Transgressive segregation reveals mechanisms of *Arabidopsis* immunity to *Brassica*-infecting races of white rust (*Albugo candida*)

Volkan Cevik[a,b], Freddy Boutrot[a], Wiebke Apel[a,c], Alexandre Robert-Seilaniantz[a,d], Oliver J. Furzer[a,e], Amey Redkar[a,f], Baptiste Castel[a], Paula X. Kover[b], David C. Prince[a,g], Eric B. Holub[h], and Jonathan D. G. Jones[a,1]

[a]The Sainsbury Laboratory, University of East Anglia, Norwich Research Park, NR4 7UH Norwich, United Kingdom; [b]The Milner Centre for Evolution, Department of Biology and Biochemistry, University of Bath, BA2 7AY Bath, United Kingdom; [c]Institute for Biology, Experimental Biophysics, Humboldt-Universität zu Berlin, 10115 Berlin, Germany; [d]Institute for Genetics, Environment and Plant Protection, Agrocampus Ouest, Institut National de la Recherche Agronomique, Universite de Rennes, 35650 Le Rheu, France; [e]Department of Biology, University of North Carolina, Chapel Hill, NC 27599; [f]Department of Genetics, University of Cordoba, 14071 Cordoba, Spain; [g]School of Biological Sciences, University of East Anglia, Norwich Research Park, NR4 7TJ Norwich, United Kingdom; and [h]Warwick Crop Centre, School of Life Sciences, University of Warwick, CV35 9EF Wellesbourne, United Kingdom

Contributed by Jonathan D. G. Jones, December 19, 2018 (sent for review August 6, 2018; reviewed by Ralph Panstruga and Guido Van den Ackerveken)

Arabidopsis thaliana **accessions are universally resistant at the adult leaf stage to white rust (*Albugo candida*) races that infect the crop species *Brassica juncea* and *Brassica oleracea*. We used transgressive segregation in recombinant inbred lines to test if this apparent species-wide (nonhost) resistance in *A. thaliana* is due to natural pyramiding of multiple *Resistance* (*R*) genes. We screened 593 inbred lines from an *Arabidopsis* multiparent advanced generation intercross (MAGIC) mapping population, derived from 19 resistant parental accessions, and identified two transgressive segregants that are susceptible to the pathogen. These were crossed to each MAGIC parent, and analysis of resulting F_2 progeny followed by positional cloning showed that resistance to an isolate of *A. candida* race 2 (Ac2V) can be explained in each accession by at least one of four genes encoding nucleotide-binding, leucine-rich repeat (NLR) immune receptors. An additional gene was identified that confers resistance to an isolate of *A. candida* race 9 (AcBoT) that infects *B. oleracea*. Thus, effector-triggered immunity conferred by distinct NLR-encoding genes in multiple *A. thaliana* accessions provides species-wide resistance to these crop pathogens.**

Arabidopsis thaliana | oomycete | *Albugo candida* | nonhost resistance | Brassicaceae

Plants and animals are colonized by diverse pathogens and parasites, and their mechanisms of immunity are of broad significance. Plants have two layers of cell-autonomous innate immunity (1–3). Pathogen molecules such as flagellin and chitin are perceived by cell surface pattern recognition receptors (PRRs). Activation of PRRs results in pattern-triggered immunity (PTI) that restricts microbial growth (4, 5). Most plant pathogens translocate pathogenicity proteins, called effectors, into host cells; many of these suppress PTI, facilitating colonization (6–8). Genetic variation for disease resistance within a plant species is often explained by allelic variation in *Resistance* (*R*) genes that encode nucleotide-binding, leucine-rich repeat (NLR) immune receptors. Effector recognition leads to effector-triggered immunity (ETI) (1). Many NLRs carry either Toll/Interleukin-1 receptor/Resistance (TIR-NLRs) or coiled-coil (CC) domains at their N-termini (CC-NLRs) (9–11) and can activate ETI either by directly detecting an effector (12–19) or indirectly through "guarding" host proteins that are modified by effectors (20–22). Unlike CC-NLRs, the function of TIR-NLR proteins requires EDS1 (ENHANCED DISEASE SUSCEPTIBILITY 1), which encodes a lipase-like protein, and forms functional heterodimers in *Arabidopsis* with the related proteins PAD4 (PHYTOALEXIN-DEFICIENT 4) or SAG101 (SENESCENCE-ASSOCIATED GENE 101) (23–25).

Plants are challenged by many potential pathogens but most plants are resistant to most pathogens, and disease is rare. Resistance of a particular plant species against all isolates of a pathogen that can infect other plant species is known as nonhost resistance (NHR) (26). The molecular mechanisms underlying NHR are poorly understood; if all accessions of a species are resistant, genetic analysis of NHR is difficult (27, 28). Conceivably, NHR or species-level resistance could involve PTI (if effectors cannot suppress PTI), ETI (if effectors do not evade detection), and/or other mechanisms (28, 29). Fundamental insights into this question are of broad interest. NHR genes that confer complete immunity in a nonhost might confer resistance in susceptible crops and elevate resistance to important crop diseases.

To investigate NHR, we studied *Albugo candida*, an obligate biotrophic oomycete plant pathogen that causes white blister rust

Significance

Most plants resist most plant pathogens. Barley resists wheat-infecting powdery mildew races (and vice versa), and both barley and wheat resist potato late blight. Such "nonhost" resistance could result because the pathogen fails to suppress defense or triggers innate immunity due to failure to evade detection. *Albugo candida* causes white rust on most Brassicaceae, and we investigated *Arabidopsis* NHR to *Brassica*-infecting races. Transgressive segregation for resistance in *Arabidopsis* recombinant inbred lines revealed genes encoding nucleotide-binding, leucine-rich repeat (NLR) immune receptors. Some of these NLR-encoding genes confer resistance to white rust in *Brassica* sp. This genetic method thus provides a route to reveal resistance genes for crops, widening the pool from which such genes might be obtained.

Author contributions: V.C. and J.D.G.J. designed research; V.C., F.B., W.A., A.R.-S., O.J.F., A.R., B.C., and D.C.P. performed research; P.X.K. and E.B.H. contributed new reagents/analytic tools; V.C., F.B., W.A., A.R.-S., O.J.F., A.R., B.C., and P.X.K. analyzed data; and V.C. and J.D.G.J. wrote the paper.

Reviewers: R.P., Rheinisch-Westfälische Technische Hochschule Aachen University; and G.V.d.A., Utrecht University.

Conflict of interest statement: J.D.G.J., O.J.F., and G.V.d.A. with 31 others are coauthors on a 2014 review article. J.D.G.J. and R.P. are coauthors on a 2014 research article (PubMed identifier: 25211078) but did not collaborate directly for this article.

Data deposition: The sequences reported in this paper have been deposited in the GenBank database: *WRR4B*$^{Ws-2}$ (accession no. MK034466), *WRR4B*$^{Col-0}$ (MK034465), *WRR8*$^{Sf-2}$ (MK034463), *WRR9*$^{Hi-0}$ (MK034464), and *WRR12*$^{Ler-0}$ (MK034462). Illumina reads for REN-Seq data produced for this study have been deposited in the European Nucleotide Archive (ENA) under accession no. PRJEB26457. SMRT RenSeq sequence reads for *Arabidopsis* accession Can-0 for this study have been deposited in the ENA under accession no. PRJEB26457.

[1]To whom correspondence should be addressed. Email: jonathan.jones@tsl.ac.uk.

This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1812911116/-/DCSupplemental.

