Natural genetic resources of *Arabidopsis thaliana* reveal a high prevalence and unexpected phenotypic plasticity of *RPW8*-mediated powdery mildew resistance

Katharina Göllner[1], Patrick Schweizer[2], Yuling Bai[3] and Ralph Panstruga[1]

[1]Max-Planck Institute for Plant Breeding Research, Department of Plant–Microbe Interactions, Carl-von-Linné-Weg 10, 50829 Köln, Germany; [2]Leibniz-Institute of Plant Genetics and Crop Plant Research (IPK), Department of Cytogenetics and Transcriptome Analysis, Corrensstraße 3, 06466 Gatersleben, Germany; [3]Laboratory of Plant Breeding, Plant Sciences Group, Wageningen University, Droevendaalsesteeg 1, 6708 PB Wageningen, the Netherlands

Author for correspondence:
Ralph Panstruga
Tel: +49 (0)221 5062 316
Fax: +49 (0)221 5062 353
Email: panstrug@mpiz-koeln.mpg.de

Received: *3 July 2007*
Accepted: *17 September 2007*

Summary

- Here, an approach based on natural genetic variation was adopted to analyse powdery mildew resistance in *Arabidopsis thaliana*.
- Accessions resistant to multiple powdery mildew species were crossed with the susceptible Col-0 ecotype and inheritance of resistance was analysed. Histochemical staining was used to visualize archetypal plant defence responses such as callose deposition, hydrogen peroxide accumulation and host cell death in a subset of these ecotypes.
- In six accessions, resistance was likely of polygenic origin while 10 accessions exhibited evidence for a single recessively or semi-dominantly inherited resistance locus. Resistance in the latter accessions was mainly manifested at the terminal stage of the fungal life cycle by a failure of abundant conidiophore production. The resistance locus of several of these ecotypes was mapped to a genomic region containing the previously analysed atypical *RPW8* powdery mildew resistance genes. Gene silencing revealed that members of the *RPW8* locus were responsible for resistance to *Golovinomyces orontii* in seven accessions.
- These results suggest that broad-spectrum powdery mildew resistance in *A. thaliana* is predominantly of polygenic origin or based on *RPW8* function. The findings shed new light on the natural variation of inheritance, phenotypic expression and pathogen range of *RPW8*-conditioned powdery mildew resistance.

Key words: disease resistance, gene silencing, natural genetic variation, powdery mildew, RPW8.

New Phytologist (2008) **177**: 725–742

© The Authors (2008). Journal compilation © *New Phytologist* (2008)

doi: 10.1111/j.1469-8137.2007.02339.x

Introduction

The dicotyledonous reference species *Arabidopsis thaliana* exhibits a wide range of genetic variation among wild accessions. Recent studies exploiting this natural variation led to the identification and functional analysis of genes underlying ecologically relevant processes and complex traits. They provided new insights into aspects of genome evolution, geographic population structure and selective mechanisms, which shape complex trait variation in natural populations (reviewed in Mitchell-Olds & Schmitt, 2006). Polymorphisms between different *Arabidopsis* accessions have also been proven to represent important resources for the identification of gene function and the elucidation of genetic pathway structure. In this context, the adoption of recombinant inbred lines (RILs) of divergent parental ecotypes has been of great importance for mapping complex traits (Koornneef *et al.*, 2004).

Powdery mildews are parasitic Ascomycete fungi that cause widespread plant diseases resulting in severe losses of plant growth and yield (Agrios, 1988). These phytopathogens are

obligate biotrophs that require living host cells for proliferation and reproduction. Four powdery mildew species are known to establish compatible interactions with *A. thaliana*: *Golovinomyces* (formerly *Erysiphe*) *cruciferarum* (Koch & Slusarenko, 1990), *Golovinomyces* (formerly *Erysiphe*) *cichoracearum* (Adam & Somerville, 1996), *Golovinomyces orontii* (Plotnikova *et al.*, 1998), and *Oidium* (*neo-*)*lycopersici* (Xiao *et al.*, 2001). In many plant species resistance to powdery mildews is conferred by dominantly or semi-dominantly inherited genes which provide race- or isolate-specific protection against the fungal parasite. Examples of this type of disease resistance comprise the allelic series of *Mla* genes in barley (reviewed in Jørgensen, 1994), *Run1* in grapevine (Donald *et al.*, 2002; Barker *et al.*, 2005), the *Ol* genes in tomato (Huang *et al.*, 2000, Bai *et al.*, 2005) and *Rpp1* in rose (Linde & Debener, 2003; Linde *et al.*, 2004). Race-specific pathogen resistance is frequently mediated by proteins of the nucleotide binding site leucine-rich repeat (NB-LRR) type, which are widely assumed to guard host proteins from manipulation by invading pathogens (van der Biezen & Jones, 1998). Members of the NB-LRR polypeptide family are also encoded by alleles of the barley *Mla* powdery mildew resistance locus (Halterman *et al.*, 2001; Zhou *et al.*, 2001). However, to date, any evidence for isolate-specific powdery mildew resistance in *Arabidopsis* is lacking. Instead, known sources of resistance in *Arabidopsis* comprise induced resistance and natural broad-spectrum resistance conferred by alleles of the *RPW8* locus. The former group includes the set of *powdery mildew resistant* (*pmr*) mutants recovered from a forward genetic screen in the Col-0 ecotype (Vogel & Somerville, 2000; Vogel *et al.*, 2002, 2004) as well as the *enhanced disease resistance 1* (*edr1*) mutant (Frye & Innes, 1998) and the cellulose synthase mutant *cev1* (Ellis & Turner, 2001).

Natural resistance to powdery mildews in *Arabidopsis* was observed in several accessions and either shown or suggested to be mediated by the dominantly inherited *RPW8* locus in three ecotypes, Kas-1, Wa-1 and Ms-0 (Schiff *et al.*, 2001; Wilson *et al.*, 2001; Xiao *et al.*, 2001). The *RPW8* locus of Ms-0 comprises two dominantly inherited polymorphic *R* genes, *RPW8.1* and *RPW8.2*, which control resistance to a broad range of powdery mildew pathogens (Xiao *et al.*, 2001). Additionally, the Ms-0 *RPW8* locus harbours the sequence-related paralogues *HR1*, *HR2* and *HR3*, which do not contribute to resistance. *RPW8.1* and *RPW8.2* encode proteins that are structurally different from other resistance (R) proteins identified to date since the only recognizable polypeptide features are a predicted coiled-coil (CC) and an assumed transmembrane (TM) domain. Resistance mediated by these genes involves activation of defence responses which are activated via a salicylic acid (SA) amplification loop and ultimately result in a hypersensitive response (HR) of the attacked cell (Xiao *et al.*, 2003a, 2005). In Kas-1, three independent resistance loci were identified. The strongest of them, *RPW10*, is presumably identical to *RPW8* (Wilson *et al.*, 2001). Similarly, in Wa-1, two loci were identified, also the strongest (*RPW13*) probably identical to *RPW8*. Based on comprehensive comparative DNA sequence analysis and correlation with phenotypic powdery mildew resistance it was proposed that *RPW8* represents the main source of broad-spectrum resistance to powdery mildews in *Arabidopsis* (Xiao *et al.*, 2004). However, given the assumed dosage-dependency of RPW8 function and its requirement for a range of components of basal defence (Xiao *et al.*, 2005) a mere biocomputational analysis of coding sequences has limited predictive power for the actual contribution of RPW8 family members to powdery mildew resistance in individual accessions of diverse biogeographical origin. Experimental data are thus desirable to support the previous biocomputational predictions on RPW8 function (Xiao *et al.*, 2004).

In the present study, natural genetic variation of *Arabidopsis* was used as a resource to screen for potentially novel types of resistance to powdery mildews. Extensive genetic analysis revealed *RPW8* and polygenic resistance as major sources of powdery mildew resistance in many *Arabidopsis* accessions. Based on the assessment of classical defence markers such as the accumulation of reactive oxygen species, callose deposition and host cell death, our analysis indicates that the phenotypic expression of *RPW8*-mediated resistance varies considerably among accessions.

Materials and Methods

Growth conditions of *Arabidopsis* plants and inoculation procedures

Plants were sown on soil substrate, stratified for 2 d at 4°C in darkness and transferred for 4–5 wk at a day/night cycle of 10 : 14 h to a light chamber with 22°C : 20°C day/night temperature and a relative humidity of 60%. For mapping experiments, F_2 plants of the respective mapping population were grown in 96-well trays on soil together with the respective parents as controls. Nottingham *Arabidopsis* Stock Centre (NASC; http://arabidopsis.info/) designations of plant lines are listed in Table 1. Additional lines used were: Bay-0 (N954), Kas-1 (N903), Ms-0 (N905), *pmr6-3* (Vogel *et al.*, 2002) and the core set of 165 recombinant inbred lines Bay × Shahdara (Sha; N57921; Loudet *et al.*, 2002).

