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Short communication

WRR4, a broad-spectrum TIR-NB-LRR gene from *Arabidopsis thaliana* that confers white rust resistance in transgenic oilseed brassica crops

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SUMMARY

White blister rust caused by *Albugo candida* (Pers.) Kuntze is a common and often devastating disease of oilseed and vegetable brassica crops worldwide. Physiological races of the parasite have been described, including races 2, 7 and 9 from *Brassica juncea*, *B. rapa* and *B. oleracea*, respectively, and race 4 from *Capsella bursa-pastoris* (the type host). A gene named *WRR4* has been characterized recently from polygenic resistance in the wild brassica relative *Arabidopsis thaliana* (accession Columbia) that confers broad-spectrum white rust resistance (*WRR*) to all four of the above *Al. candida* races. This gene encodes a TIR-NB-LRR (Toll-like/interleukin-1 receptor-nucleotide binding-leucine-rich repeat) protein which, as with other known functional members in this subclass of intracellular receptor-like proteins, requires the expression of the lipase-like defence regulator, enhanced disease susceptibility 1 (*EDS1*). Thus, we used RNA interference-mediated suppression of EDS1 in a white rust-resistant breeding line of *B. napus* (transformed with a construct designed from the *A. thaliana EDS1* gene) to determine whether defence signalling via EDS1 is functionally intact in this oilseed brassica. The eds1-suppressed lines were fully susceptible following inoculation with either race 2 or 7 isolates of *Al. candida*. We then transformed white rust-susceptible cultivars of *B. juncea* (susceptible to race 2) and *B. napus* (susceptible to race 7) with the *WRR4* gene from *A. thaliana*. The WRR4-transformed lines were resistant to the corresponding *Al. candida* race for each host species. The combined data indicate that *WRR4* could potentially provide a novel source of white rust resistance in oilseed and vegetable brassica crops.

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INTRODUCTION

Arabidopsis thaliana has been an important genetic resource for the investigation of the molecular basis of genes that specify natural variation in disease resistance to bacterial, fungal, viral and oomycete pathogens of plants (Eulgem, 2005; Holub, 2001, 2008; Ryan *et al*., 2007). As in most plant species, a majority of these so-called resistance (*R*) genes in *A. thaliana* encode intracellular receptor-like proteins that are characterized by a central nucleotide-binding (NB) domain and a C-terminal leucine-rich repeat (LRR) domain. They can be further grouped into two subclasses on the basis of either a TIR (similar to animal Toll-like/interleukin-1 receptors) or coiled-coil (CC) domain at the N-terminus (Jones and Jones, 1997).

Most *R* genes in *A. thaliana* confer disease resistance to a narrow spectrum of variants within a corresponding pathogen species, and therefore may never be cost-effective for commercial investment leading to the development of transgenic crops. However, important exceptions of broad-spectrum disease resistance have been reported across a wide taxonomic range of plants, including *A. thaliana*, in which a single, dominantly expressed R protein confers resistance to all known races of a pathogen. In an early report, a membrane-bound receptor-like kinase, designated Xa21, was described in rice, which is effective against 29 diverse isolates of the bacterium *Xanthomonas oryzae* pv. *oryzae* (Wang *et al*., 1996). Two genes in *A. thaliana*, designated *RFO1* and *RPW8*, encode other non-NB-LRR proteins that confer resistance against diverse collections of *Fusarium* or powdery mildew fungi, respectively (Diener and Ausubel, 2005; Xiao *et al*., 2001). In the Solanaceae, two broad-spectrum CC-NB-LRR proteins have been discovered, including Bs2 in pepper, which confers black spot resistance to *X. campestris* pv. *vesicatoria*, and RB (also named Rpi-blb1) from the wild species *Solanum bulbocastanum*, which confers late blight resistance to current known races of the oomycete pathogen *Phytophthora infestans* in the USA and Europe (Song *et al*., 2003; van der

Vossen *et al*., 2003). Similarly, TIR-NB-LRR genes have been reported, including *WRR4* from *A. thaliana*, which confers resistance to four races of the oomycete *Albugo candida* (white blister rust) that occur naturally on other wild and domesticated host species, including *Capsella bursa-pastoris* (the type host), *Brassica rapa*, *B. juncea* and *B. oleracea* (Borhan *et al*., 2008); *RLM3* from *A. thaliana*, which confers resistance to several necrotrophic fungi (Staal *et al*., 2008); and *RCT1* from *Medicago truncatula* which confers resistance to races of the anthracnose fungi *Colletotrichum trifolii* and *C. destructivum* (Yang *et al*., 2008).