Published online January 28, 2019.

disease in Brassicaceae. In contrast to *A. candida*, *Albugo laibachii* has specialized to cause white rust only on *Arabidopsis* (30). The asexual life cycle of *A. candida* starts with the release of biflagellate motile zoospores from sporangia. Zoospores target host stomata where they encyst and germinate into a germ tube followed by colonization of mesophyll cells by branched hyphae, which also give rise to a specialized feeding structure called an haustorium. Infection culminates in formation of zoosporangia-bearing white pustules that rupture the epidermis; these constitute the visible symptoms of the disease (31). *A. candida* forms many physiological races, each of which specialize on different host species (32–36). Some races of *A. candida* such as Race 2 cause severe annual losses of oilseed mustard (*Brassica juncea*) in India, Canada, and Australia. *Albugo* spp. infection induces a strongly immuno-compromised state in host plants, which can enable avirulent races to colonize and reproduce in the same tissue (37). Sex between different cocolonizing races in the same host could be an important source of new recombinant races (32). Comparative genomics has revealed extensive genetic exchange between races of *A. candida* (34), and this genetic exchange could result in races with novel repertoires of effector alleles that, in turn, might enable colonization of new hosts. Therefore, understanding the underlying mechanism of NHR in different *Brassica* species could inform breeding for resistance to *A. candida*.

Here, we investigate adult plant resistance to *A. candida* Race 2 (Ac2V) in diverse *Arabidopsis thaliana* accessions. While all *Arabidopsis* accessions are resistant to Ac2V, some *A. candida* strains can grow on *Arabidopsis*, but although this pathosystem does not involve NHR to the whole *A. candida* species complex, it is nonetheless instructive. We hypothesized that resistance in *A. thaliana* to Ac2V is due to multiple *R* genes, but the *R* gene repertoire in different *Arabidopsis* accessions might be distinct, creating the potential for transgressive segregation for susceptibility in recombinant inbreds or other segregating progeny from interaccession crosses. We screened a population of "MAGIC" inbred lines (38). These lines result from intercrosses of 19 parents, followed by random intercrossing, and then selfing. These lines have been extensively genotyped (39). We inoculated 593 lines and identified two transgressive segregant inbreds (MAGIC.329 and MAGIC.23) that are susceptible in true leaves to Ac2V. However, none of the MAGIC lines tested, nor the 19 parental accessions, are fully susceptible to Race 9 (AcBoT) collected from *Brassica oleracea*.

We defined three loci that contribute resistance to Ac2V, including a known locus, *White Rust Resistance 4* (*WRR4*) on chromosome 1 (40). *WRR4* carries two paralogs, *WRR4A* and *WRR4B*, that can each confer resistance. We also defined *WRR8* and *WRR9*. To investigate AcBoT resistance in *Arabidopsis*, we intercrossed MAGIC.329 with MAGIC.23. Screening of selfed progeny from this cross revealed fully susceptible plants at a frequency suggesting that resistance in the two parents is conferred by distinct genes. Using RenSeq (Resistance gene Enrichment Sequencing) (41), we identified *WRR12* (previously reported as SOC3) as a gene on chromosome 1 that confers AcBoT resistance (42). These data provide insights into the genetic basis of resistance that restricts pathogen host range and open up a greater subset of the gene pool of crop relatives as a source of genes for crop protection.

Results

Identification of Ac2V-Susceptible MAGIC Lines. All of 107 previously tested wild-type *Arabidopsis* accessions are resistant to *B. juncea*-infecting *A. candida* race Ac2V, but a Ws-2-*eds1* mutant is susceptible (34). To test if resistance in different *Arabidopsis* accessions is due to distinct resistance gene loci, we evaluated MAGIC lines derived from 19 different *Arabidopsis* accessions (38). We tested Ac2V resistance in 593 MAGIC lines at adult leaf stage with four replicates and identified 10 MAGIC lines that showed either a chlorotic phenotype or different levels of susceptibility. Eight of these 10 lines showed strong chlorotic as well as necrotic patches on infected leaves, although two of these eight lines (MAGIC.453 and MAGIC.485) supported occasional pustule formation (Fig. 1). We regularly observed pustules on the two most susceptible MAGIC lines (MAGIC.23 and MAGIC.329) with Ac2V (Fig. 1). After inoculation with Ac2V, pustules appear 7–10 d after infection (dpi) with MAGIC.329 but later (12–14 dpi) with MAGIC.23 (Fig. 1). However, MAGIC.23 and MAGIC.329 are not as susceptible as Ws-2-*eds1* or Col-*eds1-2* plants.

Genetic Segregation of Resistance and Susceptibility Phenotypes in F_2 Progeny Derived from Crosses Between MAGIC Parents and Susceptible MAGIC.329 Line. Identification of susceptible lines enables genetic analysis of resistance in *Arabidopsis* against Ac2V. We crossed MAGIC.329 with each of the 19 MAGIC parents and selfed F_1 plants to obtain F_2 populations. We also analyzed Ws-2 (also known as Ws, Ws-1, Ws-3, and Ws-4, but different from accession Ws-0 that is one of the MAGIC parents) (43) because of its adult plant resistance but seedling susceptibility to Ac2V. All F_1 progeny were resistant. F_2 populations were inoculated with Ac2V, and resistance or susceptibility was scored at 14 dpi. We classified F_2 progeny into three phenotypes: resistant (Green Resistant, GR), partially resistant with chlorosis or necrosis but no pustules (Necrotic-Chlorotic Resistant, NCR), and susceptible, with pustules (Susceptible, S) (Table 1). Segregation ratios ranged from 13R:3S to 255R:1S, suggesting that different *Arabidopsis* accessions carry two to four unlinked *WRR* genes against Ac2V. All tested F_2 plants from the MAGIC.329 × Wu-0 cross were resistant, suggesting >4 resistance loci.

Most MAGIC Parents Carry Resistance That Maps to the *WRR4* Locus. The *Arabidopsis* $WRR4^{Col\text{-}0}$ gene (*At1g56510*) confers resistance against multiple races of *A. candida* in *Arabidopsis* and in *B. juncea* (33, 40). *WRR4* encodes a TIR-NLR protein. *A. candida*

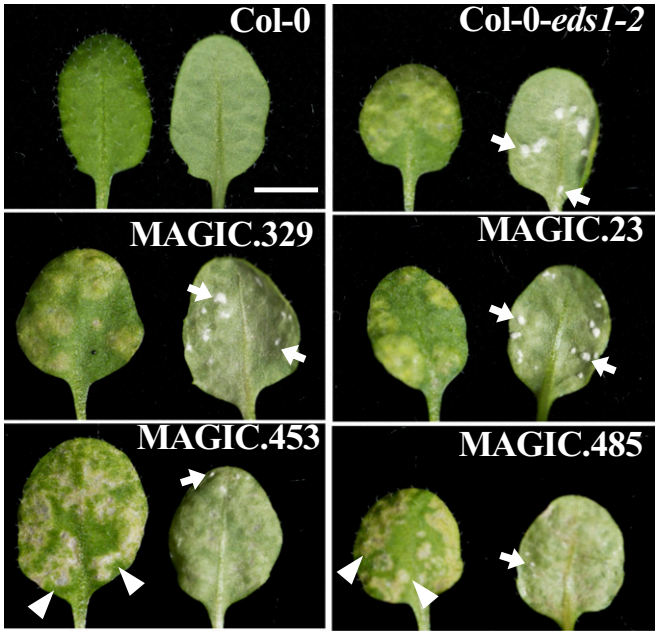

Fig. 1. Identification of transgressive segregant MAGIC lines showing different susceptibility to *B. juncea*-infecting *A. candida* race Ac2V. Different levels of susceptibility to Ac2V are observed in an *eds1-2* mutant and in four of 593 MAGIC recombinant inbred lines. Adaxial (*Left*) and abaxial (*Right*) sides of the leaves are presented. Examples of pustules (arrows) and necrotic patches (arrowheads) are indicated. Susceptibility was scored in 4-wk-old plants at 14 dpi. (Scale bars: 3 mm.)