Golovinomyces orontii was cultivated on *NahG* (Delaney *et al.*, 1994) or *eds16* (Nawrath & Metraux, 1999; Wildermuth *et al.*, 2001) mutant *A. thaliana* plants (similar day/night conditions as above and 80% humidity). Ten to eleven days postinfection (dpi), these plants were used to inoculate new plants by brush inoculation. For determination of host cell entry rates, analysis of hyphal growth with HYPHAREA (a dedicated image analysis software; Seiffert & Schweizer, 2005; see also later) and conidiophore production, lower inoculation densities were used to avoid overlapping colonies. The Wageningen isolate of *O. neolycopersici* was maintained on the susceptible tomato cultivar Moneymaker as described

 © The Authors (2008). Journal compilation © *New Phytologist* (2008)

Table 1 Infection phenotypes of selected *Arabidopsis thaliana* accessions upon challenge with four distinct powdery mildew species

		Origin		DR score[1]				
Accession	NASC[2] code	City	Country	*Go*[3]	*Gci*[3]	*Gcr*[3]	*On*[3]	F_1[4]
Ang-0	N948	Angleur	Belgium	0	0	2	0–1	2
Bu-0	N1006	Burghaun/Rhön	Germany	0–1	3	0	n.t.	2
Bu-3	N1010	Burghaun/Rhön	Germany	0	0	2	n.t.	2
Bu-15	N1034	Burghaun/Rhön	Germany	0	2	0–1	n.t.	1–2
C24	N906	Coimbra	Portugal	0	0	0	n.t.	2
Co-1	N1084	Coimbra	Portugal	0	0	0	0	3
Do-0	N1112	Donsbach/Westerwald	Germany	0	3	0	2	3
Ei-4	N1126	Eifel	Germany	0	0	0	n.t.	1–2
La-1	N970	Landsberg-Warthe	Germany	0	0	0	1	2–3
Nok-3	N1404	Noordwijk	Netherlands	0	0	0	0	3
Nw-0	N1408	Neuweilnau	Germany	0	0	0	n.t.	1–2
Ob-0	N1418	Oberursel/Hasen	Germany	0–1	0	3	n.t.	3
Ove-0	N1434	Ovelgoenne	Germany	0–1	2	0	n.t.	3
Petergof	N926	Petergof	Russia	0	0	0	n.t.	3
Pla-3	N1464	Playa de Aro	Spain	0	0	0	n.t.	3
Pla-4	N1466	Playa de Aro	Spain	0	2	0	n.t.	0
Rak-2	N1484	Raksice	Czech Republic	0	2	0	n.t.	1–2
Sha	N929	Palmiro-Alay	Tajikistan	0	0	0	3	2
Sorbo	N931	Tajikistan	Tajikistan	0	0	0	3	2
Ts-7	N1562	Tossa de Mar	Spain	0	0	0	n.t.	1–2
Uk-1	N1574	Umkirch	Germany	0	0	0	n.t.	2
Wt-2	N1606	Wietze	Germany	0	0	0	n.t.	3
Wt-3	N1609	Wietze	Germany	0–1	3	0	n.t.	2

[1]Disease reaction score: 0 fully, and 1 intermediate resistant; 2 intermediate, and 3 fully susceptible; n.t., not tested.
[2]NASC, Nottingham *Arabidopsis* Stock Centre.
[3]*Go*, *Golovinomyces orontii*; *Gci*, *Golovinomyces cichoracearum*; *Gcr*, *Golovinomyces cruciferarum*; *On*, *Oidium neolycopersici*; please note that DR scores for *Gci* and *Gcr* were taken from Adam *et al*. (1999).
[4]DR score of F_1 progeny of a crossing with the susceptible Col-0 ecotype upon inoculation with *G. orontii*.

by Bai *et al.* (2005) and spray-inoculated at a density of 5×10^4 to 2×10^5 spores ml^{-1}.

Determination of infection phenotypes

Between 7 and 9 dpi (*G. orontii*) or 8 and 15 dpi (*O. neolycopersici*), infection phenotypes were scored macroscopically at two or three time-points. Plants were assigned disease reaction (DR) scores ranging from 3 to 0. A DR score of 3 describes fully susceptible plants, indicating that strong pathogen growth was observed while DR score of 0 refers to fully resistant plants, on which no fungal structures and disease symptoms could be detected macroscopically. A DR score of 2 denotes intermediate susceptible plants, and DR score of 1 designates intermediate resistant plants, which show only little fungal structures on the leaf surface.

During the screening of *A. thaliana* ecotypes, at least five plants per ecotype were inoculated with *G. orontii*. Accessions and RILs scored as resistant in the first round were analysed in a second experiment to confirm the observed phenotype. Inoculations were performed together with the susceptible Col-0 ecotype as a reference and control. Resistant accessions were also analysed microscopically. Assessment of *O. neolycopersici* infection phenotypes is based on 10 plants per ecotype and six plants per RIL individual.

Histological analysis

Whole leaves of 4–5-wk-old plants were cleared in an alcoholic lactophenol solution (one volume of phenol–glycerol–lactic acid–water (1 : 1 : 1 : 1) and two volumes of ethanol) for a minimum of 4 d. For staining of epiphytic fungal infection structures they were washed in 50% ethanol and then incubated for approx. 1 min in a 0.25% (in ethanol) Coomassie Brilliant Blue R250 (Fluka, Buchs, Switzerland) solution followed by washing twice in water. For microscopic analysis, leaves were mounted in 50% glycerol and observed under bright-field conditions.

Host cell entry

Five to seven inoculated leaves of three plants per line were harvested at 48 h postinfection (hpi) followed by clearing and subsequent Coomassie Brilliant Blue staining.

© The Authors (2008). Journal compilation © *New Phytologist* (2008)

Analysis of fungal growth with HYPHAREA

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 24, 48 and 63 hpi. At least 20 images of single colonies per line and time-point were taken and analysed with the previously described HYPHAREA pattern recognition software (Seiffert & Schweizer, 2005). Briefly, this program calculates the number of pixels on micrographs of Coomassie Brilliant Blue-stained powdery mildew colonies, yielding a quantitative approximation of fungal growth.

Quantification of conidiophores per colony

Three plants per line were inoculated with *G. orontii* using a low inoculation density to avoid overlap of fungal colonies. At 7 dpi, four leaves per plant (three plants per line) were harvested and destained in lactophenol solution. For analysis, fungal structures were stained with Coomassie Brilliant Blue and leaves were analysed by light microscopy at low magnification and conidiophores of at least 20 different colonies per line were counted with WCIF IMAGE J software using the cell counter plug-in (http://www.uhnresearch.ca/facilities/wcif/imagej; the IMAGE J plug-in was written by Kurt De Vos, University of Sheffield).

3,3-Diaminobenzidine (DAB) staining

At 48 hpi three leaves of each plant (two plants per line) were harvested and stained with 3,3-diaminobenzidine (DAB; Sigma-Aldrich, Germany; Thordal-Christensen *et al.*, 1997). After clearing of leaves by boiling in 70% ethanol for 5 min, fungal structures were stained with Coomassie Brilliant Blue and leaves were analysed by light microscopy. Between 54 and 214 fungal colonies were analysed per plant line.

Trypan Blue staining

At 7 dpi four leaves per plant (two plants per line) were harvested and boiled in Trypan Blue (Sigma-Aldrich, Germany) solution (Peterhänsel *et al.*, 1997). Subsequently the leaves were destained in chloral hydrate (Sigma-Aldrich, Taufkirchen, Germany) and analysed by light microscopy to visualize dead cells as well as fungal structures.

Aniline Blue staining

Three leaves per plant (three plants per line) were destained in lactophenol solution and then, after washing in 50% ethanol, incubated for 2 d in Aniline Blue (Sigma-Aldrich, Germany) solution (Adam & Somerville, 1996). After staining of fungal structures with Coomassie Brilliant Blue, leaves were analysed microscopically with UV-light excitation.

Nucleic acid preparation

Genomic plant DNA was prepared following the protocol from Edwards *et al.* (1991) or from Xin *et al.* (2003) (for plants of mapping populations). RNA extraction was performed with a combination of two methods, from step 2 to 7 with Tri-Reagent (Sigma-Aldrich) according to the manufactures instructions, afterwards continued with the RNeasy Kit (Qiagen, Hilden, Germany).

DNA sequencing

DNA sequences were determined by the Automatische DNA-Isolierung und Sequenzierung (ADIS-Unit) at the Max-Planck Institute for Plant Breeding Research on Applied Biosystems (Weiterstadt, Germany) Abi Prism 377 and 3700 sequencers using Big Dye-terminator chemistry. Polymerase chain reaction (PCR) products were purified with either the Nucleospin (Macherey-Nagel, Düren, Germany) or Qiagen extract kits.

Mapping

For a first rough localization of the target gene in the genome, resistant F_2 plants of a mapping population were analysed with the simple sequence length polymorphism (SSLP) marker set described in Lukowitz *et al.* (2000). When there was a putative association with markers on a chromosome, additional F_2 plants were screened with these and further markers. When the target gene region was restricted to *c.* 5 Mb, fine mapping was initiated. Genomic DNA from approx. 1000 F_2 plants was analysed with two flanking markers to identify recombinant plants. These were inoculated with *G. orontii* to determine the infection phenotype. Sequences of mapping primers are listed in the Supplementary Material, Table S5.

Mapping with RILs

Two to six individuals per line of the Bay × Shahdara RIL population (NASC designation N57921) were inoculated with *G. orontii* or *O. neolycopersici* to determine infection phenotypes. Results were analysed with WINQTL cartographer software (Wang *et al.*, 2006; Composite Interval Mapping, CIM) on the basis of published marker data for the RIL population (Loudet *et al.*, 2002). For *G. orontii*, the resulting target gene region was further analysed with additional PCR-based markers (see the Supplementary Material, Table S5).