WRR4 provides an important example to test the transgenic use in crops of an *R* gene that was originally derived from *A. thaliana*. *Albugo candida* (Pers.) Kuntze is an economically destructive crop pathogen. This biotrophic oomycete causes white blister rust in all vegetable and oilseed brassica crops, such as *B. rapa* (Chinese cabbage and turnip rape; diploid A genome), *B. oleracea* (cabbage, kale, broccoli and cauliflower; diploid C genome), *B. juncea* (oilseed mustard; allotetraploid A and B genomes) and *B. napus* (oilseed rape; allotetraploid A and C genomes) (Fan *et al*., 1983; Harper and Pittman, 1974; Kumari *et al*., 1970; Petrie, 1988; Pound and Williams, 1963). The parasite emerges from infected tissue as white rust pustules to release asexual zoosporangia. These pustules can emerge on all aerial parts of the host, and are often associated with abnormal growth in surrounding tissue, stimulated by a hormonal imbalance, such as a common severe symptom called a 'staghead' that occurs when the pathogen invades a floral stem. Susceptible oilseed crops (*B. napus* and *B. juncea*) are particularly vulnerable to floral infections, with devastating losses (30%–60% yield reduction) commonly occurring in North America, Australia, India and China (Bernier, 1972; Li *et al*., 1996, 2007; Petrie, 1973). Oilseed *B. juncea* has better drought tolerance and more durable stem canker resistance (*Leptosphaeria maculans*) than oilseed *B. napus* (Marcroft *et al*., 2002), and therefore canola-quality *B. juncea* varieties are being developed to extend production in low-rainfall areas of Australia and North America (Li *et al*., 2007). Unfortunately, white rust looms as a major threat to *B. juncea* production in these areas.

Physiological races of *Al. candida* have been described which each have narrow host ranges, including races 2, 7 and 9 from *B. juncea*, *B. rapa* and *B. oleracea*, respectively (Hill *et al*., 1988; Pound and Williams, 1963). On the basis of the responses of different cultivars of *B. rapa* and *B. juncea*, races 2 and 7 have been further divided into pathotypes Ac2a, Ac2v, Ac7a and Ac7v (Rimmer *et al*., 2000). For example, Ac2a and Ac2v could be differentiated on the basis of being virulent on *B. juncea* cv. Burgonde and Cutlass, respectively. Similarly, A7a and Ac7v are virulent on *B. rapa* cv. Torch and Reward (Rimmer *et al*., 2000). The type specimen of *Al. candida* was collected from the invasive species *Capsella-bursa pastoris*, and was later designated as race 4. These races are highly specialized; however, they are not necessarily restricted to the host species from which they were originally collected. For example, isolates of race 7 collected from *B. rapa* can cause disease in many cultivars of *B. napus* under field conditions (Bernier, 1972; Fan *et al*., 1983). Similarly, a low frequency (less than 10%) of *A. thaliana* accessions are susceptible to standard isolates of *Al. candida* races 2, 4 and 7 in a conducive laboratory environment (Borhan *et al*., 2008; E. B. Holub, unpublished work). In contrast, *A. thaliana* is universally a nonhost of *Al. candida* race 9. It is important to note here that a molecular taxonomic distinction has been made between *Al. candida* and a species now called *Albugo laibachii* which commonly causes white rust in *A. thaliana* under natural field conditions (Holub *et al*., 1995; Rehmany *et al*., 2000; Thines *et al*., 2009; Voglmayr and Riethmüller, 2006). We have previously referred to this common parasite of wild *A. thaliana* as *Al. candida* ssp. *arabidopsis* (Borhan *et al*., 2008).

WRR4 was identified as a gene in *A. thaliana* Columbia (Col-0), which confers full immunity to *Al. candida* races 2, 4 and 7 when stably transformed into the accession Wassilewskija (Ws-3); this accession is susceptible in the laboratory to all three races (Borhan *et al*., 2008). *WRR4* also improved the partial resistance of Ws-3 to race 9, conferring full immunity in transgenic lines. RNA interference (RNAi) suppression of the defence regulator protein enhanced disease susceptibility 1 (EDS1) conferred full susceptibility to race 2, indicating that WRR4 is fully dependent on the expression of this lipase-like protein (Borhan *et al*., 2008; Parker *et al*., 1996). However, the same eds1-suppressed lines exhibited enhanced colonization to varying degrees by races 4, 7 and 9, but still exhibited residual resistance that restricted the formation of rust pustules (least restricted with race 7, most restricted with race 9). This indicates that Col-0 contains additional *WRR* genes which are independent of EDS1.