Table 1. Genetic segregation of resistance and susceptibility phenotypes in F_2 populations between MAGIC.329 and MAGIC parents as well as Ws-2

F_2 population	Interaction			Expected ratio, (R:S)	No. of loci	*P*
	R, GR	R, CNR	S			
MAGIC.329 x Bur-0	135	24	1	63:1	3	0.34
MAGIC.329 x Can-0	155	41	4	63:1	3	0.61
MAGIC.329 x Col-0	147	10	30	13:3	2*	0.34
MAGIC.329 x Ct-1	140	18	4	63:1	3	0.35
MAGIC.329 x Edi-0	500	16	2	255:1	4	0.98
MAGIC.329 x Hi-0	151	32	23	15:1	2†	0.0036
MAGIC.329 x Kn-0	76	79	10	15:1	2	0.92
MAGIC.329 x Ler-0	228	11	16	15:1	2	0.20
MAGIC.329 x Mt-0	154	10	3	63:1	3	0.81
MAGIC.329 x No-0	53	60	1	63:1	3	0.55
MAGIC.329 x Oy-0	206	27	11	15:1	2	0.26
MAGIC.329 x Po-0	74	26	4	15:1	2	0.31
MAGIC.329 x Rsch-4	165	25	32	13:3	2*	0.1
MAGIC.329 x Sf-2	134	115	16	15:1	2	0.07
MAGIC.329 x Tsu-0	223	23	21	15:1	2	0.27
MAGIC.329 x Wil-2	205	69	5	63:1	3	0.75
MAGIC.329 x Ws-0	126	32	11	15:1	2	0.89
MAGIC.329 x Ws-2	170	58	46	13:3	2*	0.40
MAGIC.329 x Wu-0	200	0	0	NT	NT	NT
MAGIC.329 x Zu-0	110	9	2	63:1	3	0.93

GR, green resistant; NCR, necrotic-chlorotic resistant; NT, not tested; *P*, probability value following χ^2 test; R, resistant; S, susceptible.
*One dominant and one recessive gene.
†Two linked genes.

infects by entry of a germ tube into stomata and production of a primary vesicle under an epidermal cell. *WRR4* arrests the development of the pathogen in this epidermal cell, which undergoes a hypersensitive response (HR) (40). As these HR symptoms are not visible macroscopically, we classify this phenotype as GR. We scored susceptible F_2 individuals using markers at the *WRR4* locus (Dataset S1) and observed cosegregation between Ac2V resistance and *WRR4* for all of the *Arabidopsis* accessions tested except Sf-2 and Wil-2 (*SI Appendix*, Table S1). Cosegregation of Ws-2 resistance with the *WRR4* locus was unexpected, as the *WRR4* gene is absent in Ws-2 (*SI Appendix*, Fig. S1 and Dataset S2), suggesting that at least one more gene at the *WRR4* locus could confer Ac2V resistance.

Ac2V Resistance in Ws-2 Is Conferred by the *WRR4A* Paralog *WRR4B*. Previously, cotyledons of Ws-2 seedlings were found to be susceptible to Ac2V and Ac7V (a *Brassica rapa*-infecting race) but not to AcBoT (a *B. oleracea*-infecting race) (40). However, Ws-2 leaves are fully resistant (GR) to Ac2V and Ac7V. An F_2 population derived from MAGIC.329 × Ws-2 segregated as 13 GR/NCR: 3 S ($P = 0.40$), suggesting one dominant and one recessive or haplo-insufficient Ac2V resistance gene in Ws-2. All Ac2V-susceptible individuals from the MAGIC.329 × Ws-2 F_2 lacked the Ws-2 alleles of the markers at the *WRR4* locus. By screening susceptible F_2 individuals with additional molecular markers (Dataset S1), we found no other loci linked to Ac2V resistance. To improve definition of the resistance locus, we identified 672 Ac2V-susceptible F_2 plants. We found two recombinants with the molecular marker corresponding to *At1g56040* and only one recombinant with the marker corresponding to *At1g57670*. These markers delineated the locus to ~397 kb (*SI Appendix*, Fig. S2*A*). *WRR4* maps to this interval in Col-0 but is deleted in Ws-2 (*SI Appendix*, Fig. S1). We therefore cloned two other *WRR4* paralogs *At1g56520* and *At1g56540* from Ws-2 and transformed them into MAGIC.329. For each construct, we tested Ac2V resistance in 48 independent T_1 plants and in homozygous T_3 lines. All plants transformed with $At1g56520^{Ws\text{-}2}$ were susceptible to Ac2V (*SI Appendix*, Fig. S3*A*), but plants with $At1g56540^{Ws\text{-}2}$ were all resistant (GR) (Fig. 2*C*). We named this gene *WRR4B*. We also cloned the Col-0 allele of *WRR4B*, transformed it into MAGIC.329, and found it also confers resistance to Ac2V (Fig. 2*D*). This suggests that in addition to the broad-spectrum *A. candida* resistance gene $WRR4^{Col\text{-}0}$ (hereafter $WRR4A^{Col\text{-}0}$), the *WRR4B* allele of Col-0 functions against Ac2V.

Ac2V Resistance in Sf-2 Is Conferred by a Resistance Gene, *WRR8*. Analysis of MAGIC line DNA sequences indicates that the MAGIC.329 WRR4 haplotype derives from Sf-2 (39). As MAGIC.329 is susceptible to Ac2V, this suggests that Sf-2 lacks functional *WRR4A* and *WRR4B* alleles. Screening of susceptible MAGIC.329 × Sf-2 F_2 progeny confirmed that resistance is unlinked to *WRR4*. We genotyped susceptible F_2 individuals derived from a MAGIC.329 × Sf-2 cross. A single locus was revealed on chromosome 5 between molecular markers derived from *At5g45400* and *At5g47130* (*SI Appendix*, Fig. S2*B*). Fine mapping using 576 additional susceptible F_2 individuals revealed an interval between markers derived from *At5g46250* (one recombinant) and *At5g46310* (four recombinants) that carries two TIR-NLR–encoding genes *At5g46260* and *At5g46270* in Col-0. We cloned both genes from *Arabidopsis* accession Sf-2, transformed them into MAGIC.329, inoculated T_1 plants with Ac2V, and found that transgenic plants carrying $At5g46260^{Sf\text{-}2}$ were all susceptible (48 of 48), but most plants carrying $At5g46270^{Sf\text{-}2}$ showed chlorotic resistance (40 of 48) to Ac2V (Fig. 2*E* and *SI Appendix*, Fig. S3*B*). *At5g46270* thus corresponds to *WRR8* in Sf-2.