Vector construction and plant transformation

RPW8.1 and *RPW8.2* cDNAs were amplified with the two Gateway-compatible primer pairs R81_F plus R81_R and R82_F plus R82_R, respectively (Supplementary material Table S5). For double-stranded RNA interference (dsRNAi)-mediated transcript depletion of *RPW8.1* and *RPW8.2*, selected

 © The Authors (2008). Journal compilation © *New Phytologist* (2008)

Arabidopsis accessions were transformed with the pJawohl8 gene silencing vector (GenBank accession number AF408413) containing the cDNA of either *RPW8.1* or *RPW8.2* as inverted repeats. The Gateway kit from Invitrogen (Carlsbad, CA) was used to introduce the respective cDNA sequences into the binary pJawohl8 vector (kindly provided by Dr Bekir Ülker) and plants were transformed via *Agrobacterium*-mediated transformation (Clough & Bent, 1998). The bulked seeds of T_0 plants were sown on soil and transformed T_1 seedlings were selected after 1–2 wk by spraying with a 1% solution of the herbicide glufosinate ammonium (Basta; Hoechst Schering AgrEvo, Frankfurt, Germany).

Reverse transcription and real-time PCR

The Superscript II Kit from Invitrogen was used to transcribe 400–800 ng RNA to cDNA. DNA levels during the PCR run on ABI Prism 7700 were visualized with Brilliant SybrGreen (Stratagene, Heidelberg, Germany) according to the manufacturer's instructions. Each sample was represented by three replicates from the same cDNA preparation. Calculations of relative transcript levels were performed based on the ΔΔCt method and normalized to the reference gene actin as suggested by Pfaffl (2001). Primers for *RPW8.1* were 5UTR-R81a and 3UTR-R81a, for *RPW8.2* and 5UTR-R82a and 3UTR-R82a, as well as Actin1_F and Actin1_R for actin (see the Supplementary Material, Table S5). The PCR-cycler conditions were as follows: 95°C for 2 min, 49 cycles of 95°C for 30 s, 58°C for 30 s and 72°C for 1–2 min, then 72°C 3 min.

Results

Selection of accessions and genetic analysis of inheritance of resistance

To preselect for ecotypes with a broad range of powdery mildew resistance, 64 accessions which were resistant to either one or to both of the previously tested powdery mildew species (*G. cichoracearum*, *G. cruciferarum*; Adam *et al.*, 1999) were analysed for their infection phenotypes with a third powdery mildew species, *G. orontii*. A group of 23 accessions were macroscopically resistant to *G. orontii* and therefore chosen for further analysis (Table 1). Of these, 13 accessions (57%) showed broad-spectrum resistance to all three powdery mildew species while the remaining ecotypes exhibited various constellations of resistance to the three powdery mildews (Table 1; please note that DR scores for *G. cichoracearum* and *G. cruciferarum* were not assessed in this work but taken from Adam *et al.*, 1999). The selected accessions originate from different parts of the world with a strong emphasis on Europe, reflecting the bias of the currently available stock collections. With three ecotypes from Spain (Pla-3, Pla-4 and Ts-7), two from Portugal (C24, Co-1) and two from central Asia (Sha and Sorbo from Tajikistan), accessions originating from regions representing the suggested refugia of *A. thaliana* during the last ice age (Sharbel *et al.*, 2000, Schmid *et al.*, 2006) were included in the set.

To determine the mode of inheritance of disease resistance, the selected resistant accessions were crossed with the susceptible Col-0 ecotype (serving as the pollen donor). All but one of the F_1 populations were macroscopically susceptible (Table 1), although to different degrees. Progeny of *c.* 40% of the accessions showed full susceptibility comparable to Col-0 (disease reaction (DR) score 3 (Adam *et al.*, 1999; see also the Materials and Methods section); Co-1, Do-0, La-1, Nok-3, Ob-0, Ove-0, Petergof, Pla-3 and Wt-2), while *c.* 55% were intermediately susceptible (DR score 2 or 1-2 for Ang-0, Bu-0, Bu-3, Bu-15, C24, Ei-4, Nw-0, Rak-2, Sha, Sorbo, Ts-7, Uk-1, Wt-3). Only one macroscopically resistant F_1 population (derived from Pla-4; Table 1) was identified by this approach.

To assess whether the resistance trait is supposedly monogenic or determined by more than one gene, segregation of resistance was tracked in the respective F_2 generations. We focused on accessions with fully or intermediately susceptible F_1 progeny (DR score 3 or 2; total of 16 ecotypes; Table 2) to avoid rediscovery of *RPW8*, which has been reported to be dominantly inherited in accession Ms-0 (Xiao *et al.*, 2001). Based on χ^2 test analysis, segregation of resistance was compatible ($P \geq 0.05$) with a 3:1 (susceptible : resistant) segregation pattern for 10 accessions (Table 2). For four of these 10 accessions (Pla-3, Sha, Sorbo, Uk-1), segregation of resistance also agreed with an 1 : 2 : 1 (susceptible : intermediate susceptible : resistant) ratio (Table 2). In the case of six accessions (Ang-0, Bu-0, Bu-3, Ob-0, Petergof and Wt-2), none of these segregation patterns was supported by χ^2 analysis (Table 2). Resistance in these accessions is probably of multigenic origin, resulting in complex segregation. Accessions compatible with either 3:1 or 1:2:1 segregation (i.e. $P \geq 0.05$) were selected for further analysis. Altogether, a total of 10 accessions were chosen which showed the following characteristics: (1) resistance to at least two different powdery mildew species, one of them being *G. orontii*; (2) the F_1 progeny of a cross with the susceptible Col-0 ecotype was macroscopically susceptible; and (3) the respective F_2 progeny segregated according to Mendel's laws for a monogenic trait in either a recessive or intermediate hereditary path.

Comparative microscopic analysis of fungal pathogenesis on resistant accessions

To determine whether resistance occurs at the same stage of fungal pathogenesis and to unravel whether quantitative differences in resistance can be observed, a subset of the 10 selected accessions was microscopically analysed following challenge with *G. orontii*. Since hyphal growth requires host cell penetration and establishment of the fungal feeding organ (haustorium), formation of secondary powdery mildew hyphae is a reliable indicator of successful plant cell penetration and

© The Authors (2008). Journal compilation © *New Phytologist* (2008)

Table 2 Segregation of resistance to *Golovinomyces orontii* in selected *Arabidopsis thaliana* accessions

		Number of F_2 plants in each group					Segregation 3 : 1					Segregation 1 : 2 : 1							
							Observed		Expected			Observed			Expected				
Accession	Resistance spectrum[1]	DR[2] 3	DR 2	DR 1	DR 0	Total	S	R	S	R	P (χ^2 test)	S	I	R	S	I	R	P (χ^2 test)	Mapping
Ang-0	*Go*, *Gci*	13	17	29	32	91	59	32	68.25	22.75	0.0251	13	46	32	22.75	45.5	22.75	0.018826	No success
Bu-0	*Go*, *Gcr*	4	12	34	40	90	50	40	67.5	22.5	2E-05	4	46	40	22.5	45	22.5	5.45E-07	n.p.
Bu-3	*Go*, *Gci*	13	5	21	53	92	39	53	69	23	5E-13	13	26	53	23	46	23	4.68E-12	n.p.
C24	*Go*, *Gci*, *Gcr*	7	39	19	15	80	65	15	60	20	0.1967	7	58	15	20	40	20	0.000136	No success
Co-1	*Go*, *Gci*, *Gcr*	8	35	24	23	90	67	23	67.5	22.5	0.9031	8	59	23	22.5	45	22.5	0.001054	Chromosome III
Do-0	*Go*, *Gcr*	5	38	32	16	91	75	16	68.25	22.75	0.1022	5	70	16	22.75	45.5	22.75	4.93E-07	Chromosome III
La-1[3]	*Go*, *Gci*, *Gcr*	140	n.d.	n.d.	44	184	140	44	138	46	0.7335	140	0	44	46	92	46	1.96E-62	Chromosome III
Nok-3	*Go*, *Gci*, *Gcr*	44	29	1	18	92	74	18	69	23	0.2286	44	30	18	23	46	23	2.47E-06	Chromosome III
Ob-0	*Go*, *Gci*	60	17	10	5	92	87	5	69	23	1E-05	60	27	5	23	46	23	2.05E-18	n.p.
Ove-0[3]	*Go*, *Gcr*	73	n.d.	n.d.	19	92	73	19	69	23	0.3355	73	0	19	23	46	23	1.81E-34	No success
Petergof[3]	*Go*, *Gci*, *Gcr*	88	n.d.	n.d.	5	93	88	5	69.75	23.25	1E-05	88	0	5	23.25	46.5	23.25	4.31E-53	n.p.
Pla-3	*Go*, *Gci*, *Gcr*	31	25	18	17	91	74	17	68.25	22.75	0.1639	31	43	17	22.75	45.5	22.75	0.101144	n.p.
Sha	*Go*, *Gci*, *Gcr*	13	28	0	13	54	41	13	40.5	13.5	0.8751	13	28	13	13.5	27	13.5	0.96364	Chromosome III
Sorbo	*Go*, *Gci*, *Gcr*	18	15	4	12	49	37	12	36.75	12.25	0.9343	18	19	12	12.25	24.5	12.25	0.139542	Chromosome III
Uk-1	*Go*, *Gci*, *Gcr*	11	17	14	7	49	42	7	36.75	12.25	0.0833	11	31	7	12.25	24.5	12.25	0.128604	n.p.
Wt-2[3]	*Go*, *Gci*, *Gcr*	60	n.d.	n.d.	32	92	60	32	69	23	0.0302	60	0	32	23	46	23	2.1E-24	No success

R, resistant; S, susceptible; I, intermediate. n.d., not determined; n.p., not performed.

[1]*Go*, resistant to *G. orontii*, *Gci* resistant to *Golovinomyces cichoracearum*, *Gcr* resistant to *Golovinomyces cruciferarum*; please note that infection phenotypes for *Gci* and *Gcr* were taken from Adam *et al*. (1999).

[2]DR, disease reaction score: 0 fully, and 1 intermediate resistant; 2 intermediate, and 3 fully susceptible.

