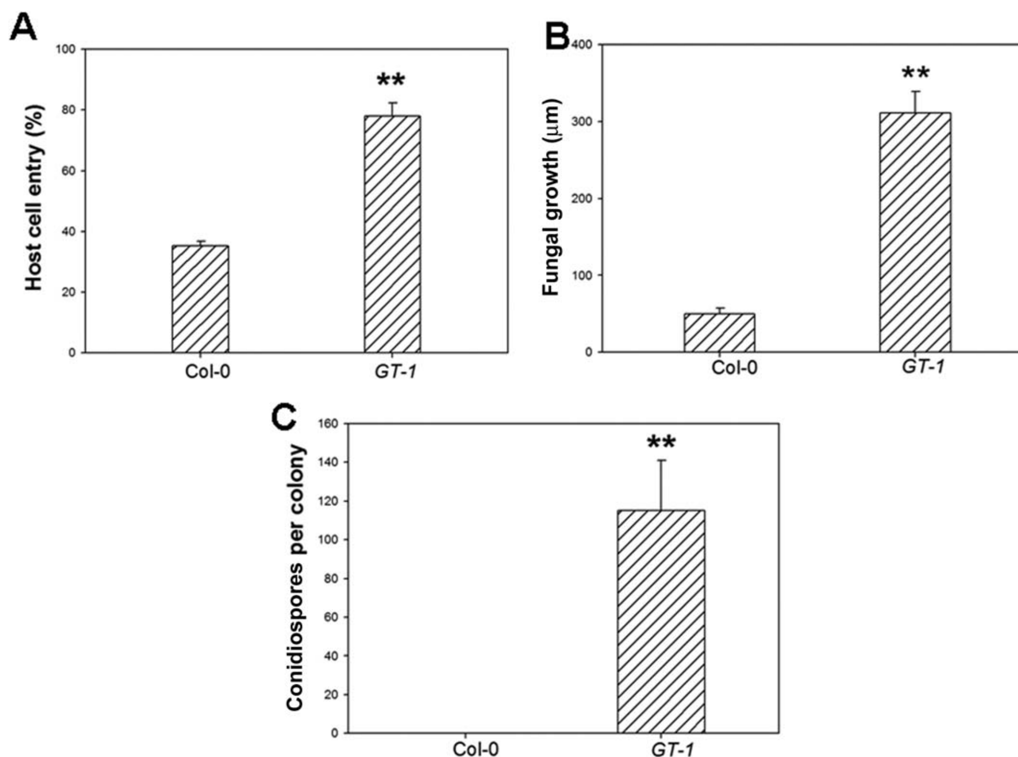

Fig. 2 Quantitative analysis of *Oidium heveae* HN1106 infection on wild-type Col-0 and *Hevea brasiliensis GT-1* leaves. (A) Quantitative assessment of host cell entry rates. Data represent the mean ± standard deviation (SD) of three experiments, each based on five to seven leaves per plant line. **Significant difference from Col-0 (Student's *t*-test, $P < 0.01$). (B) Quantitative analysis of hyphal growth of *O. heveae* HN1106 at 48 h post-inoculation (hpi). Leaves were stained with Coomassie brilliant blue at 48 hpi, and hyphal lengths per colony were measured from photographs using MIE 3.1 software. At least 10 colonies were measured for hyphal length. **Significant difference from Col-0 (Student's *t*-test, $P < 0.01$). (C) The numbers of conidiospores per colony were counted at 10 days post-inoculation (dpi). Data represent the mean ± SD of at least 20 fungal colonies per plant line. **Significant difference from Col-0 (Student's *t*-test, $P < 0.01$). These experiments were repeated twice with similar results.

rar1, but weakly in *eds1* and *pad4* mutants. Taken together, these results indicate that *EDS1*, *PAD4* and *RAR1* are required for *O. heveae* HN1106-triggered resistance responses.

SA is required for resistance to *O. heveae* HN1106 in *Arabidopsis*

The SA-dependent pathway is usually required for R protein-mediated resistance in *Arabidopsis* (Bartsch *et al.*, 2010; Rusterucci *et al.*, 2001). To test whether *O. heveae* HN1106-induced defence responses and pathogen resistance are SA dependent, we inoculated *sid2*, *npr1*, *NahG* and Col-0 plants with *O. heveae* HN1106. Similar to *rar1* mutants, the *sid2*, *npr1* and *NahG* plants displayed higher penetration ratios (Fig. 5A) and fungal growth (Fig. 5B) than did Col-0. By 10 dpi, *O. heveae* HN1106 had induced typical chlorosis symptoms on wild-type Col-0, but no symptoms on *sid2*, *npr1* and *NahG* plants (Fig. 6A). The hyphal networks were slightly denser on *sid2*, *npr1* and *NahG* leaves than on those of the wild-type (Fig. 6B); however, conidiospores were not detected on *sid2*, *npr1* and *NahG* leaves (Fig. 6B), indicating that *O. heveae* HN1106 cannot complete its life cycle on plants with compromised SA signalling. Consistent with the disease symptoms, the levels of cell death (Fig. 6C), H_2O_2 production (Fig. 6D), callose deposition (Fig. 5C) and *PR1* gene expression (Fig. 5D) triggered by *O. heveae* HN1106 were significantly lower in *sid2*, *npr1* and *NahG* plants than in the wild-type. These results indicate that SA is required for the cell death response associated with *O. heveae* HN1106 infection, and contributes to the penetration and hyphal growth resistance against the pathogen.

Callose deposition contributes to penetration resistance to *O. heveae* HN1106 in *Arabidopsis*

Callose deposition has been reported to be involved in penetration resistance in incompatible interactions between *Arabidopsis* and powdery mildews (Nishimura *et al.*, 2003). To test whether callose deposition is required for penetration resistance to *O. heveae* HN1106, the callose synthase mutant *powdery mildew resistant 4* (*pmr4*) and Col-0 were inoculated with *O. heveae* HN1106. At 1 dpi, the ratio of fungal entry was 72% on the *pmr4* mutant