Cloning of *WRR9* from *Arabidopsis* Accession Hi-0. The *WRR4* locus in the *Arabidopsis* accession Hi-0 is linked to Ac2V resistance. Using 352 susceptible F_2 individuals derived from a MAGIC.329 × Hi-0 cross, we found an additional resistance locus (*WRR9*) on chromosome 1, distinct from *WRR4*. *WRR9* lies between *At1g57670* (one recombinant in 352 plants) and *At1g63820* (one recombinant in

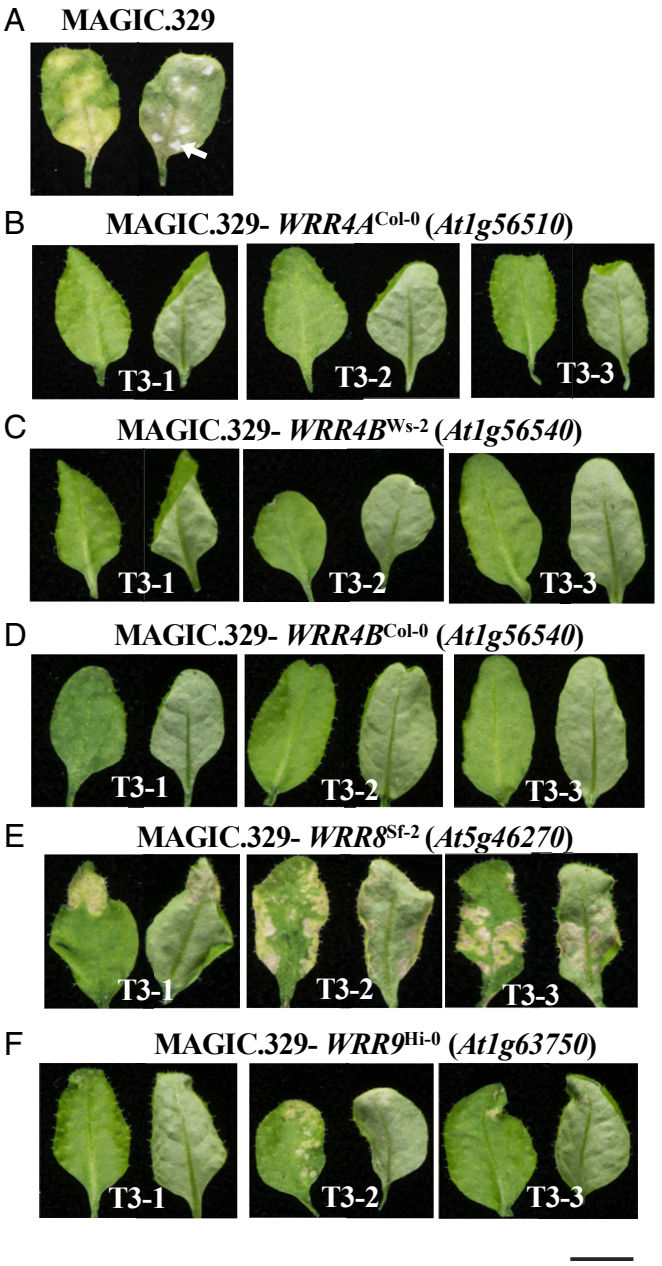

Fig. 2. Distinct *WRR* genes confer resistance to Ac2V in the susceptible MAGIC.329 line. (*A*) Nontransformed MAGIC.329 line. (*B–F*) Independent homozygous T_3 MAGIC.329 lines transformed with the genomic clones of $WRR4A^{Col\text{-}0}$ (*At1g56510*) (*B*), $WRR4B^{Ws\text{-}2}$ (*At1g56540*) (*C*), $WRR4B^{Col\text{-}0}$ (*At1g56540*) (*D*), $WRR8^{Sf\text{-}2}$ (*At5g46270*) (*E*), and $WRR9^{Hi\text{-}0}$ (*At1g63750*) (*F*). Interaction phenotypes were assayed at 12 dpi. Examples of pustules (arrows) are indicated. (Scale bar: 5 mm.)

352 plants) (*SI Appendix*, Fig. S2*C*). We thus defined three TIR-NLR *WRR9* candidate genes *At1g63730*, *At1g63740*, and *At1g63750*. We cloned all three genes from Hi-0, transformed into MAGIC.329, and tested T_1 plants with Ac2V. All of the plants transformed with $At1g63730^{Hi\text{-}0}$ and $At1g63740^{Hi\text{-}0}$ were susceptible, but 43 of 48 transgenic T_1 plants with $At1g63750^{Hi\text{-}0}$ were resistant to Ac2V (Fig. 2*F*). We infer *WRR9* corresponds to *At1g63750*.

***WRR4B* but Not *WRR8* and *WRR9* Confer Resistance to Ac2V in *B. juncea*.** $WRR4A^{Col\text{-}0}$ confers resistance to two different races of *A. candida* in *B. juncea* and *Brassica napus* (33). We transformed *WRR4B, WRR8*, and *WRR9* into *B. juncea*, obtained two independent transgenic *B. juncea* plants with $WRR4B^{Ws\text{-}2}$ but only one transgenic plant with the $WRR4B^{Col\text{-}0}$, and tested T_2 plants derived from these lines. *WRR4B* transgenic *B. juncea* lines showed green to chlorotic resistance to Ac2V (Fig. 3), resembling the *Arabidopsis* phenotype (Fig. 2 *C* and *D*). We obtained two and four independent transgenic *B. juncea* plants with $WRR8^{Sf\text{-}2}$ and $WRR9^{Hi\text{-}0}$, respectively. Following inoculation with Ac2V, the T_2 plants obtained from these independent transgenic lines were all fully susceptible to the pathogen (*SI Appendix*, Fig. S4), although reverse transcription–PCR (RT-PCR) revealed that $WRR8^{Sf\text{-}2}$ and $WRR9^{Hi\text{-}0}$ were expressed in these lines (*SI Appendix*, Fig. S5).

Transgressive Segregation for AcBoT Susceptibility in a MAGIC.329 × MAGIC.23 F_2 Reveals WRR12, an Additional TIR-NLR for AcBoT Resistance. MAGIC.329 and MAGIC.23 are resistant or partially resistant, respectively, to *B. oleracea*-infecting *A. candida* race AcBoT. To identify potential transgressive segregants susceptible to AcBoT, we crossed MAGIC.329 × MAGIC.23 and obtained F_2 progeny. Inoculation of this F_2 with AcBoT revealed fully susceptible individuals. The F_2 population segregated as 15 GR or NCR: 1 S (200GR+34CR:19S) ($P = 0.41$), suggesting a single dominant *WRR* gene is present in each parent. To test if AcBoT-susceptible F_2 lines are also susceptible to other *Brassica*-infecting *A. candida* races, we obtained F_4 plants derived from independent susceptible F_2 lines. We named these plants as "Double MAGIC" (DM) lines. We found that DM lines are also fully susceptible to *A. candida* races Ac2V and Ac7V (*SI Appendix*, Fig. S6).

To identify the underlying genes conferring resistance to AcBoT in MAGIC.329 and MAGIC.23, we collected ∼200 fully susceptible F_2 individuals following AcBoT inoculation. To accelerate the cloning, we conducted RenSeq (41) on DNA of the resistant parents MAGIC.329 and MAGIC.23 as well as bulked susceptible DNA (BS) obtained from the fully susceptible F_2 individuals. MiSeq reads obtained from the parents and from BS were used to identify polymorphisms and linkage by mapping the reads to the Col-0 reference genome. This revealed a single locus where the resistance gene from MAGIC.329 is located (*SI Appendix*, Table S2). We named this gene *WRR12* and found that, in MAGIC.329, this genomic region was introgressed from Ler-0, whereas the nonfunctional allele in MAGIC.23 was introgressed from Wu-0. We found no additional locus linked to the resistance in MAGIC.23, suggesting that its partial resistance could be multigenic. Three genes within the *WRR12* locus cosegregate with resistance (*SI Appendix*, Table S2): the TIR-NLR gene *At1g17600* and TIR-NB–only genes *At1g17610* and *At1g17615* (*SI Appendix*, Fig. S7). *At1g17600* was previously designated *SUSA1* or *SOC3* and implicated in cold-induced activation of defense by an allele of *At1g17610* (CHS1) (42, 44, 45). Recently, SOC3/CHS1 was proposed to "guard" an immune-regulating E3 ligase SAUL1 (46).