The purpose of the research described in this article was to determine whether *WRR4* from *A. thaliana* could confer white rust resistance in a brassica species. We began transgenic experiments using RNAi-mediated suppression of EDS1 in a white rust-resistant breeding line of *B. napus* (transformed with a construct designed from the *A. thaliana EDS1* gene) to confirm beforehand whether defence signalling via EDS1 was functionally intact in this crop species. We then transformed white rust-susceptible cultivars of *B. napus* (susceptible to race 7) and *B. juncea* (susceptible to race 2) with the *WRR4* gene from *A. thaliana*.

RESULTS

Resistance to *Al. candida* races 2 and 7 in *B. napus* is dependent on EDS1

We searched a *B. napus* expressed sequence tag (EST) database containing nearly 150 000 ESTs (generated at Saskatoon

Research Centre, Agriculture and Agri-Food Canada) for sequences that shared homology with *A. thaliana* EDS1 (referred to as At.EDS1). Eight *B. napus* ESTs were identified, and sequence assembly resulted in a single full-length open reading frame (ORF) of 1800 bp that encodes a lipase-like protein (referred to as Bn.EDS1). The DNA sequences of At.EDS1 and Bn.EDS1 share 71% identity when aligned, whereas the protein sequences share 61% identity (Figs S1 and S2). We also identified an EST of 634 bp from the *B. juncea* cv. Cutlass EST database (35 738 ESTs) (M. H. Borhan *et al*., unpublished work) with homology to the Arabidopsis *EDS1* ORF (BLASTN: *e*-value 0). The *B. juncea EDS1*-like EST was most similar to a *B. oleracea EDS1*-like gene (AJ620884) in the National Center for Biotechnology Information (NCBI) database (BLASTN: *e*-value $1e^{-129}$), and also a *B. napus EDS1*-like gene (EU6941108) (BLASTN: *e*-value $9e^{-72}$). The *B. juncea* EST showed 81.8% identity with the *B. napus EDS1* gene and 73.5% identity with the Arabidopsis *EDS1* gene.

Given the close homology between these two genes, we used the At.EDS1-RNAi construct described by Borhan *et al.* (2008) to suppress EDS1 in a white rust-resistant, doubled haploid breeding line of *B. napus* (DH12075). Three independent RNAi-transformed lines were identified using the herbicide selection marker phosphinotricin and confirmed by polymerase chain reaction (PCR). Seven-day-old seedlings of each T_1 line were inoculated with *Al. candida* isolate Ac7a, and each family segregated in a ratio of approximately 3 : 1 of resistant (no pustules) to susceptible (large, profuse pustules) phenotypes (Table 1; Fig. 1). We then tested T_2 lines of the susceptible T_1 generation from each independent transformant. These lines were uniformly susceptible to *Al. candida* isolate Ac2v (Table 1). The combined data from the RNAi suppression lines indicate that the natural white rust resistance in DH12075 to *Al. candida* races 2 and 7 is dependent on EDS1 expression.

Table 1 RNA interference suppression of enhanced disease susceptibility 1 (EDS1) and white rust resistance in *Brassica napus*.

Host generation	*Albugo* isolate	Line no.	Total	R	S
T_1	Ac7a	211	30	19	11
		415	37	27	10
		416	40	32	8
T_2	Ac2v	211–305	37	0	37
		416–240	47	0	47

EDS1 is a lipase-like protein that is commonly required for disease resistance conferred by TIR-NB-LRR (Toll-like/interleukin-1 receptor-nucleotide binding-leucine-rich repeat) *R* genes in *Arabidopsis thaliana*. Suppression of EDS1 was achieved using a construct designed from the *A. thaliana EDS1* gene. Transgenic lines were tested for susceptibility to *Albugo candida* races 2 (isolate Ac2v) and 7 (Ac7a) as follows: T_1 seedlings of three independent lines were assessed for resistance (R) or susceptibility (S) to Ac7a at 9 days after inoculation (dai), and T_2 seedlings derived from homozygous Ac7a-susceptible T_1 lines were assessed for response to Ac2v at 9 dai.

WRR4 from *A. thaliana* confers full resistance in transgenic *B. napus* and *B. juncea*

To test whether the *WRR4* gene from *A. thaliana* can confer white rust resistance in *B. napus* and *B. juncea*, we transformed a susceptible accession of each oilseed crop, including the *B. juncea* cv. Cutlass, which is susceptible to Ac2v, and the *B. napus* breeding line ACS-N32, which is susceptible to Ac7a. *Arabidopsis thaliana* WRR4 was expressed under its native promoter as described by Borhan *et al.* (2008).