[3]Intermediate and fully susceptible plants were grouped together for the F_2 progeny of these accessions.

 © The Authors (2008). Journal compilation © New Phytologist (2008)

haustorium differentiation, an early event of powdery mildew pathogenesis. The susceptible ecotype Col-0, the penetration resistant *Atmlo2 Atmlo6 Atmlo12* triple mutant (*Atmlo2/6/12*; Consonni *et al*., 2006), the resistant *pmr6*-3 mutant for which resistance to powdery mildew was reported to occur at later stages of fungal development (Vogel *et al*., 2002) as well as Ms-0, which shows resistance to several powdery mildews based on presence of the atypical *R* gene *RPW8* (Xiao *et al*., 2001), served as controls. In the susceptible Col-0 ecotype a host cell entry rate of 81% was observed, while resistant Ms-0 revealed reduced fungal entry (30%; Fig. 1a). As previously demonstrated (Consonni *et al*., 2006), fungal sporelings were not able to enter any epidermal cell of the highly resistant *Atmlo2/6/12* triple mutant (entry rate 0%; Fig. 1a). Compared with the Col-0 ecotype, the selected macroscopically resistant accessions as well as the *pmr6-3* mutant showed moderately reduced levels of fungal entry (37–68%; Fig. 1a). These data indicate that, unlike on the penetration resistant *Atmlo2/6/12* triple mutant, fungal pathogenesis is not terminated early on the resistant accessions.

After establishment of the first haustorium the fungal pathogen differentiates secondary hyphae, which in turn attempt to penetrate neighbouring host cells to establish further haustoria enabling expansion of fungal colonies. To comparatively track hyphal growth rates we measured the average size of fungal micro-colonies in a time course experiment. To this end, the recently developed HYPHAREA software (Seiffert & Schweizer, 2005) was employed for semi-automatic quantification of stained fungal microcolony dimensions at 24, 48 and 63 h hpi (for details see the Materials and Methods section). The selected resistant accessions Do-0, Sha and Sorbo, individuals of the F_1 progeny of Sha × Col-0 and Sorbo × Col-0 crossings, as well as the controls Col-0 (susceptible) and Ms-0 (*RPW8* genotype, resistant) were analysed in this experiment. Except for Do-0 and Sha × Col-0 F_1 progeny, which showed somewhat reduced (Do-0) or enhanced (Sha × Col-0 F_1) hyphal growth at 48 hpi, there were no major differences in hyphal expansion between the tested lines at early fungal colony development (24 hpi and 48 hpi; Fig. 1b). Compared with susceptible Col-0, at 63 hpi the area of fungal microcolonies was significantly smaller in resistant accessions Ms-0 and Do-0 (Fig. 1b). Interestingly, resistant Sha did not exhibit any reduction in hyphal growth, while Sorbo and the respective F_1 progenies of Sha × Col-0 and Sorbo × Col-0 crossings showed only a minor reduction in microcolony areas (Fig. 1b). Similar to powdery mildew entry rates discussed earlier (Fig. 1a), the pace of fungal microcolony development does not differ greatly between the susceptible Col-0 ecotype, susceptible F_1 progenies and the tested resistant accessions, suggesting that resistance becomes effective at even later stages of fungal pathogenesis.

Around 7 dpi *G. orontii* reproduces on susceptible plants by producing numerous conidiophores per colony, each typically carrying three to five conidiospores (Plotnikova *et al*.,

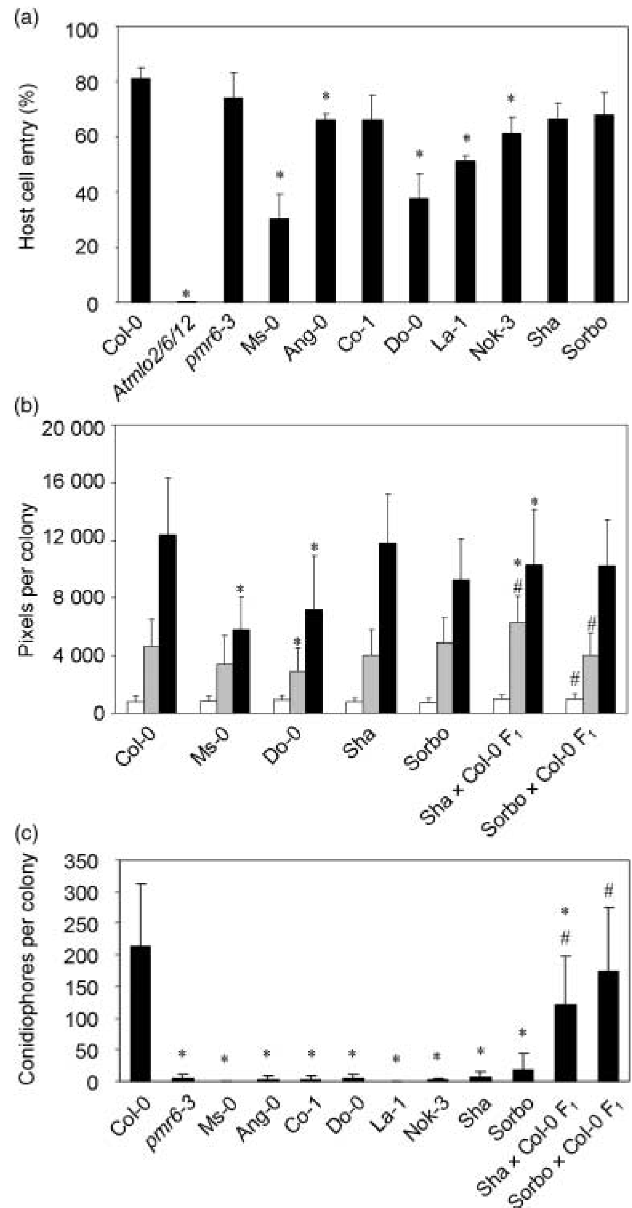


Fig. 1 Quantitative analysis of the development of *Golovinomyces orontii* infection structures on selected resistant *Arabidopsis thaliana* accessions. (a) Quantitative assessment of host cell entry rates. Data represent mean ± SD of three experiments, each based on five to seven leaves per plant line. *, Significant difference from Col-0 (Student's *t*-test, $P < 0.01$). (b) Quantitative analysis of hyphal growth between 24 h and 63 h postinfection (hpi). Microscopic images were taken at 24 (open bars), 48 (tinted bars) and 63 (closed bars) hpi with *G. orontii*. The number of pixels per fungal colony was determined with the HYPHAREA software (see the Materials and Methods section). Data represent mean ± SD of at least 20 micrographs per plant line and time-point. *, Significant difference from Col-0 (Student's *t*-test, $P < 0.01$); #, significant difference of F_1 plants from their respective resistant parental ecotype (Student's *t*-test, $P < 0.01$). (c) Production of conidiophores per colony at 7 d postinfection (dpi) with *G. orontii*. Data represent mean ± SD of at least 20 fungal colonies per plant line. *, Significant difference from Col-0; #, significant difference of F_1 plants from their respective resistant parental ecotype (Student's *t*-test, $P < 0.01$).

© The Authors (2008). Journal compilation © *New Phytologist* (2008)

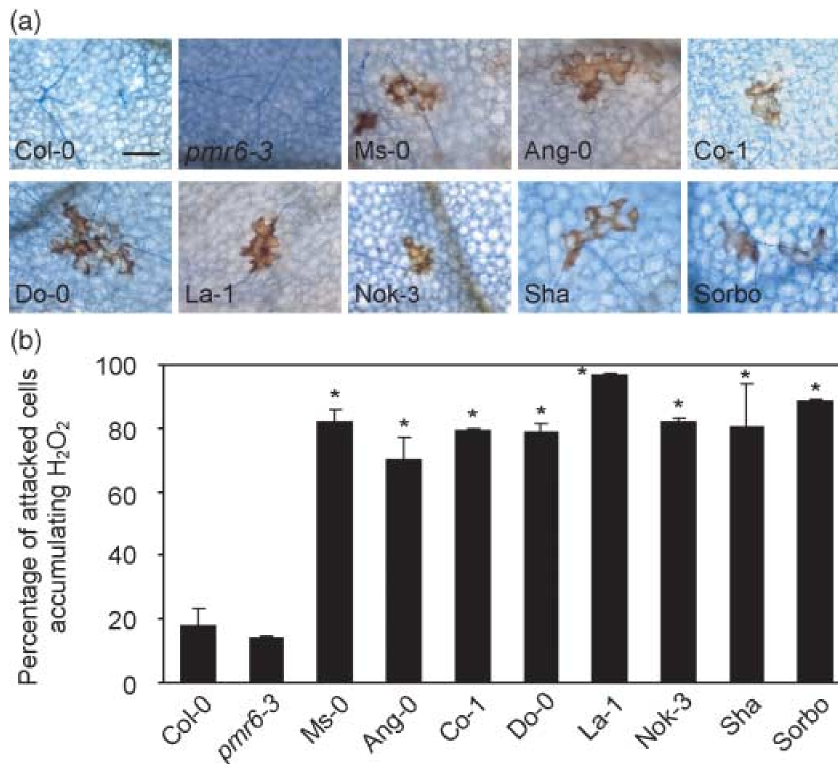


Fig. 2 Local hydrogen peroxide (H_2O_2) accumulation in response to powdery mildew attack. *Arabidopsis thaliana* leaves were harvested at 48 h post infection (hpi) with *Golovinomyces orontii* and following 3,3′-diaminobenzidine (DAB) staining analysed by light microscopy. (a) Representative micrographs of DAB-stained leaf samples. Bar, 100 μm. (b) Quantification of attacked epidermal cells showing local H_2O_2 accumulation. Data represent mean ± SD of at least 100 interaction sites. *, Significant difference from Col-0 (Student's *t*-test, $P < 0.01$).