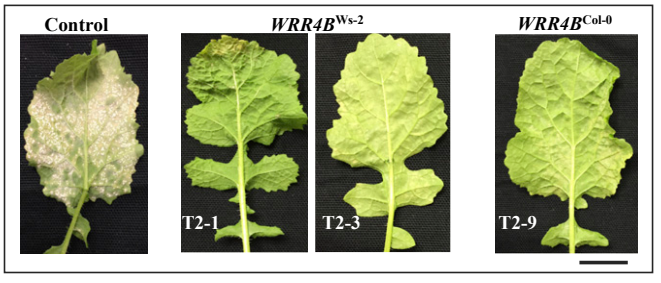

Fig. 3. *Arabidopsis WRR* genes provide resistance to *A. candida* race Ac2V in *B. juncea*. Col-0 and Ws-2 alleles of *WRR4B* provide resistance to Ac2V in transgenic *B. juncea*. Nontransgenic control plants and independent T_2 plants transformed with the indicated *WRR* genes were inoculated with Ac2V. The pictures were taken at 15 dpi. (Scale bar: 10 mm.)

TN2 (*At1g17615*) was reported to be required for the enhanced disease resistance phenotype in *exo70B1* mutant *Arabidopsis* plants (47).

The *At1g17600* allele from Wu-0 in MAGIC.23 (and only this allele; Dataset S2) carries a ∼4-kb transposon insertion (*SI Appendix*, Fig. S7 and Dataset S2), suggesting that it is nonfunctional, and that the Ler-0 allele in MAGIC.329 is a strong candidate for *WRR12*-mediated resistance. We cloned *At1g17600* from MAGIC.329 and transformed into line DM10, one of the DM lines. Independent T_1 transgenic plants were screened with *A. candida* race AcBoT. All 24 T_1 transgenic DM10 plants were resistant to AcBoT. This suggests that $At1g17600^{Ler\text{-}0}$ corresponds to *WRR12* (Fig. 4). We also transformed $WRR4B^{Col\text{-}0}$, $WRR8^{Sf\text{-}2}$, and $WRR8^{Hi\text{-}0}$ into DM10 to determine if these genes confer resistance to AcBoT in *Arabidopsis*. We found all $WRR4B^{Col\text{-}0}$ transgenic T_1 plants (eight of eight) were resistant to AcBoT, while seven of eight $WRR8^{Sf\text{-}2}$ transgenic plants showed resistance to the pathogen. In contrast, $WRR9^{Hi\text{-}0}$ transgenic DM10 lines (nine of nine) were fully susceptible to AcBoT (Fig. 4).

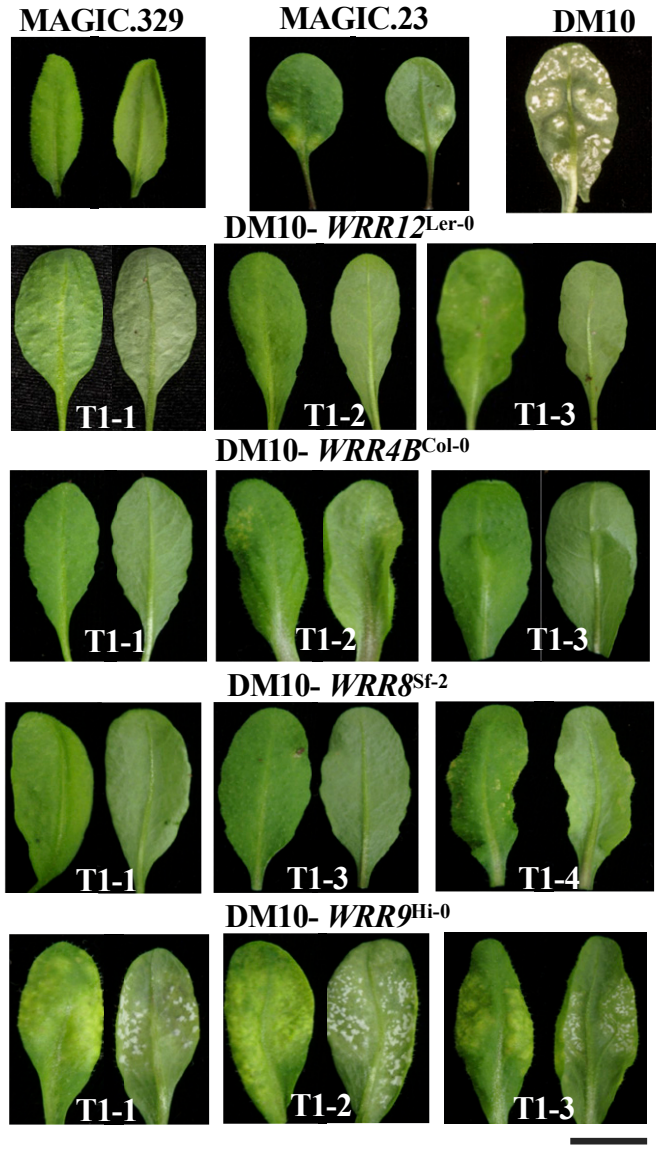

Fig. 4. $WRR12^{Ler\text{-}0}$, $WRR4B^{Col\text{-}0}$, $WRR8^{Sf\text{-}2}$, but not $WRR9^{Hi\text{-}0}$ confer resistance to *B. oleracea*-infecting *A. candida* race AcBoT in *Arabidopsis*. MAGIC.329 and MAGIC.23 are resistant or partially resistant, respectively, to AcBoT. DM10 lines were transformed with $WRR12^{Ler\text{-}0}$ (*At1g17600*), $WRR4B^{Col\text{-}0}$, $WRR8^{Sf\text{-}2}$, and $WRR9^{Hi\text{-}0}$ and interaction phenotypes were assayed in independent T_1 plants at 20 dpi. (Scale bar: 10 mm.)

In addition, we transformed *B. oleracea* DH1012 with $WRR4A^{Col\text{-}0}$, $WRR4B^{Col\text{-}0}$, and $WRR4B^{Ws\text{-}2}$, as well as $At1g56520^{Col\text{-}0}$ as a negative control and inoculated independent T_1 transgenic *B. oleracea* lines with AcBoT. T_1 transgenic plants with $WRR4A^{Col\text{-}0}$ (15 of 16), $WRR4B^{Ws\text{-}2}$ (13 of 19), and $WRR4B^{Col\text{-}0}$ (two of two) were fully resistant to AcBoT, whereas transgenic plants with $At1g56520^{Col\text{-}0}$ (four of four) were fully susceptible (*SI Appendix*, Fig. S8).