Seven independent transgenic lines of *B. juncea* cv. Cutlass were identified containing a single insertion of *WRR4* from *A. thaliana* using the selectable herbicide marker phosphinotricin. We confirmed the number of insertions by PCR and Southern blots (Table 2). T_2 seeds were harvested from each transgenic line. Seven-day-old T_2 seedlings were inoculated with Ac2v; some segregated in a resistant to susceptible ratio of *c.* 3 : 1 (Table 2), confirming the single insertion of *WRR4* in each line. Growth of the parasite in a resistant transformant was usually localized to one to three penetrated cells at the site of infection (Fig. 2). However, a necrotic patch of cells was occasionally visible macroscopically which, under the microscope, was associated with more extensive growth of hyphae. A minute pustule rarely developed from these confined, necrosis-inducing colonies of *Al. candida.* The necrotic patch phenotype may be indicative of a heterozygous genotype; however, this was not confirmed.

Similarly, three transgenic lines from *B. napus* line ACS-N32 were obtained containing the *WRR4* gene from *A. thaliana.* T_2 progeny from these lines were inoculated with *Al. candida* isolate Ac7a and segregated for resistance (Table 2). As in *B. juncea*, WRR4-mediated resistance was typically associated with the restriction of the parasite to the site of infection (Fig. 3).

DISCUSSION

Two decades of *A. thaliana* molecular biology have been central in shaping our current understanding of innate defence in plants and have also had an impact on the understanding of infectious disease in animals (Chisholm *et al*., 2006; Dangl and Jones, 2001; Holub, 2007; Jones *et al*., 2008). Early contributions to crop improvement came from a precedent that a virulent bacterial pathogen could be genetically modified to deliver an avirulence effector from a crop pathogen, and then used as a physiological probe to identify the matching receptor-like *R* gene from a nonhost of the crop pathogen (Gassmann *et al*., 1999; Holub, 2001; Warren *et al*., 1998). This method holds great promise for the transient delivery of avirulence effectors from filamentous pathogens (Rentel *et al*., 2008; Sohn *et al*., 2007; Vleeshouwers *et al*., 2008). More importantly, the use of molecular markers derived from conserved domains in the most