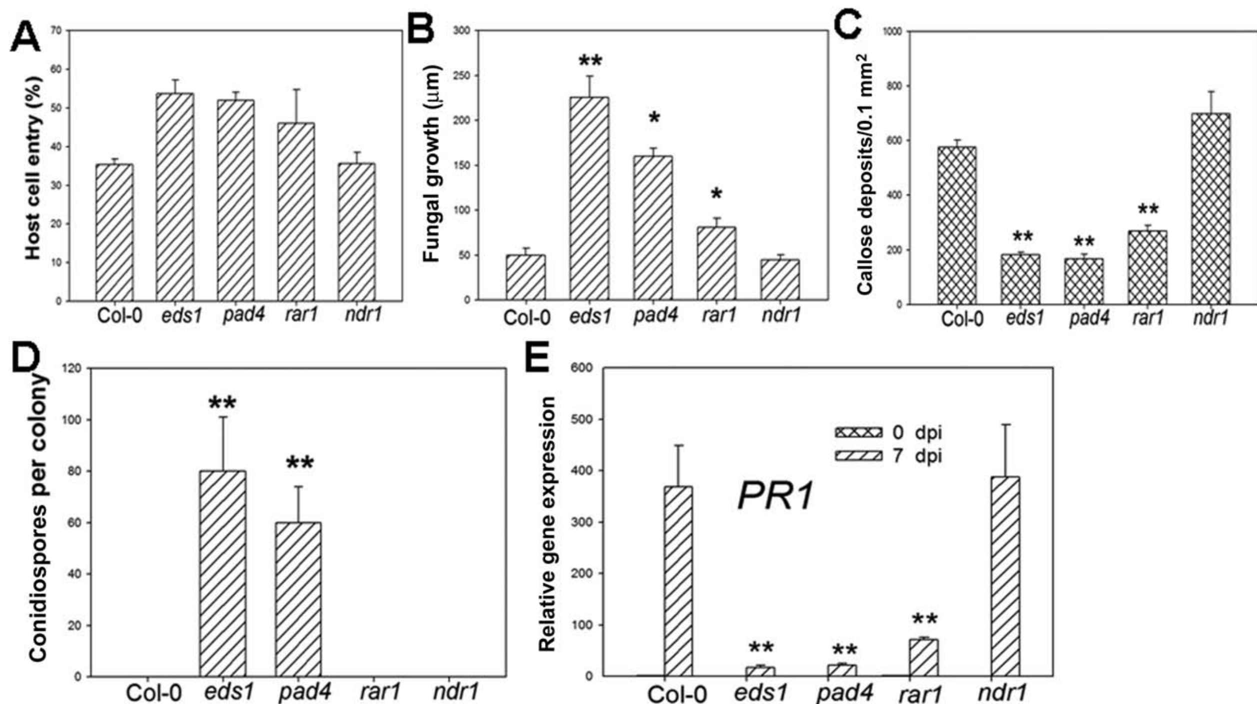

Fig. 3 *EDS1* (*Enhanced Disease Susceptibility 1*) and *PAD4* (*Phytoalexin Deficient 4*) are required for *Oidium heveae* HN1106-triggered disease resistance and defence responses in *Arabidopsis*. Four-week-old *Arabidopsis* wild-type (WT) Col-0 and mutants were inoculated with *O. heveae* HN1106. (A) Quantitative assessment of host cell entry rates. Data represent the mean ± standard deviation (SD) of three experiments, each based on five to seven leaves per plant line. (B) Quantitative analysis of hyphal growth of *O. heveae* HN1106 at 48 h post-inoculation (hpi). Leaves were stained with Coomassie brilliant blue at 48 hpi, and hyphal lengths per colony were measured from photographs using MIE 3.1 software. At least 10 colonies were measured for hyphal length. *Significant difference from Col-0 (Student's *t*-test, $P < 0.05$). **Significant difference from Col-0 (Student's *t*-test, $P < 0.01$). (C) Average numbers of callose deposits per microscope field of 0.1 mm^2 on Col-0 and mutant leaves determined at 7 days post-inoculation (dpi). **Significant difference from Col-0 (Student's *t*-test, $P < 0.01$). (D) The numbers of conidiospores per colony were counted at 10 dpi. Data represent the mean ± SD of at least 20 fungal colonies per plant line. **Significant difference from Col-0 (Student's *t*-test, $P < 0.01$). (E) The abundance of *PR1* (*Pathogenesis-Related 1*) mRNA was determined at the indicated time points by quantitative polymerase chain reaction. Data represent the mean ± SD of three RNA replicates. **Significant difference from Col-0 (Student's *t*-test, $P < 0.01$). These experiments were repeated twice with similar results.

(Fig. 7E), but 35% on Col-0 (Fig. 7E), suggesting that callose deposition contributes to the penetration resistance to *O. heveae* HN1106. By 5 dpi, chlorosis symptoms were present in *pmr4* mutants, but not in Col-0 (Fig. 7A). This could be a result of the increased entry rate of *O. heveae* HN1106 in *pmr4* mutants. Consistent with the phenotypes, *O. heveae* HN1106 triggers a higher level of cell death (Fig. 7B) and *PR* gene expression (Fig. 7C, D) in *pmr4* than in Col-0 at 5 dpi.

The *Arabidopsis* accession Lag2-2 is susceptible to *O. heveae* HN1106

Arabidopsis accessions exhibit natural genetic variation; although the Col-0 accession is resistant to *O. heveae* HN1106, other accessions may be susceptible. To test this hypothesis, a total of 47 *Arabidopsis* accessions obtained from the Nottingham *Arabidopsis* Stock Centre (NASC) and Col-0 as a control were challenged with *O. heveae* HN1106. We found that 37 *Arabidopsis* accessions, including Shigu-2 (Fig. 8A, B) and Col-0 (Fig. 8A, B), were resistant, showing characteristic chlorosis symptoms, cell death, limited fungal growth and the absence of conidiospores (Table 1). By contrast, nine *Arabidopsis* accessions, including HKT2-4 (Fig. 8A, B), displayed reduced disease symptoms, such as a sparse hyphal network, no apparent cell death and no formation of conidiospores (Table 1). Only the Lag2-2 accession showed typical powdery mildew symptoms, as indicated by strong hyphal growth and conidiospore formation (Fig. 8A, B). These results indicate that *O. heveae* HN1106 and Lag2-2 exhibit a compatible interaction.

To further determine whether the susceptibility of Lag2-2 is specific to *O. heveae* strain HN1106, *Arabidopsis* Lag2-2 plants were inoculated with two other *O. heveae* strains, HN1208 and YN7834, isolated in Hainan and YunNan, China, respectively. By 10 dpi, Lag2-2 seemed to be equally susceptible to HN1208 and HN1106 infection (Fig. S4, see Supporting Information). However, the *Arabidopsis* accession was found to be resistant to YN7834, because YN7834 only developed an apparent hyphal network, but

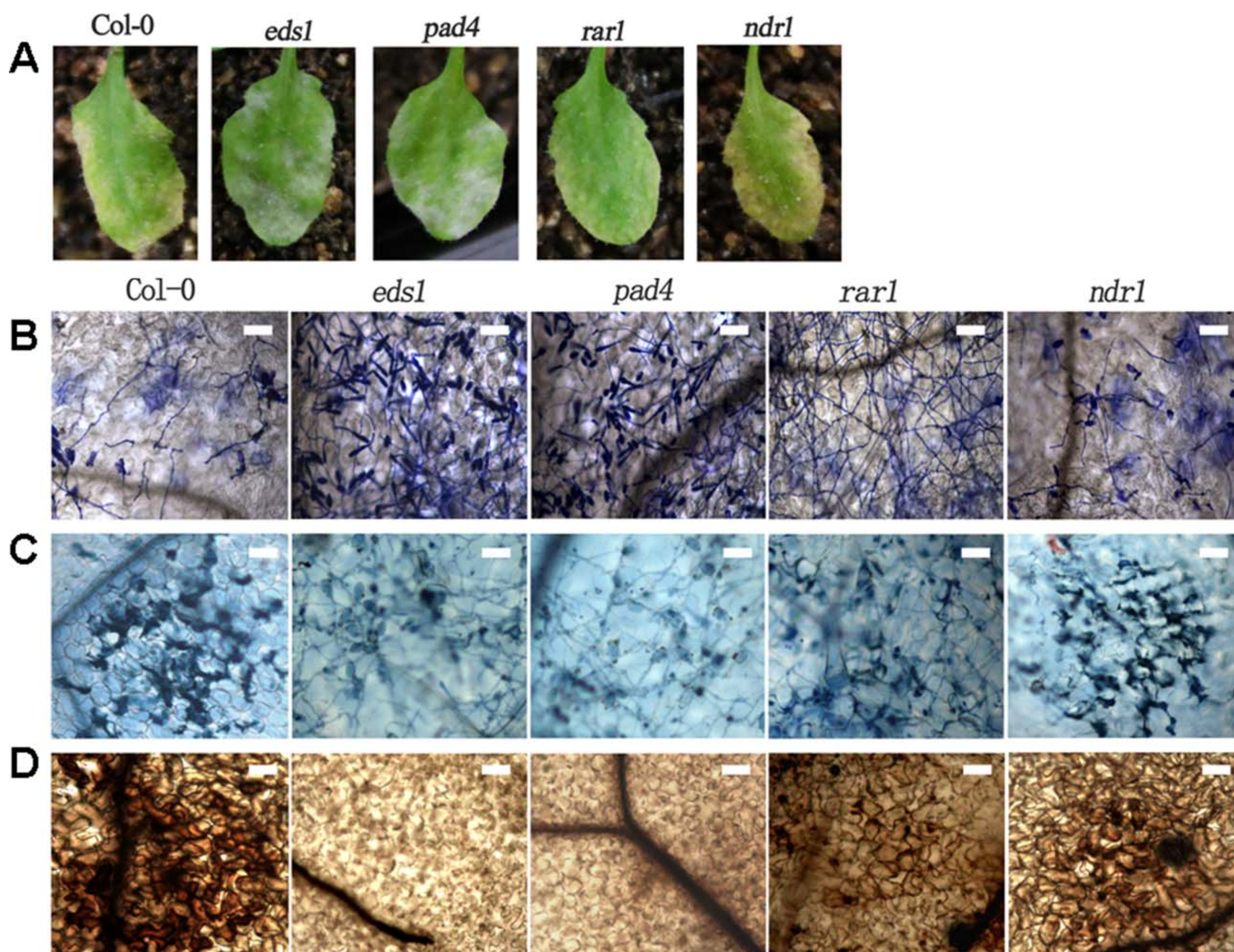

Fig. 4 Symptoms and light micrographs of *Arabidopsis* Col-0 and *eds1* (*enhanced disease susceptibility 1*), *pad4* (*phytoalexin deficient 4*), *rar1* (*required for Mla12 resistance*) and *ndr1* (*non-race-specific disease resistance 1*) mutants infected with *Oidium heveae* HN1106. Four-week-old *Arabidopsis* wild-type (WT) Col-0 and mutants were inoculated with *O. heveae* HN1106. (A) Symptoms were photographed at 10 days post-inoculation (dpi). (B) Light microscopy images were taken after fungal structures had been stained with Coomassie brilliant blue at 10 dpi. Bar, 50 μm. (C) Infected leaves were stained with trypan blue at 10 dpi. Bar, 50 μm. (D) Infected leaves were stained with 3,3′-diaminobenzidine (DAB) at 7 dpi. Bar, 50 μm. These experiments were repeated twice with similar results.

no conidiospores (Fig. S4), suggesting that the susceptibility of Lag2-2 is specific to *O. heveae* strains HN1106 and HN1208.