***WRR* Gene Haplotypes in MAGIC Parents.** To determine the distribution and sequence variation of *WRR4A*, *WRR4B*, *WRR8*, *WRR9*, and *WRR12* genes, the MAGIC parents as well as Ws-2 were sequenced using SMRT RenSeq (48). The sequences of the *WRR* alleles from each accession were identified by blastn (49) against the SMRT RenSeq assemblies. Blastn hits showing less than 95% identity were not considered to be alleles of the *WRR* genes. We used the Augustus gene prediction server (50) to obtain predicted protein sequences of the *WRR* alleles. We identified *WRR4A* alleles in all MAGIC parent accessions except Ws-2, Edi-0, and No-0 (Dataset S2). We also identified *WRR4B* alleles in all *Arabidopsis* accessions except Tsu-0 in the RenSeq assemblies. Both Sf-2 and Wil-2 (source of the *WRR4* haplotypes in MAGIC.329 and MAGIC.23, respectively) lack functional *WRR4A* and *WRR4B* genes. We identified RenSeq assemblies for *WRR4A* and *WRR4B* in both *Arabidopsis* accessions, and the lack of functional *WRR4A* and *WRR4B* in Sf-2 and Wil-2 is not due to deletion (Dataset S2). Although the Sf-2 *WRR4A* region was not clearly resolved in the de novo assembly, by aligning the RenSeq reads to the Col-0 genome, we confirmed a single base deletion, also observed in the *Arabidopsis* 1001 genomes browser, at nucleotide position 177 that results in an early stop codon, explaining why the Sf-2 *WRR4A* allele is nonfunctional.

Blastn analysis revealed that all of the *Arabidopsis* accessions contain *WRR8* and *WRR9* alleles except for *WRR9* in Ler-0. However, as for the *WRR4A* and *WRR4B* alleles, some of the assemblies did not cover full-length *WRR8* and *WRR9*. This is most likely due to partial SMRT RenSeq assemblies or incomplete capture.

We also identified *WRR12* alleles in MAGIC parents and Ws-2. All lines carried an apparently functional allele, except for Wu-0.

Discussion

NHR in one plant species can be defined as complete resistance to pathogens that infect another species (26). Multiple mechanisms, such as preformed antimicrobial metabolites, and induced defenses such as PTI and ETI, could contribute to NHR (51, 52). A better understanding of the mechanisms of NHR could reveal additional genes that confer resistance in crops to plant pathogens.

We investigated NHR in *Arabidopsis* against *Brassica*-infecting *A. candida* races. All *Arabidopsis* accessions tested are resistant to *B. juncea*-infecting race Ac2V, *B. rapa*-infecting race Ac7V, and *B. oleracea*-infecting race AcBoT (ref. 34, this study). However, we found that both Col-0-*eds1-2* (53) and Ws-2-*eds1* (34) are susceptible to all three *A. candida* races, suggesting that NHR to these races might involve TIR-NLR genes (23). We further hypothesized that resistance in different *Arabidopsis* accessions could be mediated by distinct resistance genes. Therefore, we screened MAGIC lines derived from 19 different *Arabidopsis* parents (38) and identified transgressive segregant lines that are susceptible to Ac2V. These susceptible plants enabled us to perform genetic analysis to identify resistance genes in multiple *Arabidopsis* accessions.

We defined three *WRR* (*WRR4B*$^{Col-0}$, *WRR8*$^{Sf-2}$, and *WRR9*$^{Hi-0}$) genes against Ac2V, and a gene, *WRR12* (*SOC3*), conferring NHR to AcBoT, in addition to the previously identified broad spectrum resistance gene *WRR4A*$^{Col-0}$. Other investigations have revealed additional *WRR* genes, but we focus in this paper on resistances at the *WRR4*, *8*, *9*, and *12* loci. A point mutation in *At1g17610*, the neighboring gene of *WRR12* encoding a TIR-NB protein, results in *chilling sensitive 1* (*CHS1*), with an autoactive defense phenotype (44). This phenotype could be suppressed by mutations in *WRR12*, which was therefore named *suppressor of chilling sensitive 1–3* (*SOC3*). SOC3 and CHS1 can associate physically (42).

A phylogenetic analysis using an alignment of the NB-ARC region of TNLs in *Arabidopsis* accession Col-0 reveals that WRR4, WRR4B, and WRR9 are monophyletic, suggesting they shared a more recent common ancestor than with WRR8 (*SI Appendix*, Fig. S9). This analysis also reveals that WRR12 and CHS1 are located in neighboring expanded clades, many members of which are part of divergently transcribed pairs in the Col-0 genome (*SI Appendix*, Fig. S9). This suggests that multiple duplications of an ancestral WRR12/CHS1 pair occurred, similar to the expansion that occurred of RPS4/RRS1-like pairs (refs. 54 and 55 and *SI Appendix*, Fig. S9).

Neither *WRR8* nor *WRR9* confer resistance to Ac2V in *B. juncea*, although these genes confer resistance in *Arabidopsis*. *WRR8* also confers resistance to AcBoT in *Arabidopsis*. This could be due to the fact that WRR8- and WRR9-mediated resistance involves a guardee or decoy that is present in *Arabidopsis* but absent or divergent in *Brassica* sp. Indeed, recent publications show that *WRR12*/*SOC3* and *CHS1* form a gene pair and that WRR12/SOC3, together with CHS1, monitors the homeostasis of E3 ligase SAUL1, a potential guardee that we hypothesize might be targeted by *A. candida* effector(s) (42, 46).

F_2 individuals from crosses between MAGIC.329 and Col-0, Rsch-4, or Ws-2 segregated at a ratio of 13:3, suggesting one dominant and one recessive or haplo-insufficient gene. Identification of a second resistance locus in these F_2s will require genotyping fully resistant individuals that lack resistant *WRR4* haplotypes. Crosses between MAGIC.329 and Oy-0 or Sf-2 show a 15:1 segregation in the F_2, suggesting two independent dominant resistance loci, but genotyping susceptible plants revealed only one locus. How many more *WRR* genes might there be in *Arabidopsis*? All F_2 individuals resulting from selfing the F_1 between MAGIC.329 × Wu-0 are resistant, suggesting that Wu-0 likely contains >4 resistance loci, so additional loci for resistance to Ac2V and AcBoT likely remain to be discovered.

Our data suggest that *Arabidopsis* NHR against *Brassica*-infecting *A. candida* races is primarily mediated via ETI, consistent with the expectation that ETI is more likely to contribute to NHR if there is a close evolutionary relationship between the host and nonhost plant species (29). ETI may contribute to NHR in other plant pathosystems. For example, various RxLR effectors from *Phytophthora infestans* trigger a HR in the nonhost pepper (56). NHR to *P. capsici* in various *Nicotiana* species likely involves PcAvr3a1 effector recognition (57). *Pseudomonas syringae* AvrRps4 homologs (HopK1$_{DC3000}$ and AvrRps4$_{Pph1448A}$) trigger HR in lettuce, and this HR phenotype cosegregates with a NLR locus RGC4 (58). NHR against wheat stripe rust (*Puccinia striiformis* f. sp. *tritici*) in barley or in *Brachypodium distachyon* was mapped to *Rps6* or *Yrr2* loci, respectively. Both intervals were shown to contain NLR genes, suggesting that NLRs may contribute to NHR against wheat stripe rust in barley and *B. distachyon* (59–61). Furthermore, nonhost resistance to *Lolium* and *Avena* isolates of *Pyricularia oryzae* in wheat was shown to be mediated by two resistance genes, *Rwt3* and *Rwt4*, and the emergence of wheat blast was attributed to a host jump as a result of widespread growth of *rwt3* wheat (62).