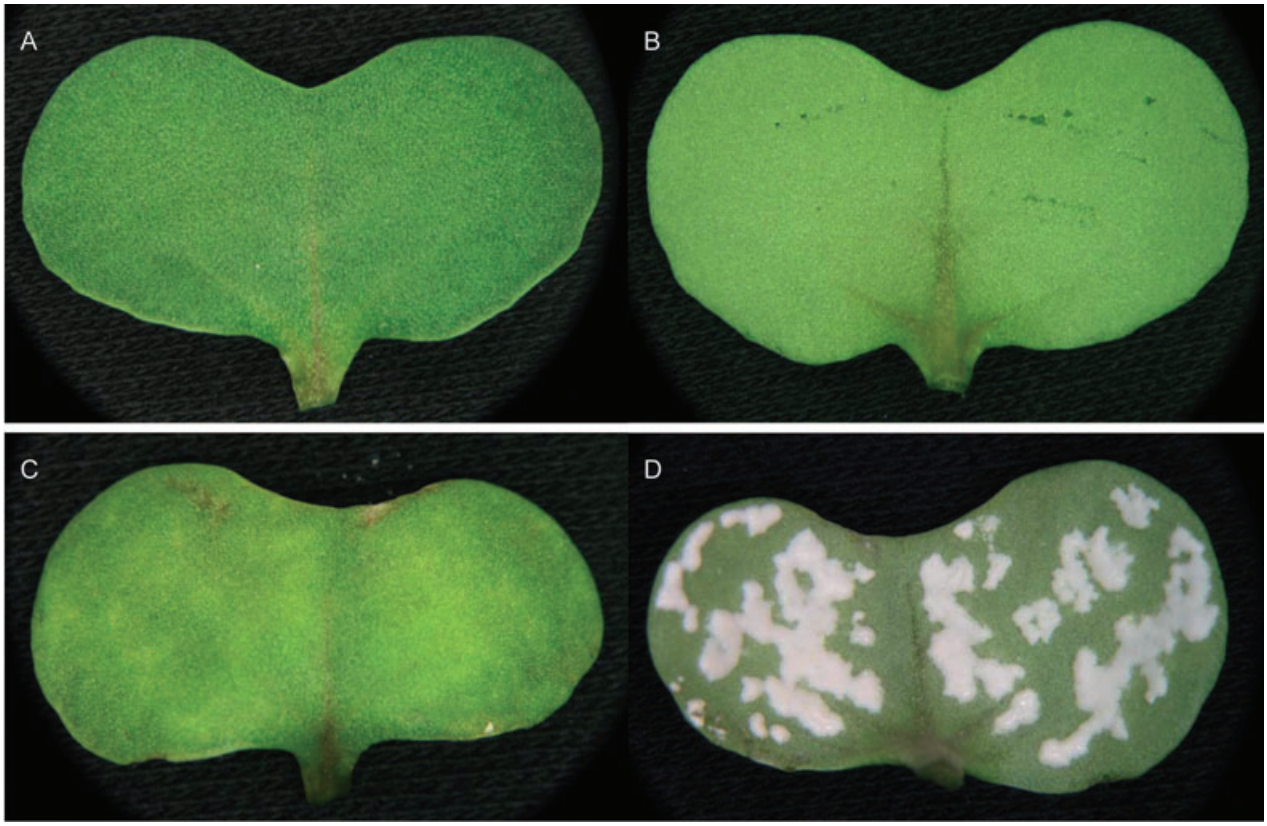

Fig. 1 White rust resistance to two avirulent isolates of *Albugo candida* (Ac2V and Ac7A) is suppressed in *Brassica napus* line DH12075 with RNA interference (RNAi) suppression of the lipase-like defence gene enhanced disease susceptibility 1 (*EDS1*). One-week-old seedlings were infected with either isolate, and interaction phenotypes (for Ac7A, shown here) were photographed 10 days later. (A, B) Top and bottom, respectively, of a fully resistant wild-type cotyledon. (C, D) Top and bottom of a cotyledon from a fully susceptible EDS1-RNAi-suppressed line.

Table 2 Segregation of white rust resistance amongst progeny of *Brassica juncea* cv. Cutlass and *B. napus* line ACS-N32 following transformation with the *WRR4* TIR-NB-LRR (Toll-like/interleukin-1 receptor-nucleotide binding-leucine-rich repeat) gene from *Arabidopsis thaliana*.

Brassica species	Line no.	No. of T_1 insertions	Total T_2	R	S	χ^2	*P*
B. juncea	1	1	42	30	12	0.28	0.59
	2	2	41	32	9	0.20	0.65
	3	2	35	23	12	1.60	0.20
	4	1	41	37	4	5.08	0.02
	5	4	9	9	0	3.00	0.08
	6	1	40	27	13	1.20	0.27
	7	1	40	31	9	0.13	0.71
	8	1	41	27	14	1.82	0.17
B. napus	1	4	41	37	4	5.08	0.02
	2	1	39	22	17	7.18	0.007
	3	1	25	18	7	0.12	0.72

The number of transgene insertions in the T_1 generation was determined by Southern blotting. The numbers of resistant (R; green cotyledon and no rust pustules) and susceptible (S; large pustules formed profusely on the underside of cotyledons) T_2 seedlings were recorded at 10 days after inoculation.

common NB-LRR, *R* and other essential defence response genes enables the marker-assisted selection of homologues in crops (Aarts *et al*., 1998; Botella *et al*., 1997; Leister *et al*., 1996; McHale *et al*., 2009; Shen *et al*., 1998). Lettuce provides a superb illustration of how even minor crops will benefit from this combined 'model-to-crop' knowledge transfer (Caldwell and Michelmore, 2009; McHale *et al*., 2009; Wroblewski *et al*., 2009).

Surprisingly, the direct use of *A. thaliana R* genes in transgenic crops has not been demonstrated, despite several examples of genes that could potentially confer broad-spectrum resistance to