DISCUSSION

Powdery mildew is an important disease affecting rubber plantations worldwide. However, studies of the molecular mechanism underlying the interaction between *H. brasiliensis* and *O. heveae* have been hampered largely by the lack of genetic background and methodology for these species. The model plant *A. thaliana* has been widely used to investigate the molecular mechanisms underlying powdery mildew disease. Several isolates of powdery mildew, *Golovinomyces cichoracearum* (Adam and Somerville, 1996), *G. cruciferarum* (Koch and Slusarenko, 1990), *G. orontii* (Plotnikova *et al*., 1998) and *Oidium neolycopersici* (Xiao *et al*., 2001), can infect *Arabidopsis*. Microscopy analysis showed that *O. heveae* HN1106 exhibited similar pathogenicity on *H. brasiliensis* and *Arabidopsis eds1* mutants. In addition, mature conidia developed from *Arabidopsis eds1* mutant leaves can successfully infect *H. brasiliensis*, suggesting that studies of the interaction between *Arabidopsis* and *O. heveae* might further our understanding of powdery mildew disease in economically important rubber trees.

The interactions between plants and powdery mildew can be divided into non-host, compatible and incompatible interactions. Non-host resistance usually occurs in plant resistance against some non-adapted powdery mildews. Post-penetration resistance, mediated by EDS1 or PAD4, and penetration resistance, conferred by PENETRATION2 (PEN2) or PEN3, limit invasion, growth and asexual reproduction of the non-adapted powdery mildew

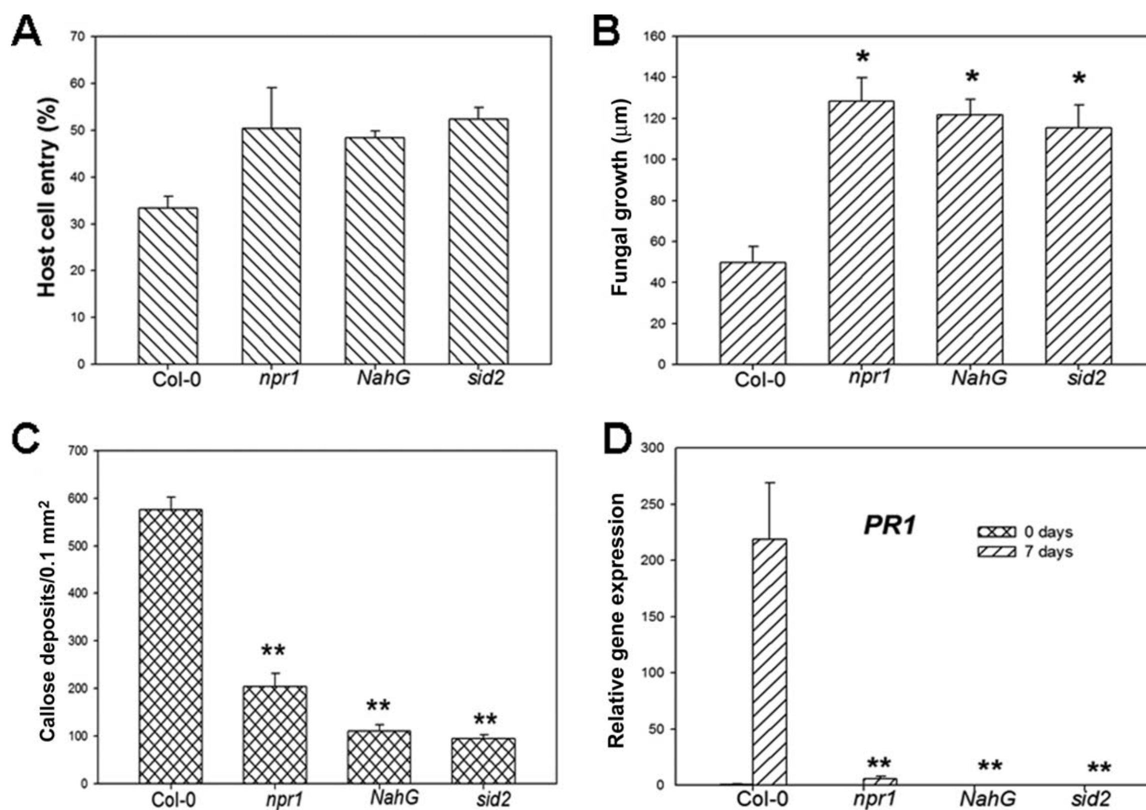

Fig. 5 Salicylic acid (SA) contributes to the penetration and hyphal growth resistance and defence responses against *Oidium heveae* HN1106. Four-week-old *Arabidopsis* wild-type Col-0 and mutants were inoculated with *O. heveae* HN1106. (A) Quantitative assessment of host cell entry rates. Data represent the mean ± standard deviation (SD) of three experiments, each based on five to seven leaves per plant line. (B) Quantitative analysis of hyphal growth of *O. heveae* HN1106 at 48 h post-inoculation (hpi). Leaves were stained with Coomassie brilliant blue at 48 hpi, and hyphal lengths per colony were measured from photographs using MIE 3.1 software. At least 10 colonies were measured for hyphal length. *Significant difference from Col-0 (Student's *t*-test, $P < 0.05$). (C) Average numbers of callose deposits per microscopic field of 0.1 mm^2 on Col-0 and mutant leaves determined at 7 days post-inoculation (dpi). **Significant difference from Col-0 (Student's *t*-test, $P < 0.01$). (D) The abundance of *PR1* (*Pathogenesis-Related 1*) mRNA was determined at the indicated time points by quantitative polymerase chain reaction. Data represent the mean ± SD of three RNA replicates. **Significant difference from Col-0 (Student's *t*-test, $P < 0.01$). These experiments were repeated twice with similar results.

Erysiphe pisi on Col-0 *Arabidopsis* (Stein *et al.*, 2006). In contrast with non-host interactions, penetration resistance conditioned by PEN2 or PEN3 does not appear to be required for compatible and incompatible interactions. In our study, *O. heveae* HN1106 displayed an entry ratio of about 35% on Col-0 and about 55% on the *eds1* mutant, which are significantly higher than that in non-host interactions (10%), suggesting that the interaction between *Arabidopsis* and *O. heveae* HN1106 is not a non-host interaction. Furthermore, *O. heveae* HN1106 can finish its life cycle and produce many conidiospores on *eds1* or *pad4* single mutants, indicating that post-penetration resistance is sufficient to limit infection by *O. heveae* HN1106.