1998). The number of conidiophores per colony can be used to quantitatively characterize successful pathogenesis at the terminal stage of the asexual fungal infection cycle. The number of conidiophores per colony was determined for the susceptible ecotype Col-0, the resistant accessions Ang-0, Co-1, Do-0, La-1, Nok-3, Sha, and Sorbo, as well as for Sha × Col-0 F_1 and Sorbo × Col-0 F_1 plants. As further controls, the *pmr6-3* mutant and the *RPW8*-carrying ecotype Ms-0 were included in the analysis. For the latter two lines a reduced number of conidiophores has been previously found upon challenge with distinct compatible powdery mildew species (Xiao *et al.*, 1997; Vogel *et al.*, 2002). We observed a strong reduction of conidiophore formation in resistant accessions from more than 200 conidiophores per colony on susceptible Col-0 to less than 20 on Sorbo, less than 10 on *pmr6-3*, Ang-0, Co-1, Do-0, Nok-3 and Sha, and virtually complete absence of any conidiophores on Ms-0 and La-1 (Fig. 1c). For the F_1 plants of crossings Sha × Col-0 and Sorbo × Col-0 that were analysed, conidiophore formation was intermediary (56% of the Col-0 level in Sha F_1 and 81% in Sorbo F_1 progeny, respectively), indicative of an intermediate hereditary path for resistance in Sha and Sorbo rather than dominant-recessive inheritance. These results are consistent with the observed 1 : 2 : 1 (susceptible : intermediate susceptible : resistant) segregation pattern of infection phenotypes in the F_2 generation of Sha × Col-0 and Sorbo × Col-0 crossings (see earlier and Table 2). Taken together, the comparative microscopic analysis revealed that resistance in all accessions tested becomes predominantly effective at a late stage of fungal pathogenesis.

Histochemical analysis of host responses in resistant accessions upon powdery mildew challenge

A common early plant defence reaction to pathogen attack is the production of reactive oxygen species (ROS; Apel & Hirt, 2004). Leaves of selected resistant accessions and respective control lines (Col-0, *pmr6-3* and accession Ms-0 carrying *RPW8*) were inoculated with *G. orontii* and examined by DAB staining for the accumulation of hydrogen peroxide (H_2O_2) as a representative ROS. Based on earlier studies Col-0 was not expected to exhibit hydrogen peroxide production while *RPW8*-mediated powdery mildew resistance was previously found to be associated with local H_2O_2 accumulation (Xiao *et al.*, 2001). At 48 hpi all resistant accessions tested showed abundant local H_2O_2 production (70% to 97% of attacked host cells in resistant accessions compared to less than 20% in susceptible Col-0 and resistant *pmr6*; Fig. 2). The fact that extensive hydrogen peroxide accumulation did not correlate with reduced fungal entry rates and/or fungal microcolony size at this time-point (e.g. Sha and Sorbo; see earlier and Fig. 1a,b) suggests that the local generation of H_2O_2 has no direct impact on host cell entry success and/or early expansion of fungal hyphae.

Owing to the biotrophic lifestyle of powdery mildews, host cell death could be an important feature of resistance by inhibiting pathogen growth via termination of nutrient supply. Host cell death is indeed a common facet of isolate-specific resistance to biotrophic pathogens, which is frequently conferred by intracellular avirulence factor recognition proteins (van der Biezen & Jones, 1998). We experimentally assessed

 © The Authors (2008). Journal compilation © *New Phytologist* (2008)

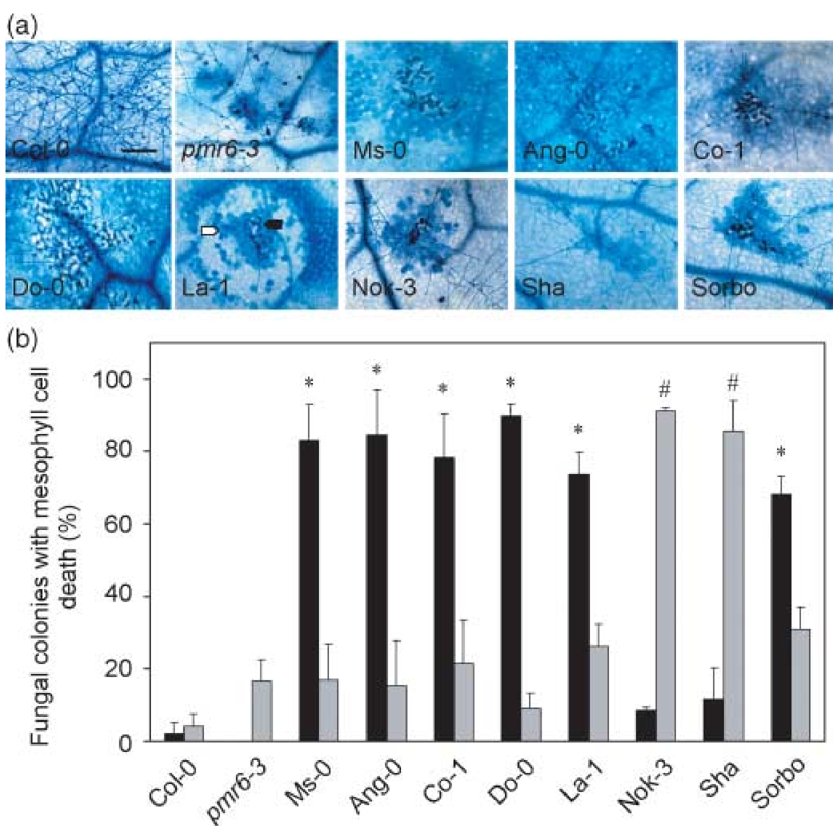


Fig. 3 Mesophyll cell death in response to powdery mildew attack. *Arabidopsis thaliana* leaves were harvested at 7 d post infection (dpi) with *Golovinomyces orontii* and following Trypan Blue staining analysed by light microscopy. (a) Representative micrographs of Trypan Blue-stained leaf samples. White arrowhead, an example of light blue mesophyll cells indicative of medium cell death; black arrowhead points towards dark blue mesophyll cells suggestive of strong cell death. Bar, 200 µm. (b) Quantitative analysis of mesophyll cell death. Data represent mean ± SD of approx. 200 interaction sites (based on two plants with three leaves each). Closed bars indicate massive, tinted bars medium cell death. * and #, significant differences from Col-0 with regard to massive and medium cell death, respectively (Student's *t*-test, $P < 0.01$).

the occurrence and extent of cell death in a range of resistant accessions and control lines by Trypan Blue staining at late stages of pathogen development (7 dpi). When unchallenged, none of the accessions tested showed any spontaneous cell death, except for some patches at leaf margins and tips, probably resulting from ordinary senescence (not shown). In inoculated leaf tissue, Trypan Blue staining was mostly observed in mesophyll cells located beneath hyphae or in circular regions below the entire colonies (Fig. 3a). Two types of stained cells could be differentiated: First, mesophyll cells that stained dark blue and seemed to be collapsed (referred to in Fig. 3b as massive cell death; La-1, black arrowhead) and second, cells that were less dark blue and did not seem to be collapsed yet (referred to as medium cell death; Fig. 3b, white arrowhead). In all resistant accessions, including Ms-0, the percentage of colonies associated with cell death (mostly 100%) was much higher compared with less than 10% in Col-0 and *c.* 17% in *pmr6-3* (Fig. 3b). The intensity of cell death, however, differed drastically between the resistant accessions: In Nok-3 and Sha, the percentage of colonies associated with dark blue-stained and collapsed cells was much lower (9% and 13%, respectively) than in other resistant accessions (68% to 90%; Fig. 3b). Intriguingly, the production of conidiophores per colony was similarly reduced in all resistant accessions, suggesting that generally the occurrence but not the extent of cell death inversely correlates with asexual fungal reproduction success (see above and Fig. 1c). However, occurrence of cell death appears to be tightly correlated with hydrogen peroxide production (compare Figs 2b and 3b).

Deposition of callose (a β-1-3 polyglucan) between the plasma membrane and the cell wall typically occurs in plants in response to various abiotic and biotic stresses (Stone & Clarke, 1992). Especially after microbial attack, callose deposition contributes to the formation of cell wall appositions (papillae) at attempted pathogen entry sites as well as to encasement of fungal penetration pegs and haustoria. Although it is thought that papillae may act as physical barriers to impede microbial penetration, callose generated by the callose synthase GLUCAN SYNTHASE-LIKE 5 (GSL5) is postulated to be important for successful infection since *gsl5* (=*pmr4*) mutant plants are resistant to powdery mildews (Jacobs *et al.*, 2003; Nishimura *et al.*, 2003). In addition to its accumulation in papillae, callose is also deposited in dying cells. We studied callose deposition in various resistant and control accessions by Aniline Blue staining (see the Materials and Methods section) at 7 dpi. In all accessions analysed the β-1,3 glucan was predominantly deposited close to penetration sites and around haustoria (Fig. 4). In all resistant accessions, callose was additionally observed in larger circular or irregular-shaped areas of epidermal and mesophyll cells underneath fungal colonies, especially extensive in La-1 and Do-0. Only in ecotype Sha was callose deposition in whole cells rather weak and roughly comparable to susceptible Col-0. Interestingly, these differences do not correlate with the variation in

© The Authors (2008). Journal compilation © *New Phytologist* (2008)

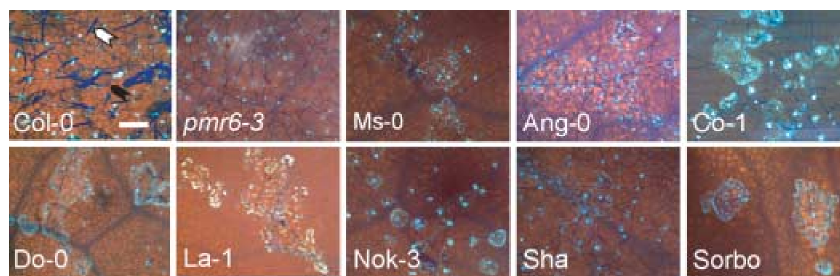


Fig. 4 Callose deposition in response to powdery mildew attack. *Arabidopsis thaliana* leaves were harvested at 7 d post infection (dpi) with *Golovinomyces orontii* and following Aniline Blue (callose) and Coomassie Brilliant Blue (fungal structures) staining analysed by light microscopy. Representative micrographs are shown. Bar, 200 μm. White arrow indicates a conidiophore; black arrow indicates a fungal hypha.

cell death intensity among the resistant accessions (see earlier and Fig. 3), suggesting that pathogen-triggered cell death and callose deposition are likely under separate genetic control.