NLR-encoding resistance genes recognize pathogen effectors. When *A. candida* races of Ac2V and Ac7V were intercrossed, and F_2 individuals obtained and inoculated on *B. rapa* (host for Ac7V but nonhost for Ac2V), a segregation ratio of three avirulent to one virulent was obtained. This supports the hypothesis that resistance to Ac2V in *B. rapa* involves resistance gene-dependent recognition of an Ac2V effector allele that is absent from or different in Ac7V (63).

Specific races of *A. candida*, usually considered a generalist pathogen, colonize a particular host species (34). Why might resistance genes in nonhost plants recognize effectors from non-adapted pathogens? Conceivably, host and nonhost plants share a common ancestor that was a host for the pathogen (56). Our data suggest that host/race specificity of *A. candida* is determined by the NLR repertoire of the host plant and the recognized effectors of the pathogen race, rather than host compatibility factors. Therefore, some of the NLRs recognizing specific races or multiple races are maintained in different Brassicaceae species. This, in turn, provides an excellent resource to identify *WRR* genes for different *Brassica* species. In summary, by using transgressive segregation to reveal susceptible lines, we were able to reveal genes that underpin resistance in *Arabidopsis* to *Brassica*-infecting *A. candida* races and show that some of these genes might be useful for elevating crop disease resistance. This strategy could also be applied to identify useful new resistance genes in other crop relatives that show NHR to crop-adapted pathogen races.

Materials and Methods

All *Arabidopsis* accessions used in this study were obtained from the Nottingham *Arabidopsis* Stock Centre. Col-0-*eds1-2* and Ws-2-*eds1* were described in refs. 32 and 48. MAGIC lines were described in ref. 38. *Arabidopsis* seeds were sown on Scotts Levington F2 compost (Scotts) and vernalized for 1 wk at 5–6 °C. Seedlings were subsequently grown in a controlled environment room (CER) with a 10-h day and a 14-h night photoperiod and at a constant temperature of 22 °C for 2 wk and then pricked-out into "Arabidopsis mix" [Scotts Levington F2 compost-grit (6:1, vol/vol), 0.03% (m/v) Intercept insecticide] and returned to the CER. *B. juncea* seeds were sown on Scotts Levington F2 compost. Seedlings were subsequently grown in a controlled environment room (CER) with a 10-h day and a 14-h night photoperiod and at a constant temperature of 22 °C for 1 wk and then pricked-out into Arabidopsis mix and returned to the CER. Detailed information is provided in *SI Appendix, Supplementary Materials and Methods*.

ACKNOWLEDGMENTS. We thank Matthew Smoker and Jodie Taylor for help with *Arabidopsis* and *Brassica* transformation and the members of the Gordon and Betty Moore Foundation (GBMF)-funded Ren-seq Team—Felix Bemm, Anna-Lena Van de Weyer, Freddy Monteiro, Jeff Dangl, and Detlef Weigel—for their help in generating the RenSeq NLR database. F.B., W.A., A.R.-S., and D.C.P. were supported by European Research Council Advanced Investigator Grant 233376 (ALBUGON) (to J.D.G.J.); V.C. was supported by the Biotechnology and Biological Sciences Research Council (BBSRC) Grant BB/L011646/1; O.J.F. was supported by BBSRC Grant BB/M003809/1; A.R. was supported by European Molecular Biology Organization Long-Term Fellowship ALTF-842-2015. The authors also were supported by GBMF Grant GBMF4725.

1. Jones JD, Dangl JL (2006) The plant immune system. *Nature* 444:323–329.
2. Dangl JL, Jones JD (2001) Plant pathogens and integrated defence responses to infection. *Nature* 411:826–833.
3. Dodds PN, Rathjen JP (2010) Plant immunity: Towards an integrated view of plant-pathogen interactions. *Nat Rev Genet* 11:539–548.
4. Macho AP, Zipfel C (2014) Plant PRRs and the activation of innate immune signaling. *Mol Cell* 54:263–272.
5. Boutrot F, Zipfel C (2017) Function, discovery, and exploitation of plant pattern recognition receptors for broad-spectrum disease resistance. *Annu Rev Phytopathol* 55: 257–286.
6. Rovenich H, Boshoven JC, Thomma BP (2014) Filamentous pathogen effector functions: Of pathogens, hosts and microbiomes. *Curr Opin Plant Biol* 20:96–103.
7. Dangl JL, Horvath DM, Staskawicz BJ (2013) Pivoting the plant immune system from dissection to deployment. *Science* 341:746–751.
8. Deslandes L, Rivas S (2012) Catch me if you can: Bacterial effectors and plant targets. *Trends Plant Sci* 17:644–655.
9. Maekawa T, Kufer TA, Schulze-Lefert P (2011) NLR functions in plant and animal immune systems: So far and yet so close. *Nat Immunol* 12:817–826.
10. Cui H, Tsuda K, Parker JE (2015) Effector-triggered immunity: From pathogen perception to robust defense. *Annu Rev Plant Biol* 66:487–511.