NBS-LRR-type R proteins that respond to powdery mildews have been characterized in many plant species, such as the *Mla* genes in barley (Shen *et al.*, 2007), *Ol* genes in *Solanum lycopersicum* (tomato; Bai *et al.*, 2005), *Run1* in *Vitis vinifera* (grapevine; Barker *et al.*, 2005) and *Rpp1* in *Rosa multiflora* (rose; Linde *et al.*, 2004). *Golovinomyces cruciferarum* is an incompatible pathogen on *Arabidopsis* ecotype Ms-0. However, no canonical powdery mildew *R* genes have been identified in *Arabidopsis* to date. Thus, *Arabidopsis* might not have co-evolved with *G. cruciferarum* long enough to select for canonical *R* genes. The *RPW8* locus of Ms-0 was found to be involved in disease resistance to *G. cruciferarum* (Xiao *et al.*, 2003). RPW8 is an atypical R protein with a transmembrane (TT)-CC domain, and mediates broad-spectrum resistance against powdery mildews through *EDS1*, *PAD4* and SA signalling (Xiao *et al.*, 2001, 2005). Here, we found that *Arabidopsis* Col-0 is a resistant host of *O. heveae* HN1106, and proposed that similar mechanisms might function in *O. heveae* HN1106–Col-0 and *G. cruciferarum*–Ms-0 interactions. Nevertheless, *O. heveae* HN1106 triggers disease resistance in *Arabidopsis* Col-0 in an *EDS1*- and *PAD4*-dependent manner, which is different from the compatible interaction between *G. cruciferarum* and Col-0. Therefore, we speculate that EDS1-mediated ETI signalling, which

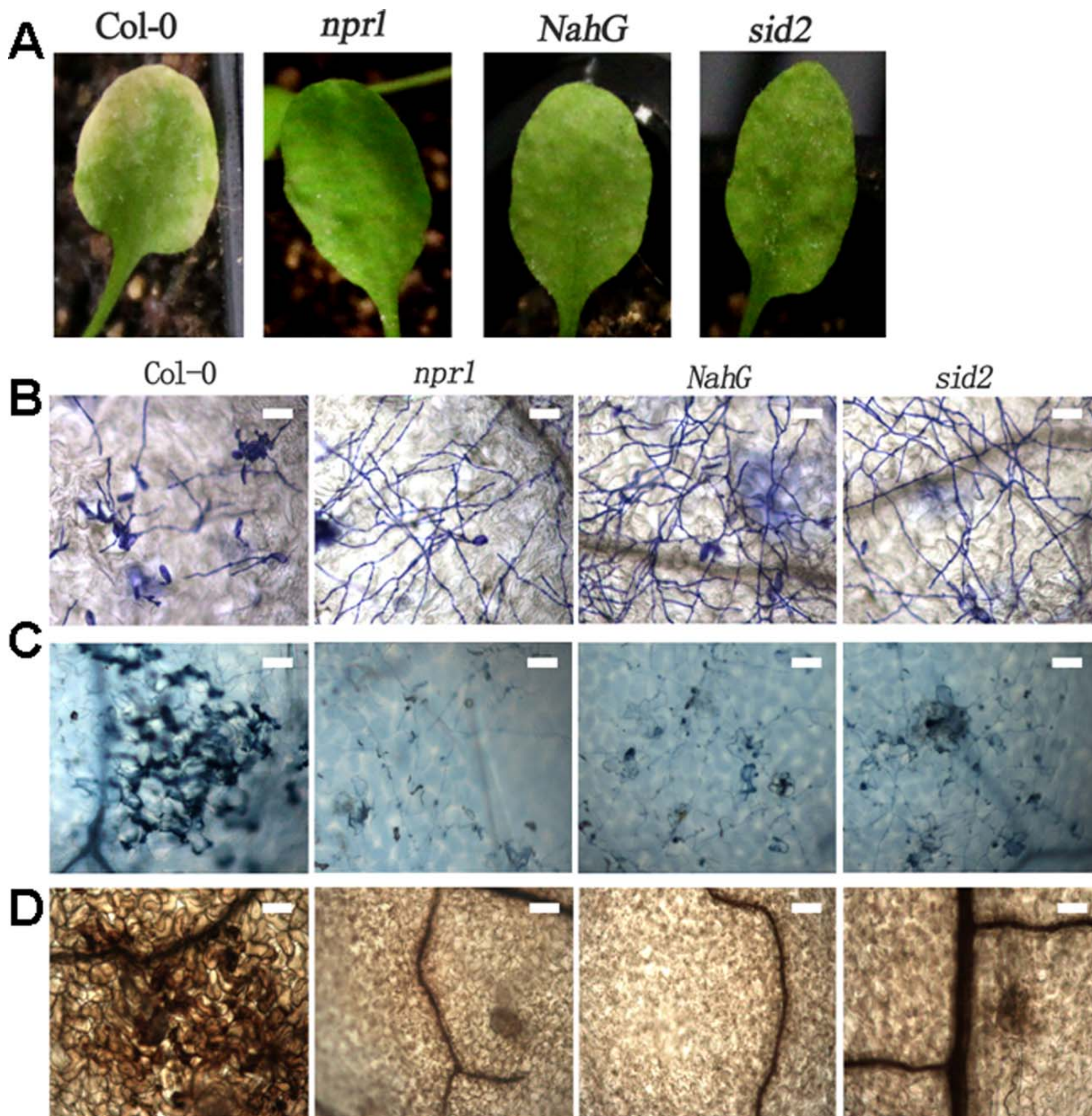

Fig. 6 Salicylic acid (SA) is required for a cell death response against *Oidium heveae* HN1106 on *Arabidopsis*. Symptoms and light micrographs of *Arabidopsis* Col-0 and *npr1* (*nonexpressor of pr1*), *NahG* and *sid2* (*SA induction deficient 2*) mutants infected with *O. heveae* HN1106. Four-week-old *Arabidopsis* wild-type (WT) Col-0 and mutants were inoculated with *O. heveae* HN1106. (A) Symptoms were photographed at 10 days post-inoculation (dpi). (B) Light microscopy images were taken after fungal structures had been stained with Coomassie brilliant blue at 10 dpi. Bar, 50 μm. (C) Infected leaves were stained with trypan blue at 10 dpi. Bar, 50 μm. (D) Infected leaves were stained with 3,3′-diaminobenzidine (DAB) at 7 dpi. Bar, 50 μm. These experiments were repeated twice with similar results.

is usually activated by TIR-NB-LRR proteins, is responsible for *O. heveae*-induced resistance in *Arabidopsis*. If this is indeed the case, canonical TIR-NB-LRR-type *R* gene(s) should be present in Col-0 in response to *O. heveae* infection. However, which TIR-NB-LRR protein(s) confers resistance to *O. heveae* HN1106 remains to be elucidated. CC-NB-LRR-type R proteins are not likely to be involved in resistance to *O. heveae*, because *NDR1* is not required for *O. heveae* HN1106-induced disease resistance and defence responses.

In plant–microbe interactions, the full output of resistance conditioned by TIR-NB-LRR-type R proteins requires both SA-dependent and SA-independent resistance pathways. In addition,