Linkage analysis reveals location of the resistance locus on the lower arm of chromosome III in some accessions

To identify the region(s) that contain(s) the locus responsible for resistance in the selected accessions, F_2 progenies of crossings of ecotypes Do-0, La-1, Nw-0, Ove-0, Sorbo and Wt-2 with Col-0 were analysed using a set of 22 SSLP markers distributed evenly throughout the genome (Lukowitz *et al.*, 2000) to detect linkage of one or several markers with the resistance phenotype. In accessions Do-0, La-1 and Sorbo resistance was linked to markers located on the lower arm of chromosome III (75–98% association; see the Supplementary Material, Table S1). A population of RILs obtained from a crossing between the intermediate susceptible ecotype Bay-0 (DR score 2) and resistant Shahdara (Sha; DR score 0; Loudet *et al.*, 2002) was employed to localize the resistance locus in the latter accession. A subset of 165 RILs assumed to represent maximal genetic diversity (Loudet *et al.*, 2002) as well as 40 additional lines were inoculated with *G. orontii*. Resistant and susceptible plants in this RIL sub-population occurred in a ratio of approx. 1 : 1 (103 resistant : 102 susceptible; see the Supplementary Material, Table S2), as expected for a monogenic trait in population of inbred lines. In addition to the parental phenotypes of Bay-0 and Sha, plants with the transgressive phenotypes DR score 1 and DR score 3 were observed (Table S2). Transgression is a frequent phenomenon of RILs and was previously shown for other traits studied with this RIL population (Loudet *et al.*, 2002). The results were analysed with WINQTL cartographer software (Wang *et al.*, 2006), revealing that resistance in Sha was similarly localized to the lower arm of chromosome III (Fig. 5a). The single peak with a LOD score of *c.* 64 further corroborates that resistance is highly likely a monogenic trait in this accession, although several loci in close proximity cannot be excluded by this approach.

Oidium neolycopersici is one of two closely related fungal species that are able to cause the powdery mildew disease on tomato (Jones *et al.*, 2001). Interestingly, resistance against *O. neolycopersici* did not map to chromosome III. Instead, two loci on chromosomes II and IV, derived from the parental ecotype Bay-0 (DR = 0) appear to collectively contribute to resistance against *O. neolycopersici* (Fig. 5c), while parental accession Sha was found to be susceptible to this fungal pathogen (DR = 2–3). Similar discrepancies between the infection phenotypes were obtained with this and the three other powdery mildew species tested in accessions Sorbo and Do-0 (Table 1), suggesting that the resistance gene residing on the lower arm of chromosome III is ineffective against the *O. neolycopersici* isolate used.

Based on the results obtained with the accessions mentioned earlier, ecotypes Ang-0, C24, Co-1 and Nok-3 were tested with markers located on chromosome III in a targeted manner (Table S1). Co-1 and Nok-3 showed a similar association (85% and 92%, respectively) of resistance to the lower arm of chromosome III. By contrast, association of markers with resistance in Ang-0 and C24 was *c.* 50%, indicative of free segregation. Though Co-1 and C24 are regarded as the same genotype (Schmid *et al.*, 2006, see earlier), resistance in Co-1 localized to chromosome III, while in C24 no clear association could be determined, possibly because of the small sample size of the respective F_2 population.

Accessions Sorbo and Sha were chosen for fine mapping of the resistance locus. A total of 1112 F_2 plants for Sorbo and 205 RILs for Sha were analysed to identify those with at least one recombination event between two markers located on the lower arm of chromosome III. The target gene region in Sorbo could be restricted to a 0.73 Mb interval between markers Sorb34 (18.24 Mb) and Sorb54 (18.77 Mb; Fig. 5b). Although for most plants the results of the phenotypic analysis were consistent with the respective genotype, in few cases contradictions between genotype and phenotype remained even within F_3 families (not shown). In parallel, the population of RILs was genotyped with polymorphic markers deduced from the segregating Sorbo × Col-0 F_2 progeny to identify the target gene region in the Sha ecotype. A total of 205 RILs were analysed and indicated a target gene region of 1.28 Mb between markers Sorb50 (18.49 Mb) and Sorb42 (19.77 Mb; Fig. 5b). However, results for 18% of all RILs were in conflict with this target gene region. The percentage of contradictory plants was higher in the group of resistant RILs (DR score 0; 68% of all inconsistencies) compared with the susceptible ones (DR score 2 and 3; 32% of the discrepancies). The various contradictions observed in the Sorbo × Col-0 mapping population and the Bay-0 × Sha RIL prevented a further reliable genetic

 © The Authors (2008). Journal compilation © *New Phytologist* (2008)

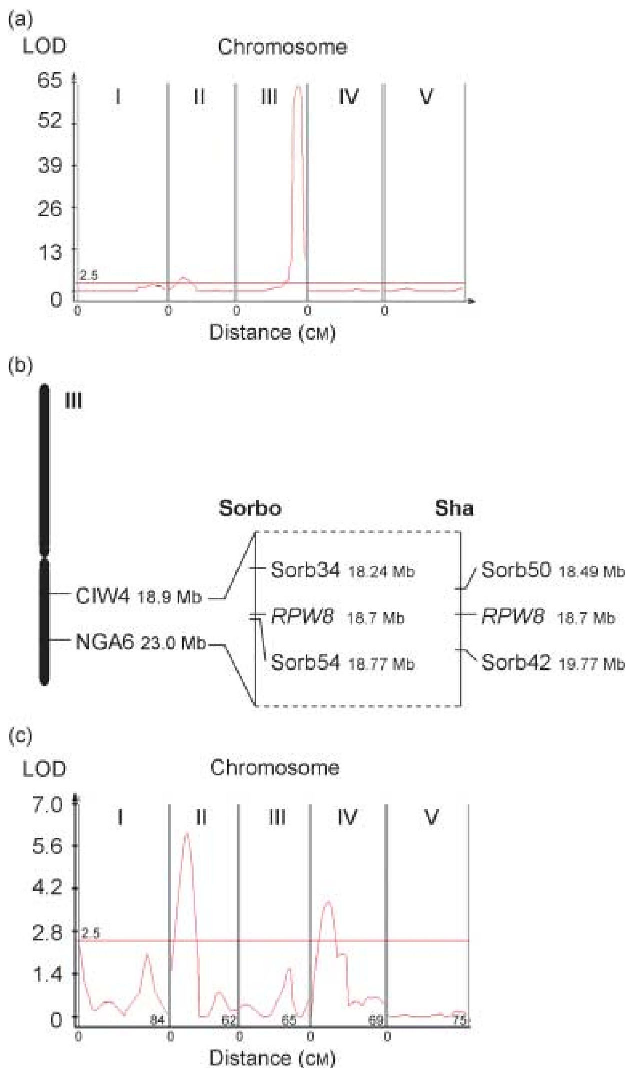


Fig. 5 Mapping of the resistance locus to the lower arm of chromosome III. (a) Analysis of Bay-0 × Sha recombinant inbred lines (RILs). Infection phenotypes and marker genotypes of the 203 RILs were analysed and results plotted with WINQTL cartographer software. The chart indicates the probable location (indicated by the peak of the red plotted line) of the resistance locus in Sha along the five *Arabidopsis* chromosomes (each separated by vertical lines). The red horizontal line at LOD score 2.5 demarks the threshold for significance. (b) Location of simple sequence length polymorphism (SSLP) markers flanking the target gene region in accessions Sha and Sorbo. Please note that distances of markers are not drawn to scale. (c) The chart indicates the probable location (indicated by the peaks of the red plotted line on chromosomes II and IV) of the *Oidium neolycopersici* resistance loci in Bay-0. The red horizontal line at LOD score 2.5 demarks the threshold for significance.

delimitation of the resistance locus. Taken together, genetic linkage analysis revealed the same region on chromosome III for the location of the resistance locus in six different accessions (Co-1, Do-0, La-1, Nok-3, Sha and Sorbo). It should, however, be stressed that in all cases tested linkage between phenotypes and genotypes was imperfect, suggesting that either additional unlinked loci contribute to or modulate the resistance response, or that other (epi-)genetic phenomena interfere with the mapping process.