11. Jacob F, Vernaldi S, Maekawa T (2013) Evolution and conservation of plant NLR functions. *Front Immunol* 4:297.
12. Césari S, et al. (2014) The NB-LRR proteins RGA4 and RGA5 interact functionally and physically to confer disease resistance. *EMBO J* 33:1941–1959.
13. Le Roux C, et al. (2015) A receptor pair with an integrated decoy converts pathogen disabling of transcription factors to immunity. *Cell* 161:1074–1088.
14. Sarris PF, et al. (2015) A plant immune receptor detects pathogen effectors that target WRKY transcription factors. *Cell* 161:1089–1100.
15. Jia Y, McAdams SA, Bryan GT, Hershey HP, Valent B (2000) Direct interaction of resistance gene and avirulence gene products confers rice blast resistance. *EMBO J* 19: 4004–4014.
16. Ueda H, Yamaguchi Y, Sano H (2006) Direct interaction between the tobacco mosaic virus helicase domain and the ATP-bound resistance protein, N factor during the hypersensitive response in tobacco plants. *Plant Mol Biol* 61:31–45.
17. Dodds PN, et al. (2006) Direct protein interaction underlies gene-for-gene specificity and coevolution of the flax resistance genes and flax rust avirulence genes. *Proc Natl Acad Sci USA* 103:8888–8893.
18. Krasileva KV, Dahlbeck D, Staskawicz BJ (2010) Activation of an Arabidopsis resistance protein is specified by the in planta association of its leucine-rich repeat domain with the cognate oomycete effector. *Plant Cell* 22:2444–2458.
19. Catanzariti AM, et al. (2010) The AvrM effector from flax rust has a structured C-terminal domain and interacts directly with the M resistance protein. *Mol Plant Microbe Interact* 23:49–57.
20. Mackey D, Holt BF, 3rd, Wiig A, Dangl JL (2002) RIN4 interacts with *Pseudomonas syringae* type III effector molecules and is required for RPM1-mediated resistance in Arabidopsis. *Cell* 108:743–754.
21. Shao F, et al. (2003) Cleavage of Arabidopsis PBS1 by a bacterial type III effector. *Science* 301:1230–1233.
22. Wang G, et al. (2015) The decoy substrate of a pathogen effector and a pseudokinase specify pathogen-induced modified-self recognition and immunity in plants. *Cell Host Microbe* 18:285–295.
23. Wiermer M, Feys BJ, Parker JE (2005) Plant immunity: The EDS1 regulatory node. *Curr Opin Plant Biol* 8:383–389.
24. Feys BJ, et al. (2005) Arabidopsis SENESCENCE-ASSOCIATED GENE101 stabilizes and signals within an ENHANCED DISEASE SUSCEPTIBILITY1 complex in plant innate immunity. *Plant Cell* 17:2601–2613.
25. Rietz S, et al. (2011) Different roles of enhanced disease susceptibility1 (EDS1) bound to and dissociated from phytoalexin Deficient4 (PAD4) in Arabidopsis immunity. *New Phytol* 191:107–119.
26. Heath MC (2000) Nonhost resistance and nonspecific plant defenses. *Curr Opin Plant Biol* 3:315–319.
27. Mysore KS, Ryu CM (2004) Nonhost resistance: How much do we know? *Trends Plant Sci* 9:97–104.
28. Niks RE, Marcel TC (2009) Nonhost and basal resistance: How to explain specificity? *New Phytol* 182:817–828.
29. Schulze-Lefert P, Panstruga R (2011) A molecular evolutionary concept connecting nonhost resistance, pathogen host range, and pathogen speciation. *Trends Plant Sci* 16:117–125.
30. Kemen E, et al. (2011) Gene gain and loss during evolution of obligate parasitism in the white rust pathogen of *Arabidopsis thaliana*. *PLoS Biol* 9:e1001094.
31. Holub EB, et al. (1995) Phenotypic and genotypic variation in the interaction between *Arabidopsis thaliana* and *Albugo candida*. *Mol Plant Microbe Interact* 8:916–928.
32. Saharan GSVP, Meena PD, Kumar A (2014) *White Rust of Crucifers: Biology, Ecology and Management* (Springer, New Delhi).
33. Borhan MH, et al. (2010) *WRR4*, a broad-spectrum TIR-NB-LRR gene from *Arabidopsis thaliana* that confers white rust resistance in transgenic oilseed Brassica crops. *Mol Plant Pathol* 11:283–291.
34. McMullan M, et al. (2015) Evidence for suppression of immunity as a driver for genomic introgressions and host range expansion in races of *Albugo candida*, a generalist parasite. *eLife* 4:e04550.
35. Ploch S, et al. (2010) Evolution of diversity in *Albugo* is driven by high host specificity and multiple speciation events on closely related Brassicaceae. *Mol Phylogenet Evol* 57:812–820.
36. Jouet A, et al. (October 5, 2018) *Albugo candida* race diversity, ploidy and host-associated microbes revealed using DNA sequence capture on diseased plants in the field. *New Phytol*, 10.1111/nph.15417.
37. Cooper AJ, et al. (2008) Basic compatibility of Albugo candida in *Arabidopsis thaliana* and *Brassica juncea* causes broad-spectrum suppression of innate immunity. *Mol Plant Microbe Interact* 21:745–756.
38. Kover PX, et al. (2009) A multiparent advanced generation inter-cross to fine-map quantitative traits in *Arabidopsis thaliana*. *PLoS Genet* 5:e1000551.
39. Gan X, et al. (2011) Multiple reference genomes and transcriptomes for *Arabidopsis thaliana*. *Nature* 477:419–423.
40. Borhan MH, et al. (2008) WRR4 encodes a TIR-NB-LRR protein that confers broad-spectrum white rust resistance in *Arabidopsis thaliana* to four physiological races of *Albugo candida*. *Mol Plant Microbe Interact* 21:757–768.
41. Jupe F, et al. (2013) Resistance gene enrichment sequencing (RenSeq) enables reannotation of the NB-LRR gene family from sequenced plant genomes and rapid mapping of resistance loci in segregating populations. *Plant J* 76:530–544.
42. Zhang Y, et al. (2017) Temperature-dependent autoimmunity mediated by *chs1* requires its neighboring TNL gene *SOC3*. *New Phytol* 213:1330–1345.
43. Anastasio AE, et al. (2011) Source verification of mis-identified *Arabidopsis thaliana* accessions. *Plant J* 67:554–566.
44. Wang Y, Zhang Y, Wang Z, Zhang X, Yang S (2013) A missense mutation in CHS1, a TIR-NB protein, induces chilling sensitivity in Arabidopsis. *Plant J* 75:553–565.
45. Zbierzak AM, et al. (2013) A TIR-NBS protein encoded by Arabidopsis *Chilling Sensitive 1* (*CHS1*) limits chloroplast damage and cell death at low temperature. *Plant J* 75: 539–552.
46. Tong M, et al. (2017) E3 ligase SAUL1 serves as a positive regulator of PAMP-triggered immunity and its homeostasis is monitored by immune receptor SOC3. *New Phytol* 215:1516–1532.
47. Zhao T, et al. (2015) A truncated NLR protein, TIR-NBS2, is required for activated defense responses in the exo70B1 mutant. *PLoS Genet* 11:e1004945.
48. Witek K, et al. (2016) Accelerated cloning of a potato late blight-resistance gene using RenSeq and SMRT sequencing. *Nat Biotechnol* 34:656–660.
49. Camacho C, et al. (2009) BLAST+: Architecture and applications. *BMC Bioinformatics* 10:421.
50. Hoff KJ, Stanke M (2013) WebAUGUSTUS–A web service for training AUGUSTUS and predicting genes in eukaryotes. *Nucleic Acids Res* 41:W123–W128.
51. Lee HA, et al. (2017) Current understandings on plant nonhost resistance. *Mol Plant Microbe Interact* 30:5–15.
52. Fan J, Doerner P (2012) Genetic and molecular basis of nonhost disease resistance: Complex, yes; silver bullet, no. *Curr Opin Plant Biol* 15:400–406.
53. Bartsch M, et al. (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.
54. Narusaka M, Kubo Y, Shiraishi T, Iwabuchi M, Narusaka Y (2009) A dual resistance gene system prevents infection by three distinct pathogens. *Plant Signal Behav* 4: 954–955.
55. Di Donato A, Andolfo G, Ferrarini A, Delledonne M, Ercolano MR (2017) Investigation of orthologous pathogen recognition gene-rich regions in solanaceous species. *Genome* 60:850–859.
56. Lee HA, et al. (2014) Multiple recognition of RXLR effectors is associated with nonhost resistance of pepper against Phytophthora infestans. *New Phytol* 203:926–938.
57. Vega-Arreguín JC, Jalloh A, Bos JI, Moffett P (2014) Recognition of an Avr3a homologue plays a major role in mediating nonhost resistance to *Phytophthora capsici* in *Nicotiana* species. *Mol Plant Microbe Interact* 27:770–780.
58. Wroblewski T, et al. (2009) Comparative large-scale analysis of interactions between several crop species and the effector repertoires from multiple pathovars of *Pseudomonas* and *Ralstonia*. *Plant Physiol* 150:1733–1749.
59. Dawson AM, et al. (2016) Isolation and fine mapping of *Rps6*: An intermediate host resistance gene in barley to wheat stripe rust. *Theor Appl Genet* 129:831–843.
60. Li K, et al. (2016) Fine mapping of barley locus Rps6 conferring resistance to wheat stripe rust. *Theor Appl Genet* 129:845–859.
61. Gilbert B, et al. (2018) Components of Brachypodium distachyon resistance to non-adapted wheat stripe rust pathogens are simply inherited. *PLoS Genet* 14:e1007636.
62. Inoue Y, et al. (2017) Evolution of the wheat blast fungus through functional losses in a host specificity determinant. *Science* 357:80–83.
63. Adhikari TB, Liu JQ, Mathur S, Wu CX, Rimmer SR (2003) Genetic and molecular analyses in crosses of race 2 and race 7 of *Albugo candida*. *Phytopathology* 93: 959–965.