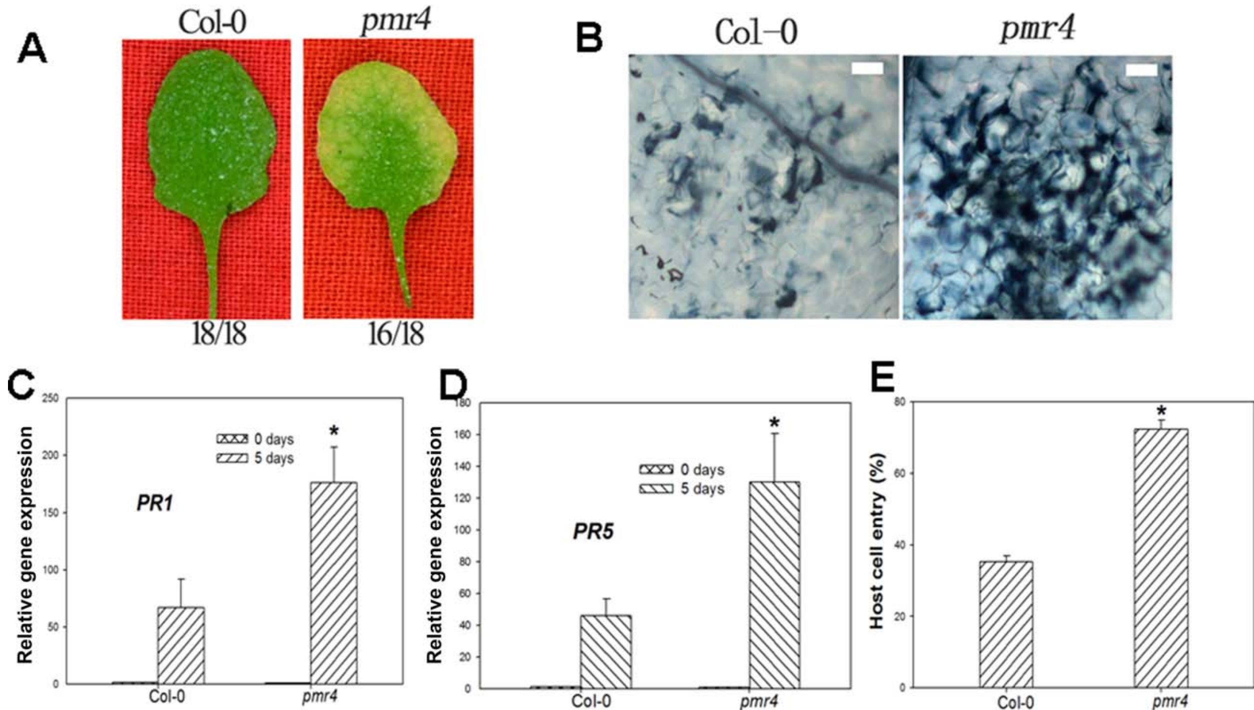

Fig. 7 *PMR4* (*Powdery Mildew Resistant 4*) contributes to the penetration resistance to *Oidium heveae* HN1106 in *Arabidopsis*. Four-week-old plants were inoculated with *O. heveae* HN1106. (A) The symptoms were examined for Col-0 and the *pmr4* mutant at 5 days post-inoculation (dpi). The numbers below each panel indicate the number of leaves surveyed (denominator) and the number of leaves showing the phenotypes (numerator). (B) Infected leaves of Col-0 and the *pmr4* mutant were stained with trypan blue at 5 dpi. Bar, 50 μm. (C) The mRNA abundance of *PR1* (*Pathogenesis-Related 1*) was determined at 0 and 5 dpi. Data represent the mean ± standard deviation (SD) of three RNA replicates. *Significant difference from Col-0 (Student's *t*-test, $P < 0.05$). (D) The mRNA abundance of *PR5* was determined at 0 and 5 dpi. Data represent the mean ± SD of three RNA replicates. *Significant difference from Col-0 (Student's *t*-test, $P < 0.05$). (E) Quantitative assessment of host cell entry rates. Data represent the mean ± SD of three experiments, each based on five to seven leaves per plant line. *Significant difference from Col-0 (Student's *t*-test, $P < 0.05$). These experiments were repeated twice with similar results.

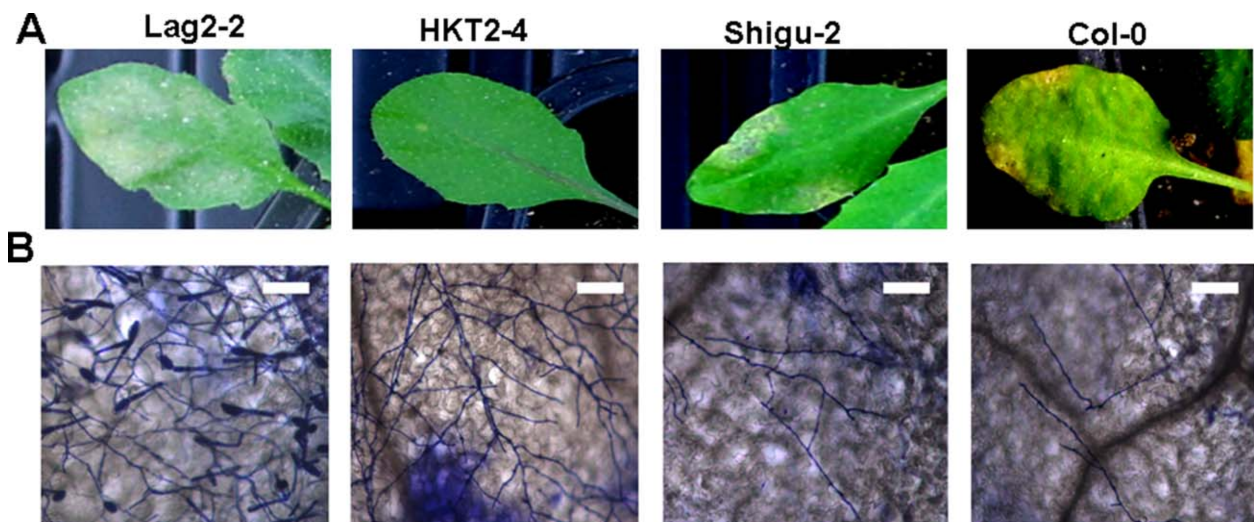

Fig. 8 Ecotype Lag2-2 is susceptible to *Oidium heveae* HN1106. Leaves from 4-week-old plants were inoculated with *O. heveae* HN1106. (A) The symptoms of *Arabidopsis* accessions Lag2-2, HKT-2, Shigu-2 and Col-0 infected with *O. heveae* HN1106 at 10 days post-inoculation (dpi). (B) Light micrographs of *O. heveae* growth in the Lag2-2, HKT-2, Shigu-2 and Col-0 ecotypes at 10 dpi. Light microscopy images were taken after visualization of the fungal structures using Coomassie brilliant blue at 10 dpi. Bar, 50 μm. These experiments were repeated twice with similar results.

Table 1 Infection phenotypes of 47 *Arabidopsis thaliana* accessions on challenge with *Oidium heveae* HN1106.

Accession	NASC code	Chlorosis symptom	Cell death	Hyphal network	Conidiospores
Altenb-2	N76353	+	+	–	–
Apost-1	N76368	+	+	–	–
Nemrut-1	N76398	+	+	–	–
Xan-1	N76387	+	+	–	–
Shigu-2	N76374	+	+	–	–
Slavi-1	N76419	+	+	–	–
Ped-0	N76415	+	+	–	–
Sha	N76382	+	+	–	–
Petro-1	N76370	+	+	–	–
Bak-2	N76392	+	+	–	–
Don-0	N76411	+	+	–	–
Lerik1-3	N76388	+	+	–	–
Nie1-2	N76402	+	+	–	–
Vie-0	N76418	+	+	–	–
Lag2-2	N76390	–	–	+	+
Tu-Scha-9	N76401	+	+	–	–
Kidr-1	N76376	+	+	–	–
Vash-1	N76391	+	+	–	–
Sij1	N76379	+	+	–	–
Dobra-1	N76369	+	+	–	–
Angel-1	N76362	+	+	–	–
Lago-1	N76367	–	–	+	–
Star-8	N76400	–	–	+	–
Agu-1	N76409	+	+	–	–
Moran-1	N76363	+	+	–	–
Yeg-1	N76394	+	+	–	–
Rovero-1	N76351	+	+	–	–
Kly1	N76385	+	+	–	–
Castelfed-4	N76355	+	+	–	–
Shigu-1	N76375	+	+	–	–
Tu-Wa1-2	N76405	+	+	–	–
Bak-7	N76393	+	+	–	–
Vezzano-2	N76349	–	–	+	–
Voeran-1	N76352	–	–	+	–
Pra-6	N76416	+	+	–	–
Istisu-1	N76389	+	+	–	–
Bolin-1	N76373	+	+	–	–
Lecho-1	N76371	–	–	+	–
Da(1)-12	N76470	–	–	+	–
Qui-0	N76417	+	+	–	–
Monte-1	N76361	–	–	+	–
Ciste-1	N76359	–	–	+	–
HKT2-4	N76404	–	–	+	–
Ler-0	N77020	+	+	–	–
Kas-1	N22636	+	+	–	–
Nossen	N3081	+	+	–	–
Ws-0	N76303	+	+	–	–

NASC, Nottingham *Arabidopsis* Stock Centre.
+, chlorosis symptom or cell death similar to Col-0, conidiospores formation and hyphal network formation. –, no chlorosis symptom, cell death, conidiospores or hyphal network formation.

both need the signalling nodes of EDS1 and PAD4. For example, the EDS1- and PAD4-mediated *Flavin-Dependent Monooxygenase 1* (*FMO 1*) signalling pathway, which is activated by the TIR-NB-LRR-type R proteins RPS4 and RPP2, represents an SA-independent resistance pathway (Bartsch *et al.*, 2006). In this study, although *Arabidopsis* lost the ability to trigger the cell death response to *O. heveae* HN1106 when SA signalling was disrupted, a modest resistance was still present, suggesting that SA-independent pathways, together with SA signalling, are required for full resistance to *O. heveae* HN1106 in *Arabidopsis*. However, whether FMO 1 signalling is involved in resistance to *O. heveae* HN1106 remains to be determined.