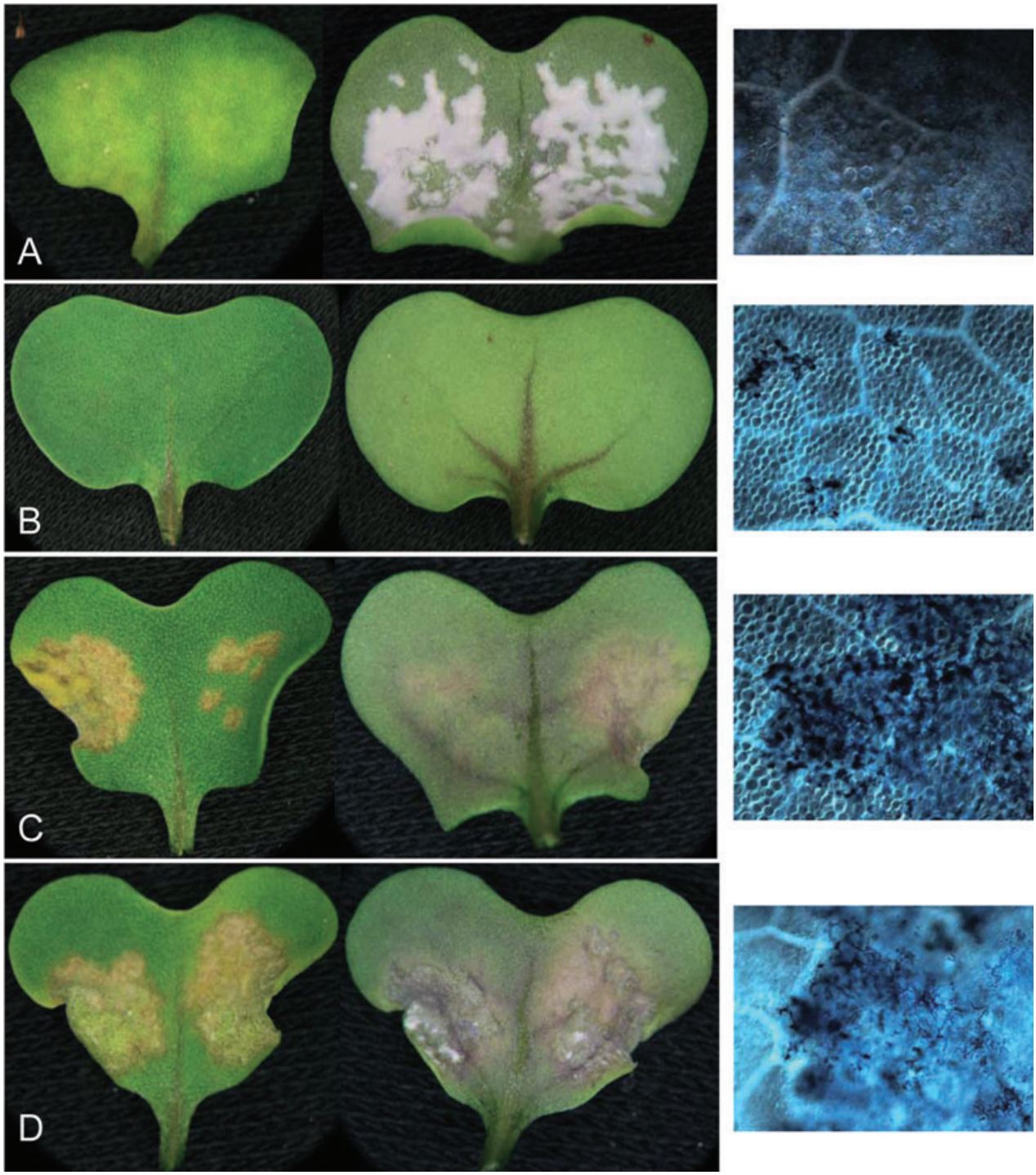

Fig. 2 *Arabidopsis thaliana WRR4* (WRR, white rust resistance) confers full resistance in the susceptible *Brassica juncea* cv. Cutlass. Photographs of cotyledons inoculated with the virulent isolate Ac2v at 10 days after inoculation. (A) Wild-type Cutlass exhibiting full susceptibility with profuse development of white pustules on the lower surface and no host cell necrosis visible microscopically (far right). (B–D) Cotyledons of *WRR4* transgenic Cutlass showing resistance phenotypes. (B) Full resistance with no formation of pustules on the upper or lower surfaces of the cotyledon; minute patches of necrotic cells are visible microscopically at the site of infection (far right). (C) Necrotic patches with no formation of pustules on the upper or lower surfaces of the cotyledon, and restriction of hyphae to cells surrounding the site of infection as shown by microscopy (far right). (D) Necrotic patch with a few minute sporadic pustules, and restriction of hyphae to cells surrounding the site of infection as shown by microscopy (far right).