Allelism tests between accessions

Although in principle resistance could be caused by different genes located in the same chromosomal region, it is more likely that the same gene is responsible for resistance in the above-mentioned ecotypes. To test this hypothesis, some accessions were crossed with each other (Sha × Sorbo, Do-0 × Sha and Do-0 × Sorbo) and F_1 as well as F_2 progeny of these crossings were macroscopically analysed upon challenge with *G. orontii*. If resistance was allelic, then F_1 and F_2 progeny were both expected to be resistant. All tested F_1 plants ($n = 5$) of each crossing were indeed resistant to *G. orontii*. Unexpectedly, in crossings of Sha × Sorbo and Do-0 × Sha, resistance segregated in the F_2 generation. In Sha × Sorbo F_2 progeny 18.5% of the plants tested ($n = 92$) were susceptible to different degrees, while in the Do-0 × Sha crossing 13.5% of the F_2 plants tested ($n = 92$) showed susceptibility (all at an intermediate level). In the F_2 progeny of crossings Do-0 × Sorbo all plants tested ($n = 44$) were resistant. Although not fully conclusive, this analysis suggests that resistance in the accessions tested is either not conferred by the same locus, or that transgression or other (epi-)genetic phenomena interfere with this approach in the accessions tested.

Candidate locus *RPW8*

A multigene locus in the target gene region known to confer resistance to several powdery mildews is *RPW8*, located at *c*. 18.8 Mb on chromosome III. This genomic location would be in accordance with the target gene interval deduced from the Col-0 × Sorbo and Col-0 × Sha F_2 progeny as well as from the Bay-0 × Sha RIL (Fig. 5). We therefore first tested presence or absence as well as transcript accumulation of *RPW8.1* and *RPW8.2* (the two polymorphic paralogues of the *RPW8* locus which have been shown to confer powdery mildew resistance; Xiao *et al*., 2001) by PCR (genomic DNA level) and reverse transcriptase (RT) PCR (cDNA level), respectively. In all resistant accessions analysed ($n = 23$), transcripts of *RPW8.1* and *RPW8.2* were detected (Table S3). Next, the population of Bay-0 × Sha RILs was used to determine whether infection phenotypes correlated with the presence or absence of *RPW8.1* and/or *RPW8.2* sequences in the individual lines. Unlike segregating F_2 populations, RILs offer the advantage of genetic stability. While both *RPW8.1* and *RPW8.2* were present in Sha, neither could be detected in Bay-0. This finding allowed testing for a correlation of the presence of *RPW8.1* and *RPW8.2* with infection phenotypes obtained upon challenge with *G. orontii*. A subset of 124 RILs with unambiguous infection phenotypes (DR scores 0, 2 or 3) were analysed by PCR for presence of *RPW8.1* or *RPW8.2*. The majority of the analysed RILs

(85%) supported the hypothesis that *RPW8* could be the locus responsible for resistance (Supplementary material Table S2). Most of the conflicts were observed for plants with DR score of 0 (only 73% association), while for plants with DR scores of 2 and 3 the association between susceptibility and absence of *RPW8* genes was high (92%).

The presence and/or absence of the *RPW8* candidate gene provides merely correlative but no functional evidence for a role of these genes in disease resistance. Because of the predicted dominant or intermediate inheritance of *RPW8*-mediated resistance, depletion of *RPW8.1* and *RPW8.2* transcript accumulation in resistant accessions by double-stranded RNA interference (dsRNAi) could provide direct proof whether resistance is mediated by *RPW8* in these ecotypes. This approach should result in susceptibility in those accessions where *RPW8* is responsible for resistance. We generated dsRNAi constructs in binary vectors supposed to target either *RPW8.1* or *RPW8.2* (for details, see the Materials and Methods section) and transformed 44 ecotypes, including the susceptible controls Col-0 and Bay-0 (assumed to express no *RPW8*-mediated resistance; negative control) as well as accessions Ms-0 and Kas-1 (accessions known or suspected to express *RPW8*-mediated powdery mildew resistance; positive controls; Wilson *et al*., 2001; Xiao *et al*., 2001) with the respective constructs.

T_1 plants from 44 different accessions (including controls) transformed with 35S::*RPW8.1*-dsRNAi and from 14 different accessions (including controls) with 35S::*RPW8.2*-dsRNAi were recovered from selection, inoculated with *G. orontii* and macroscopically screened for enhanced susceptibility. In seven ecotypes we found macroscopically susceptible T_1 plants (Table 3; see the Supplementary Material, Table S4). In four out of seven accessions with susceptible T_1 plants susceptibility was confirmed in the T_2 progeny (Fig. 6a): In several T_2 families susceptibility segregated while in some T_2 families all plants were susceptible, suggesting the presence of more than one transgene copy in the respective parental lines. The susceptible transgenic Kas-1 line indicates that depletion of *RPW8* transcript accumulation by the *RPW8.2* dsRNAi construct is successful in an accession in which *RPW8* has been previously assumed to represent the major resistance locus (Wilson *et al*., 2001). However, we failed to recover susceptible transgenic lines of the *RPW8*-containing accession Ms-0 (Table S4).

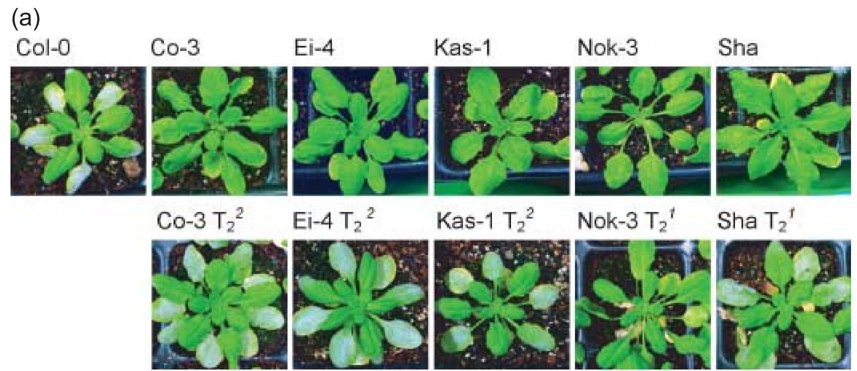
To determine whether the observed susceptibility in the transformed plants correlates with reduced *RPW8* transcript accumulation, T_1 and/or T_2 plants of selected accessions were analysed by semiquantitative RT-PCR. This uncovered severely reduced *RPW8.1* and/or *RPW8.2* transcript levels in most of the transgenic lines, except line Co-3 $1A^2$ which for unknown reasons revealed contradictory data for the powdery mildew infection phenotype and *RPW8* transcript accumulation (Fig. 6b). In some lines, gene silencing was highly efficient resulting in absence of detectable *RPW8.1* and/or *RPW8.2* transcript accumulation (Fig. 6b), suggesting that dsRNAi constructs directed against either *RPW8.1* or *RPW8.2* are capable of co-silencing multiple sequence-related *RPW8* paralogues. To corroborate these findings we performed real-time PCR using cDNA from selected transgenic lines (Fig. 6c). Alterations of *RPW8.1* transcript levels ranged from absence of detectable expression (lines Ei-4 $T_1.1J^2$ and Ei-5 $T_1.1\ i^2$) to 14-fold (lines Nok-3 $T_1.1D^1$ and Sha $T_1.1C^1$) or 50-fold (Sha $T_1.16C^1$) reduction or a slight increase in expression (line Sha $T_1.3C^1$). Results of this experiment were essentially consistent with the data from the semi-quantitative RT-PCR analysis (Fig. 6b), indicating that *RPW8* transcript accumulation is strongly reduced in most of the transgenic lines. Taken together,

Table 3 Phenotypic and molecular characteristics of *RPW8* dsRNAi lines

			dsRNAi	Transcript accumulation[1]		Phenotypic segregation in T_2 progeny with respect to *G. orontii* infection		
Accession	T_1 plant	DR[2]	Target gene	*RPW8.1*	*RPW8.2*	Susceptible	Intermediate	Resistant
Co-3	1A	2	*RPW8.2*	+	+	15	0	0
Do-0	2A	2	*RPW8.1*	–	–	n.d.[3]	n.d.	n.d.
Ei-4	1J	3	*RPW8.2*	–	–	16	0	0
Ei-5	1I	3	*RPW8.2*	–	–	n.d.	n.d.	n.d.
Kas-1	1M	3	*RPW8.2*	–	–	16	0	0
Nok-3	1D	3	*RPW8.1*	–	+	0	2	13
Sha	1C	0	*RPW8.1*	–	+	0	2	14
	3C	0	*RPW8.1*	–	+	0	2	14
	16C	2	*RPW8.1*	–	–	0	1	14
	1A	2	*RPW8.2*	–	–	4	0	10
	2A	2	*RPW8.2*	–	–	2	2	10
	3A	2	*RPW8.2*	–	–	3	2	10

[1]+, wild type-like *RPW8* transcript accumulation; –, reduced *RPW8* transcript accumulation.
[2]DR, disease reaction score: 0 fully resistant; 2 intermediate; 3 fully susceptible.
[3]n.d., not determined.