In incompatible interactions between *Arabidopsis* and some powdery mildews, callose deposition is fully responsible for penetration resistance. However, the *pmr4* mutant exhibited increased resistance to compatible powdery mildews, and this was caused by the hyperactivation of SA-mediated defence responses in the *pmr4* mutant (Nishimura *et al.*, 2003). In this study, we found that *O. heveae* HN1106 exhibits an incompatible interaction with *Arabidopsis*, and induces chlorosis symptoms and defence responses more rapidly in *pmr4* mutants than in Col-0, presumably because of the significantly higher host cell entry rate in *pmr4*. ETI is often accompanied by callose deposition at infection sites, and callose synthesis is significantly compromised in mutants in which ETI is disrupted. Here, we found that *eds1*, *pad4*, *rar1*, *npr1*, *NahG* and *sid2* mutants displayed considerably less callose deposition, which underlies the higher penetration ratio of *O. heveae* HN1106 in these mutants.

Arabidopsis Col-0 is the resistant host for *O. heveae* HN1106. Thus, we postulated that a susceptible ecotype may exist in natural *Arabidopsis* accessions. We screened 47 *Arabidopsis* accessions for resistance to *O. heveae* HN1106 and identified significant genetic diversity. Most accessions (46 of 47) were resistant to *O. heveae* HN1106, and did not support conidiospore formation. Among the 46 resistant accessions, 37 accessions showed chlorotic/necrotic flecks with collapsed hyphae at inoculation sites. Another nine resistant accessions displayed observable hyphal networks with no apparent cell death, which is consistent with a previous study, which showed that powdery mildew resistance is not always associated with a rapid HR (Adam and Somerville, 1996), indicating that resistance mechanisms other than rapid necrosis arrest pathogen development. Only one accession, Lag2-2, was susceptible to *O. heveae* HN1106. Lag2-2 was further found to be susceptible to HN1208, but resistant to *O. heveae* YN7834. We propose that the R proteins involved in *O. heveae* HN1106 and HN1208 resistance may have been lost or may display sequence divergence from their counterparts in other accessions. Furthermore, we predict that the effector proteins secreted by *O. heveae* YN7834 are also probably different from those secreted by *O. heveae* HN1106 and HN1208.

EXPERIMENTAL PROCEDURES

Arabidopsis and mutants

A full list of *A. thaliana* accessions used in this study is provided in Table 1. The common laboratory *Arabidopsis* plants used in this study included

the wild-type (Col-0) and the following mutants: *eds1-2* (backcrossed multiple times into the Col-0 background) (Aarts *et al.*, 1998), *pad4-1* (Glazebrook *et al.*, 1997), *rar1-20* (Tornero *et al.*, 2002), *ndr1-1* (Century *et al.*, 1997), *sid2-2* (Wildermuth *et al.*, 2001), *NahG* (Lawton *et al.*, 1995), *npr1-1* (Cao *et al.*, 1997) and *pmr4-1* (Nishimura *et al.*, 2003).

Powdery mildew infections

The *O. heveae* strain HN1106 was used throughout this study and was maintained in the leaves of the susceptible *H. brasiliensis* clone *GT-1*. Actively growing fungal spores (at 12–15 dpi) were used as a source of inoculum. To ensure an even inoculation density, the plants were placed under a modified settling tower (diameter, 40 cm; height, 60 cm), which was covered with a nylon mesh. Conidia from three to five infected *GT-1* leaves were dusted on top of the tower, and the inoculated plants were kept under the settling tower for 1 h before being moved to the growth chamber (Adam and Somerville, 1996).

Phylogenetic tree creation

A phylogenetic tree was created based on 11 powdery mildew ITS sequences using MEGA 5.0. The analysis preferences were as follows: test of phylogeny, bootstrap method; number of bootstrap replications, 500; number of initial trees (random addition), 2; Mp search level, 1; and maximum number of trees retained, 100.

Host cell entry

Three inoculated leaves were harvested at 1 dpi and stained with Coomassie brilliant blue. The proportion of germinated fungal sporelings that developed secondary hyphae was assessed (minimum of 50 germinated sporelings/leaf evaluated). Fungal penetration success on each plant was quantified in at least three independent experiments.

Analysis of fungal growth

Three different plants per line were inoculated with a low density of powdery mildew spores (to avoid overlap of fungal colonies) and four leaves per plant were harvested at 48 hpi. At least 20 images of single colonies per line and time point were taken and analysed with MIE 3.1 software (http://www.miesoftware.com/).

Quantification of conidiospores per colony

Eight inoculated leaves were harvested at 10 dpi and stained with Coomassie brilliant blue. Conidiospores were counted. This procedure was repeated two to four times.

DNA extraction and polymerase chain reaction (PCR) amplification

DNA was extracted from fungal spores and hyphae with 2 × CTAB (hexadecyltrimethylammonium bromide) buffer (Martin and Rygiewicz, 2005). The nuclear rDNA ITS region (644 bp), which included the 31-bp fragment at the 3′ end of the 18S (small subunit) rRNA gene, 220-bp fragment in the first ITS (ITS1), 154-bp fragment in the complete 5.8S rRNA gene, 183-bp fragment in the second ITS (ITS2) and 56-bp fragment at the 5′ end of the 28S (large subunit) rRNA gene, was amplified using the primers ITS1 (5′-TCCGTAGGTGAACCTGCGG-3′) and ITS4 (5′-TCCTCCGCTTA TTGATATGC-3′).

Callose deposition assay

The leaves of 4-week-old *Arabidopsis* plants were inoculated with *O. heveae* HN1106. Leaves were harvested at 7 dpi, cleared, stained with aniline blue (Hauck *et al.*, 2003) and mounted in 50% glycerol. The leaves were examined with a fluorescence microscope under ultraviolet light. The number of callose deposits per microscopic field of 0.1 mm^2 was calculated from six leaves using Image J software (http://www.uhnresearch.ca/wcif).

RNA isolation and real-time reverse transcription (RT)-PCR

The leaves of 4-week-old *Arabidopsis* plants were inoculated with *O. heveae* HN1106, and RNA was isolated at the indicated time points. Three to five micrograms of RNA were used for cDNA synthesis. mRNA was quantified by real-time RT-PCR using a SYBR Premix Ex Taq Kit (TaKaRa, Changping, Beijing, China). *Arabidopsis ACTIN* was used as the reference gene. Primers 5′-TACGCAGAACAACTAAGAGG-3′ and 5′-TCGTT CACATAATTCCCACG-3′ were used to amplify *PR1*, and primers 5′-TGGTG GAAGCACAGAAGTTG-3′ and 5′-GATCCATGTTTGGCTCCTTC-3′ were used to amplify *ACTIN*. Primers 5′-GCACAGAGACACACACAAAA-3′ and 5′-TGT TCCTTAGAGTGAAGTCTG-3′ were used to amplify *PR5*. The RT-PCR conditions were as follows: 95°C for 2 min, followed by 40 cycles of 95°C for 15 s, 55°C for 15 s and 72°C for 25 s. The expression level was normalized to that of the *ACTIN* control, and relative expression values were determined against uninfected samples or wild-type Col-0 using the comparative C_t method.

Trypan blue staining

The leaves of 4-week-old *Arabidopsis* plants were inoculated with *O. heveae* HN1106, and leaves were harvested at 10 dpi. Fungal structures and dead plant cells were stained with trypan blue and cleared with chloral hydrate overnight at room temperature (Frye and Innes, 1998). Cleared leaves were mounted under coverslips in 50% glycerol and examined with a microscope.

H_2O_2 production assay

The leaves of 4-week-old *Arabidopsis* plants were inoculated with *O. heveae* HN1106, and leaves were collected at 7 dpi. H_2O_2 was detected by staining with 3,3′-diaminobenzidine-HCl, pH 3.8, for 8 h, followed by clearing in 95% ethanol overnight at room temperature (Xiao *et al.*, 2003). Cleared leaves were mounted under coverslips in 50% glycerol and examined with a microscope.

Coomassie brilliant blue staining

Infected leaves were fixed and cleared in an ethanol solution containing 6.7% phenol, 6.7% lactic acid, 13.3% glycerol and 6.7% H_2O. Fungal structures in cleared leaves were visualized by staining with an ethanolic solution containing 0.6% Coomassie brilliant blue (Lipka *et al.*, 2005).