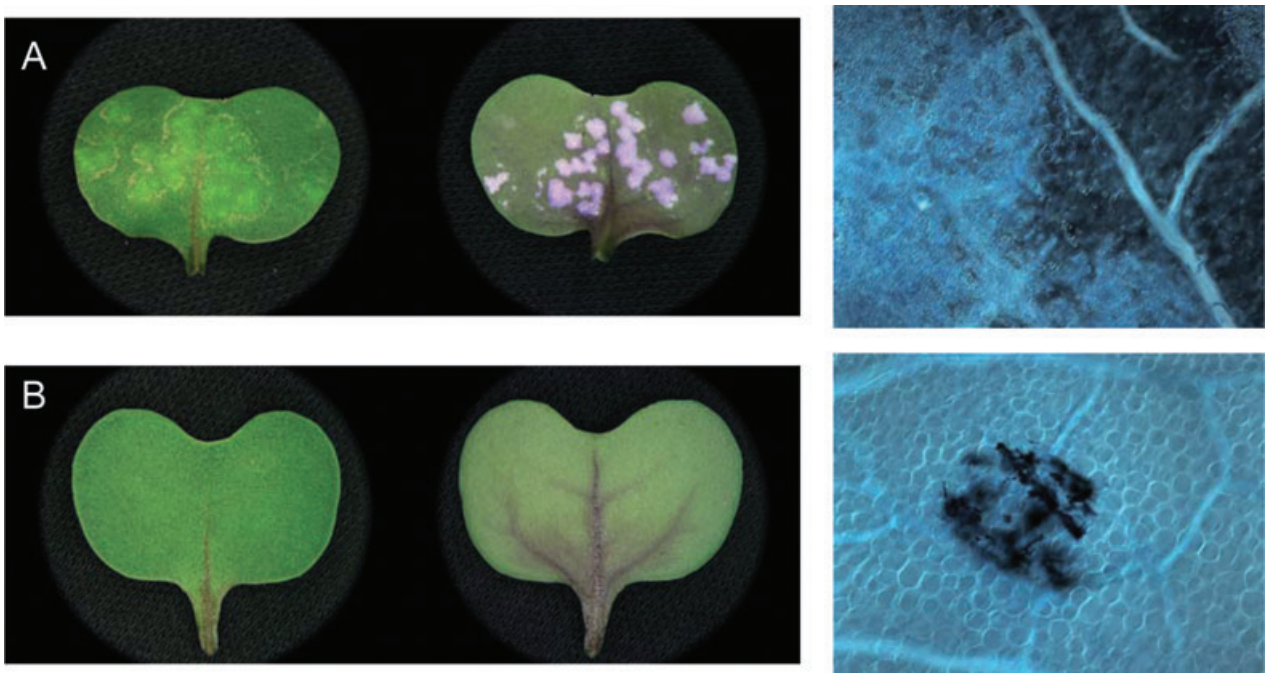

Fig. 3 The white rust resistance gene *WRR4* from *Arabidopsis thaliana* confers full resistance in a susceptible cultivar of *Brassica napus* (ACS-N32). (A) Upper and lower surfaces of a wild-type cotyledon 10 days after inoculation with the virulent isolate Ac7a, exhibiting full susceptibility with the profuse development of white pustules on the lower surface and no host cell necrosis visible microscopically (far right). (B) Upper and lower surfaces of an ACS-N32 cotyledon stably transformed with *WRR4*, indicating full resistance with no formation of pustules on the upper or lower surfaces of the cotyledon, and minute patches of necrotic cells visible microscopically at the site of infection (far right).

brassica crop pathogens (Cooley *et al*., 2000; Grant *et al*., 1995; Staal *et al*., 2006, 2008; Xiao *et al*., 2001). Thus, the observation that *WRR4* from *A. thaliana* confers white rust resistance in two oilseed brassica crops provides another important precedent for the utility of *A. thaliana* research. *Albugo candida* can cause severe yield losses in oilseed and vegetable crops of *B. juncea*, *B. oleracea* and *B. rapa*, with losses in oilseed mustard (*B. juncea*) often reaching 60% for small-holding farmers in India (Bernier, 1972; Li *et al*., 1996; Li *et al*., 2007; Petrie, 1973). *Brassica juncea* has better drought tolerance and more durable stem canker resistance (*L. maculans*) than *B. napus* oilseed rape (Marcroft *et al*., 2002), and canola-quality *B. juncea* varieties have therefore been developed to extend oilseed production in low-rainfall areas of Australia and Canada (Li *et al*., 2007). Unfortunately, white rust looms as a major threat to production in these areas.

The durability of a single broad-spectrum *R* gene for disease control in crops is not guaranteed, but will instead depend on how readily the pathogen can evolve variants to overcome host resistance (Leach *et al*., 2001). Pathogen effector molecules have been identified that correspond as the avirulence effector detected by broad-spectrum R proteins, including Bs2 from pepper and Rb from potato (Kearney and Staskawicz, 1990; Vleeshouwers *et al*., 2008). The corresponding avirulence effectors in these examples occur species wide in each respective pathogen and appear to be significantly constrained from evolution as a result of a high penalty of mutation. Thus, slow or nonevolving effectors represent a plausible expectation that warrants investigation in pathogens from the other examples of broad-spectrum R proteins, such as the predicted avirulence effector corresponding with *WRR4* that may be shared amongst physiological races of *Al. candida* (Borhan *et al*., 2008).

NB-LRR *R* genes may have evolved and expanded extensively as a gene family in plants because they typically induce defence only when it is likely to be beneficial after detection of an avirulent pathogen, and are unlikely to confer susceptibility to nontarget microorganisms. *R* genes, such as *Bs2*, *Rb*, *RLM3* and *WRR4*, provide the additional advantage of broad-spectrum disease control, and illustrate the potential for expanding the use of NB-LRR genes from wild species in crop improvement. Interestingly, a trade-off in enhanced susceptibility to nontarget pathogens has been observed for broad-spectrum resistance genes that do not encode NB-LRR proteins (Jarosch *et al*., 2003; Wang *et al*., 2007).