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REFERENCES

Aarts, N., Metz, M., Holub, E., Staskawicz, B.J., Daniels, M.J. and Parker, J.E. (1998) Different requirements for EDS1 and NDR1 by disease resistance genes define at least two R gene-mediated signaling pathways in *Arabidopsis*. *Proc. Natl. Acad. Sci. USA*, **95**, 10 306–10 311.

Adam, L. and Somerville, S.C. (1996) Genetic characterization of five powdery mildew disease resistance loci in *Arabidopsis thaliana*. *Plant J.* **9**, 341–356.

Bai, Y., van der Hulst, R., Bonnema, G., Marcel, T.C., Meijer-Dekens, F., Niks, R.E. and Lindhout, P. (2005) Tomato defense to *Oidium neolycopersici*: dominant Ol genes confer isolate-dependent resistance via a different mechanism than recessive ol-2. *Mol. Plant–Microbe Interact.* **18**, 354–362.

Barker, C.L., Donald, T., Pauquet, J., Ratnaparkhe, M.B., Bouquet, A., Adam-Blondon, A.F., Thomas, M.R. and Dry, I. (2005) Genetic and physical mapping of the grapevine powdery mildew resistance gene, Run1, using a bacterial artificial chromosome library. *Theoretical and applied genetics*, **111**, 370–377.

Bartsch, M., Gobbato, E., Bednarek, P., Debey, S., Schultze, J.L., Bautor, J. and Parker, J.E. (2006) Salicylic acid-independent ENHANCED DISEASE SUSCEPTIBILITY1 signaling in *Arabidopsis* immunity and cell death is regulated by the monooxygenase FMO1 and the Nudix hydrolase NUDT7. *Plant Cell*, **18**, 1038–1051.

Bartsch, M., Bednarek, P., Vivancos, P.D., Schneider, B., von Roepenack-Lahaye, E., Foyer, C.H., Kombrink, E., Scheel, D. and Parker, J.E. (2010) Accumulation of isochorismate-derived 2,3-dihydroxybenzoic 3-O-beta-D-xyloside in *Arabidopsis* resistance to pathogens and ageing of leaves. *J. Biol. Chem.* **285**, 25 654–25 665.

Beeley, F. (1933) *Oidium heveae*: report on the 1933 outbreak of *Hevea* leaf mildew. *J. Rubber Res. Inst. Malaysia*, **5**, 5–13.

Boller, T. and He, S.Y. (2009) Innate immunity in plants: an arms race between pattern recognition receptors in plants and effectors in microbial pathogens. *Science*, **324**, 742–744.

Cao, H., Glazebrook, J., Clarke, J.D., Volko, S. and Dong, X. (1997) The *Arabidopsis* NPR1 gene that controls systemic acquired resistance encodes a novel protein containing ankyrin repeats. *Cell*, **88**, 57–63.

Catanzariti, A.M., Dodds, P.N. and Ellis, J.G. (2007) Avirulence proteins from haustoria-forming pathogens. *FEMS Microbiol. Lett.* **269**, 181–188.

Century, K.S., Shapiro, A.D., Repetti, P.P., Dahlbeck, D., Holub, E. and Staskawicz, B.J. (1997) NDR1, a pathogen-induced component required for *Arabidopsis* disease resistance. *Science*, **278**, 1963–1965.

Consonni, C., Humphry, M.E., Hartmann, H.A., Livaja, M., Durner, J., Westphal, L., Vogel, J., Lipka, V., Kemmerling, B., Schulze-Lefert, P., Somerville, S.C. and Panstruga, R. (2006) Conserved requirement for a plant host cell protein in powdery mildew pathogenesis. *Nat. Genet.* **38**, 716–720.

Cui, H., Tsuda, K. and Parker, J.E. (2015) Effector-triggered immunity: from pathogen perception to robust defense. *Annu. Rev. Plant Biol.* **66**, 487–511.

Day, B., Dahlbeck, D. and Staskawicz, B.J. (2006) NDR1 interaction with RIN4 mediates the differential activation of multiple disease resistance pathways in *Arabidopsis*. *Plant Cell*, **18**, 2782–2791.

Delaney, T.P., Uknes, S., Vernooij, B., Friedrich, L., Weymann, K., Negrotto, D., Gaffney, T., Gut-Rella, M., Kessmann, H., Ward, E. and Ryals, J. (1994) A central role of salicylic acid in plant disease resistance. *Science*, **266**, 1247–1250.

Falk, A., Feys, B.J., Frost, L.N., Jones, J.D., Daniels, M.J. and Parker, J.E. (1999) EDS1, an essential component of R gene-mediated disease resistance in *Arabidopsis* has homology to eukaryotic lipases. *Proc. Natl. Acad. Sci. USA*, **96**, 3292–3297.

Feys, B.J., Moisan, L.J., Newman, M.A. and Parker, J.E. (2001) Direct interaction between the *Arabidopsis* disease resistance signaling proteins, EDS1 and PAD4. *EMBO J.* **20**, 5400–5411.

Feys, B.J., Wiermer, M., Bhat, R.A., Moisan, L.J., Medina-Escobar, N., Neu, C., Cabral, A. and Parker, J.E. (2005) *Arabidopsis* SENESCENCE-ASSOCIATED GENE101 stabilizes and signals within an ENHANCED DISEASE SUSCEPTIBILITY1 complex in plant innate immunity. *Plant Cell*, **17**, 2601–2613.

Frye, C.A. and Innes, R.W. (1998) An *Arabidopsis* mutant with enhanced resistance to powdery mildew. *Plant Cell*, **10**, 947–956.

Gaffney, T., Friedrich, L., Vernooij, B., Negrotto, D., Nye, G., Uknes, S., Ward, E., Kessmann, H. and Ryals, J. (1993) Requirement of salicylic acid for the induction of systemic acquired resistance. *Science*, **261**, 754–756.

Glazebrook, J., Zook, M., Mert, F., Kagan, I., Rogers, E.E., Crute, I.R., Holub, E.B., Hammerschmidt, R. and Ausubel, F.M. (1997) Phytoalexin-deficient mutants of *Arabidopsis* reveal that PAD4 encodes a regulatory factor and that four PAD genes contribute to downy mildew resistance. *Genetics*, **146**, 381–392.

Gollner, K., Schweizer, P., Bai, Y. and Panstruga, R. (2008) Natural genetic resources of *Arabidopsis thaliana* reveal a high prevalence and unexpected phenotypic plasticity of RPW8-mediated powdery mildew resistance. *New Phytol.* **177**, 725–742.

Hammond-Kosack, K.E. and Parker, J.E. (2003) Deciphering plant–pathogen communication: fresh perspectives for molecular resistance breeding. *Curr. Opin. Biotechnol.* **14**, 177–193.

Hauck, P., Thilmony, R. and He, S.Y. (2003) A *Pseudomonas syringae* type III effector suppresses cell wall-based extracellular defense in susceptible *Arabidopsis* plants. *Proc. Natl. Acad. Sci. USA*, **100**, 8577–8582.

Hubert, D.A., He, Y., McNulty, B.C., Tornero, P. and Dangl, J.L. (2009) Specific *Arabidopsis* HSP90.2 alleles recapitulate RAR1 cochaperone function in plant NB-LRR disease resistance protein regulation. *Proc. Natl. Acad. Sci. USA*, **106**, 9556–9563.

Jirage, D., Tootle, T.L., Reuber, T.L., Frost, L.N., Feys, B.J., Parker, J.E., Ausubel, F.M. and Glazebrook, J. (1999) *Arabidopsis thaliana* PAD4 encodes a lipase-like gene that is important for salicylic acid signaling. *Proc. Natl. Acad. Sci. USA*, **96**, 13 583–13 588.

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

Koch, E. and Slusarenko, A.J. (1990) Fungal pathogens of *Arabidopsis thaliana* (L.) Heynh. *Bot Helv*, **100**, 257–268.

Lawton, K., Weymann, K., Friedrich, L., Vernooij, B., Uknes, S. and Ryals, J. (1995) Systemic acquired resistance in *Arabidopsis* requires salicylic acid but not ethylene. *Mol. Plant–Microbe Interact.* **8**, 863–870.

Linde, M., Mattiesch, L. and Debener, T. (2004) Rpp1, a dominant gene providing race-specific resistance to rose powdery mildew (*Podosphaera pannosa*): molecular mapping, SCAR development and confirmation of disease resistance data. *Theor. Appl. Genet. (Theoretische und angewandte Genetik)* **109**, 1261–1266.

Lipka, V., Dittgen, J., Bednarek, P., Bhat, R., Wiermer, M., Stein, M., Landtag, J., Brandt, W., Rosahl, S., Scheel, D., Llorente, F., Molina, A., Parker, J., Somerville, S. and Schulze-Lefert, P. (2005) Pre- and postinvasion defenses both contribute to nonhost resistance in *Arabidopsis*. *Science*, **310**, 1180–1183.

Martin, K.J. and Rygiewicz, P.T. (2005) Fungal-specific PCR primers developed for analysis of the ITS region of environmental DNA extracts. *BMC Microbiol.* **5**, 28.

McDowell, J.M. and Dangl, J.L. (2000) Signal transduction in the plant immune response. *Trends Biochem. Sci.* **25**, 79–82.

Mitra, M. and Mehta, P.R. (1938) Some leaf diseases of *Hevea brasiliensis* new to India. *Indian J. Agric. Sci.* **8**, 185–188.

Moreau, M., Degrave, A., Vedel, R., Bitton, F., Patrit, O., Renou, J.P., Barny, M.A. and Fagard, M. (2012) EDS1 contributes to nonhost resistance of *Arabidopsis thaliana* against *Erwinia amylovora*. *Mol. Plant–Microbe Interact.* **25**, 421–430.

Nawrath, C., Heck, S., Parinthawong, N. and Metraux, J.P. (2002) EDS5, an essential component of salicylic acid-dependent signaling for disease resistance in *Arabidopsis*, is a member of the MATE transporter family. *Plant Cell*, **14**, 275–286.

Nishimura, M.T., Stein, M., Hou, B.H., Vogel, J.P., Edwards, H. and Somerville, S.C. (2003) Loss of a callose synthase results in salicylic acid-dependent disease resistance. *Science*, **301**, 969–972.

Parker, J.E., Holub, E.B., Frost, L.N., Falk, A., Gunn, N.D. and Daniels, M.J. (1996) Characterization of eds1, a mutation in *Arabidopsis* suppressing resistance to *Peronospora parasitica* specified by several different RPP genes. *Plant Cell*, **8**, 2033–2046.

Plotnikova, J.M., Reuber, T.L. and Ausubel, F.M. (1998) Powdery mildew pathogenesis of *Arabidopsis thaliana*. *Mycologia*, **90**, 1009–1016.

Ramonell, K., Berrocal-Lobo, M., Koh, S., Wan, J., Edwards, H., Stacey, G. and Somerville, S. 2005. Loss-of-function mutations in chitin responsive genes show increased susceptibility to the powdery mildew pathogen *Erysiphe cichoracearum*. *Plant Physiol.* **138**, 1027–1036.

Ridout, C.J., Skamnioti, P., Porritt, O., Sacristan, S., Jones, J.D. and Brown, J.K. 2006. Multiple avirulence paralogues in cereal powdery mildew fungi may contribute to parasite fitness and defeat of plant resistance. *Plant Cell*, **18**, 2402–2414.

Rietz, S., Stamm, A., Malonek, S., Wagner, S., Becker, D., Medina-Escobar, N., Vlot, A.C., Feys, B.J., Niefind, K. and Parker, J.E. 2011. Different roles of Enhanced Disease Susceptibility1 (EDS1) bound to and dissociated from Phytoalexin Deficient4 (PAD4) in *Arabidopsis* immunity. *New Phytol.* **191**, 107–119.

Rusterucci, C., Aviv, D.H., Holt, B.F., 3rd, Dangl, J.L. and Parker, J.E. 2001. The disease resistance signaling components EDS1 and PAD4 are essential regulators of the cell death pathway controlled by LSD1 in *Arabidopsis*. *Plant Cell*, **13**, 2211–2224.

Saranya, L., Sawanee, K., Edson, L., Kon, W., Baharuddin, S., Yukio, S. and T., S. 2005. Molecular phylogenetic and morphological analyses of *Oidium heveae*, a powdery mildew of rubber tree. *J. Mol. Evol.* **46**, 220–226.

Shen, Q.H., Saijo, Y., Mauch, S., Biskup, C., Bieri, S., Keller, B., Seki, H., Ulker, B., Somssich, I.E. and Schulze-Lefert, P. 2007. Nuclear activity of MLA immune receptors links isolate-specific and basal disease-resistance responses. *Science*, **315**, 1098–1103.

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

Tornero, P., Merritt, P., Sadanandom, A., Shirasu, K., Innes, R.W. and Dangl, J.L. 2002. RAR1 and NDR1 contribute quantitatively to disease resistance in *Arabidopsis*, and their relative contributions are dependent on the R gene assayed. *Plant Cell*, **14**, 1005–1015.

Venugopal, S.C., Jeong, R.D., Mandal, M.K., Zhu, S., Chandra-Shekara, A.C., Xia, Y., Hersh, M., Stromberg, A.J., Navarre, D., Kachroo, A. and Kachroo, P. 2009. Enhanced disease susceptibility 1 and salicylic acid act redundantly to regulate resistance gene-mediated signaling. *PLoS Genetics*, **5**, e1000545.

Wagner, S., Stuttmann, J., Rietz, S., Guerois, R., Brunstein, E., Bautor, J., Niefind, K. and Parker, J.E. 2013. Structural basis for signaling by exclusive EDS1 heteromeric complexes with SAG101 or PAD4 in plant innate immunity. *Cell Host Microbe*, **14**, 619–630.

Ward, E.R., Uknes, S.J., Williams, S.C., Dincher, S.S., Wiederhold, D.L., Alexander, D.C., Ahl-Goy, P., Metraux, J.P. and Ryals, J.A. 1991. Coordinate gene activity in response to agents that induce systemic acquired resistance. *Plant Cell*, **3**, 1085–1094.

Wildermuth, M.C., Dewdney, J., Wu, G. and Ausubel, F.M. 2001. Isochorismate synthase is required to synthesize salicylic acid for plant defence. *Nature*, **414**, 562–565.

Xiao, S., Ellwood, S., Calis, O., Patrick, E., Li, T., Coleman, M. and Turner, J.G. 2001. Broad-spectrum mildew resistance in *Arabidopsis* thaliana mediated by RPW8. *Science*, **291**, 118–120.

Xiao, S., Brown, S., Patrick, E., Brearley, C. and Turner, J.G. 2003. Enhanced transcription of the *Arabidopsis* disease resistance genes RPW8.1 and RPW8.2 via a salicylic acid-dependent amplification circuit is required for hypersensitive cell death. *Plant Cell*, **15**, 33–45.

Xiao, S., Calis, O., Patrick, E., Zhang, G., Charoenwattana, P., Muskett, P., Parker, J.E. and Turner, J.G. 2005. The atypical resistance gene, RPW8, recruits components of basal defence for powdery mildew resistance in *Arabidopsis*. *Plant J. Cell Mol. Biol.* **42**, 95–110.

Zhou, J.M. and Chai, J. 2008. Plant pathogenic bacterial type III effectors subdue host responses. *Curr. Opin. Microbiol.* **11**, 179–185.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's website:

Fig. S1 The internal transcribed spacer (ITS) sequence of *Oidium heveae* HN1106.

Fig. S2 Phylogenetic analysis of the *Oidium heveae* HN1106 internal transcribed spacer (ITS) sequence. A phylogenetic tree was created based on 11 powdery mildew ITS sequences using MEGA 5.0. The phylogeny reconstruction used the maximum parsimony method. The percentage bootstrap support (500 replications) is shown above the branches.

Fig. S3 Symptoms of *Hevea brasiliensis GT-1* infection with mature conidia on an *Arabidopsis eds1* mutant. Light-green phase *H. brasiliensis GT-1* leaves were inoculated with mature conidia from *Arabidopsis eds1* (*enhanced disease susceptibility 1*) mutants, and symptoms were examined at 15 days post-inoculation (dpi). These experiments were repeated twice with similar results.

Fig. S4 Ecotype Lag2-2 is resistant to *Oidium heveae* YN7834. Leaves of 4-week-old plants were inoculated with *O. heveae* strains HN1106, HN1208, and YN7834. (A) The plants were photographed at 10 days post-inoculation (dpi). (B) Light micrograph of *O. heveae* growth on ecotype Lag2-2 at 10 dpi. Light microscopy images were taken after fungal structures had been stained with Coomassie brilliant blue at 10 dpi. Bar, 50 μm. These experiments were repeated twice with similar results.