Genetic improvement of multiple agronomic traits (e.g. drought tolerance, nutrient-use efficiency, yield performance and disease resistance) in crops that have large and complex genomes will continue to benefit from underpinning investment in model plants (Bevan and Waugh, 2007). In particular, *A.*

thaliana is an excellent tool for crop scientists working with brassica crops, considering the significant synteny between the two genomes (Schranz *et al*., 2007). The results from the transgenic suppression of EDS1 and gain-of-function in resistance to *Al. candida* with *WRR4* indicate that brassica species contain the genes essential for the direct use of NB-LRR encoding other *R* genes from *A. thaliana* in brassica crops, such as the broad-spectrum stem canker resistance genes *RLM1* and *RLM3* (Staal *et al*., 2006, 2008). The advantage in both the white rust and stem canker examples is that economically devastating crop pathogens were strategically used from inception in the molecular genetics research of *A. thaliana*.

EXPERIMENTAL PROCEDURES

Pathogen handling and inoculation

Albugo candida races were propagated on the appropriate susceptible host (Rimmer *et al*., 2000). Pustules were harvested at 10–14 days after inoculation (dai) and stored at −20 °C, or used fresh to prepare the inoculum by suspending the spores in dH_2O at a concentration of 2×10^4/mL sporangia. Inoculum was incubated at 14–16 °C for 2–4 h to ensure the release of motile zoospores. This inoculum was kept on ice during the inoculation of 5–7-day-old brassica seedlings. A repeater pipette was used to place a 10-μL drop of the inoculum on each half of a cotyledon. Inoculated plants were kept at 14–16 °C under a micropropagator to maintain humidity. After 24 h, plants were transferred to a growth chamber with 12 h light, at temperatures of 18 °C during the night and 20 °C during the daytime. Cotyledon responses to *Al. candida* were scored at 7–14 dai.

Microscopy of infected tissues

To prepare inoculated tissues for microscopic observation of pathogen growth and plant response, cotyledons were excised from the seedlings at 7–14 dai, placed in a 50-mL Falcon tube submerged in trypan blue (Parker *et al*., 1996) and transferred to a boiling water bath for 20 min. After this, stain was replaced with chloral hydrate. Tissues were left in chloral hydrate for 24 h. Cleared tissues were placed on a glass slide in 50% glycerol, covered with a coverslip and observed using differential interference contrast (DIC) with a Zeiss (Oberkochen, Germany) Axio Imager Z1 microscope.

Brassica transformation

Hypocotyl explants of *B. napus* or *B. juncea* were used for *Agrobacterium tumefaciens* (GV3101-MP90)-mediated transformation (De Block *et al*., 1989). Plants were grown in tissue culture growth room conditions (22 ± 1 °C under 16 h light, 100 $\mu E/m^2/s$). Transgenic plants were selected on the basis of resistance to the herbicide phosphinotricin, transferred to soil and grown in the glasshouse (16 h light/8 h dark, 20 °C/17 °C). Transgenic plants were further confirmed by PCR. The WRR4 construct used for transformation has been described previously by Borhan *et al*. (2008).

Generation of *B. napus* EDS1-RNAi lines

The EDS1-RNAi construct described by Borhan *et al*. (2008) was transferred to the *B. napus* double haploid line DH12075, which is naturally resistant to *Al. candida* races 2 and 7. The response of seedlings of EDS1-suppressed lines was monitored at 7–14 dai with Ac2 or Ac7.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Fig. S1 Alignment of *Brassica napus* enhanced disease susceptibility 1 (EDS1) homologue and *Arabidopsis thaliana EDS1* open reading frames (ORFs). BLASTN search of *B. napus* sequence database identified eight expressed sequence tags (ESTs) with homology to the Arabidopsis *EDS1*. Assembly of these ESTs resulted in a cDNA of 2043 bp and an ORF of 1803 bp encoding a lipase-like protein. Underlined sequences show the interval of Arabidopsis *EDS1* used for the suppression of Brassica *EDS1* by RNA interference.

Fig. S2 Alignment of *Brassica napus* protein with homology to the lipase-like protein enhanced disease susceptibility 1 (EDS1) in *Arabidopsis thaliana*, which is required for several examples of TIR-NB-LRR (Toll-like/interleukin-1 receptor-nucleotide binding-leucine-rich repeat)-mediated disease resistance. The *B. napus* EDS1 open reading frame encodes a 600-amino-acid protein of 68.6 kDa with 70% similarity and 61% identity to *A. thaliana* EDS1.

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