 © The Authors (2008). Journal compilation © *New Phytologist* (2008)



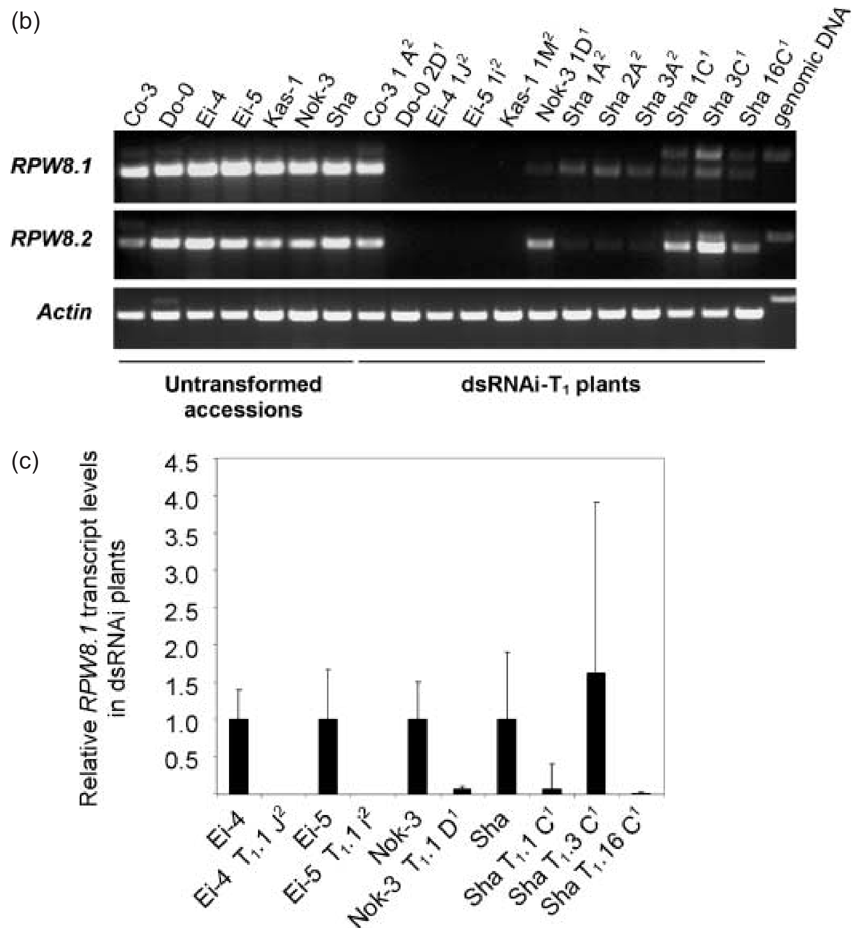


Fig. 6 dsRNAi-mediated gene silencing of *RPW8* confers susceptibility in multiple accessions. (a) Infection phenotypes of selected untransformed (upper panel) and transgenic (expressing *RPW8* dsRNAi constructs; lower panel) ecotypes at 8 days post inoculation with *Golovinomyces orontii*. [1],[2], plants transformed with a dsRNAi construct targeting either *RPW8.1* or *RPW8.2*, respectively. (b) Steady-state transcript levels in rosette leaves of unchallenged (pathogen-free) *RPW8* dsRNAi T_1 plants. Semiquantitative reverse transcriptase polymerase chain reaction (RT-PCR) was performed using *RPW8.1* (upper panel) or *RPW8.2* (lower-level)-specific primer pairs and the following templates: cDNA of untransformed control plants, cDNA of dsRNAi-T_1 plants, or genomic DNA (control, Do-0). [1],[2], plants transformed with a dsRNAi construct targeting either *RPW8.1* or *RPW8.2*, respectively. (c) Steady-state transcript levels in rosette leaves of unchallenged (pathogen-free) *RPW8* dsRNAi T_1 plants. Quantitative RT-PCR was performed using *RPW8.1*-specific primer pairs and cDNA of untransformed control plants as well as cDNA of dsRNAi T_1 plants as templates. Values were normalized to the reference gene actin and ratios were determined with the method described by Pfaffl (2001). *RPW8* expression in the parental (untransformed) accession was set as 1. [1],[2], plants transformed with a dsRNAi construct targeting either *RPW8.1* or *RPW8.2*, respectively.

the findings indicate that susceptibility in *RPW8*-silenced transgenic lines of resistant accessions largely coincides with reduced transcript levels of *RPW8.1*, *RPW8.2* or both. We conclude that *RPW8* is responsible for resistance against *G. orontii* in these accessions (Do-0, Ei-4, Ei-5, Kas-1, Nok-3 and Sha and possibly also Co-3).

Discussion

In an approach based on natural genetic variation, powdery mildew resistance was analysed in *A. thaliana* to identify novel sources of resistance for this plant-microbe interaction. While in six accessions (Ang-0, Bu-0, Bu-3, Ob-0, Petergof and Wt-2) genetic evidence points towards polygenic resistance, in further six accessions (Co-1, Do-0, La-1, Nok-3, Sha and Sorbo) a single target gene locus on the lower arm of chromosome III was identified (Table 2, see the Supplementary Material, Table S1; Fig. 5). The latter ecotypes differed in the spectrum and strength of resistance as well as the expression of defence responses such as hydrogen peroxide accumulation, callose deposition and host cell death (Table 1; Figs 1–4). Gene silencing via dsRNAi revealed that the previously characterized atypical

© The Authors (2008). Journal compilation © *New Phytologist* (2008)

R gene locus *RPW8* is responsible for resistance in three of the above-mentioned (Do-0, Nok-3 and Sha) plus four additional (Co-3, Ei-4, Ei-5, Kas-1) accessions (Fig. 6). These findings provide an overview about the natural defence arsenal of *Arabidopsis* to defeat powdery mildew attacks.

Polygenic powdery mildew resistance appears to be a common phenomenon in *Arabidopsis*

Segregation patterns of the F_2 progeny were determined for 16 resistant accessions crossed with the susceptible Col-0 ecotype. In six accessions (Ang-0, Bu-0, Bu-3, Ob-0, Petergof and Wt-2), segregation patterns of either 3 : 1 or 1 : 2 : 1 were not applicable (Table 2), indicating that resistance in these ecotypes is most likely not caused by a single gene, but is a consequence of several loci acting together (polygenic resistance). The subsequent mapping process did not yield a chromosomal location of the resistance-mediating gene in two additional accessions (C24 and Ove-0), although their segregation ratios were nominally compatible with a 3 : 1 distribution (Table 2). In these accessions, resistance could also be inherited in a more complex manner and thus be polygenic. The contribution of many different genes to the trait can result in false-positive 3 : 1 segregation pattern, especially when the scoring of infection phenotypes is performed in a qualitative rather than a quantitative manner. It was previously suggested that polygenic powdery mildew resistance is more common in *Arabidopsis* than in barley and seemingly over-represented compared to other *Arabidopsis* plant–microbe interactions (Schulze-Lefert & Vogel, 2000). Results from the current study support this notion, indicating that polygenic resistance contributes significantly to powdery mildew resistance in *Arabidopsis*. It is possible that the *RPW8* locus discussed next adds to polygenic resistance in some accessions, as previously described for Kas-1 (Wilson *et al.*, 2001) and Wa-1 (Schiff *et al.*, 2001).

RPW8 is a major resistance locus in multiple ecotypes

Based on a correlation between DNA sequence analysis of the *RPW8* locus and phenotypic disease resistance it has been previously hypothesized that *RPW8* represents the main source of natural broad-spectrum resistance to powdery mildews in *Arabidopsis* (Xiao *et al.*, 2004). In support of this hypothesis, in this study we provide strong experimental evidence based on genetic mapping and gene silencing that *RPW8* is indeed a major powdery mildew resistance locus.

Accessions with experimental genetic evidence for *RPW8*-based powdery mildew resistance originate from Portugal (Co-3), Central Europe (Ei-4 and Ei-5, Germany and Nok-3, The Netherlands), Eastern Europe (Ms-0, Russia) and Asia (Kas-1, Kashmir; Sha, Tajikistan), indicating that *RPW8*-mediated immunity is a geographically widespread phenomenon. This is consistent with the previous (Xiao *et al.*, 2004) and current (Table S3) DNA sequence analysis of the *RPW8* locus. Although our experimental data obtained upon dsRNAi-mediated gene silencing indicate that members of the *RPW8* locus confer powdery mildew resistance in at least a subset of the resistant accessions, we have no information about the precise gene organization of this locus in the various ecotypes and therefore do not know how many *RPW8* paralogues are silenced in each case. Previous detailed comparative DNA sequence analysis of the *RPW8* region in accessions Col-0 and Ms-0 revealed the presence of two functional and three nonfunctional (with respect to powdery mildew resistance) copies in Ms-0 while Col-0 harbours four nonfunctional copies (Xiao *et al.*, 2001, 2004). It is conceivable that the organization of this locus also differs between the accessions employed in the present study. The DNA sequence data obtained by PCR using oligonucleotides derived from *RPW8.1* and/or *RPW8.2* coding sequences (Table S3) must therefore treated with care.

Interestingly, despite a common major genetic source of resistance, accessions Do-0, Ms-0, Nok-3 and Sha differ considerably in the extent of disease resistance as indicated by unequal rates of host cell entry, colony expansion and conidiophore production (Fig. 1). Similarly, these ecotypes also vary with respect to commonly used histological plant defence markers such as hydrogen peroxide accumulation, host cell death and callose deposition (Figs 2–4). It is known that environmental factors such as light intensity, temperature and humidity influence *RPW8* expression, which could impinge on *RPW8*-mediated resistance (Xiao *et al.*, 2003a). Since our experiments, except those with *O. neolycopersici*, were conducted in the same conditions, environmental factors are unlikely to account for the observed phenotypic plasticity of resistance. Instead, these differences may relate to the diverse genetic backgrounds of the accessions. Alternatively, or in addition, sequence polymorphisms at the *RPW8* locus might contribute to the observed variation (see below).

In ecotype Ms-0, besides *G. cruciferarum* and *G. cichoracearum*, resistance was previously reported to be effective against *O. lycopersici* (isolate Oxford; Xiao *et al.*, 2001), a fungal species that is now referred to as *O. neolycopersici* (Kiss *et al.*, 2001). Similarly, heterologous expression of *RPW8* genes in tobacco, but not in tomato, was shown to confer enhanced resistance to the Oxford isolate of *O. (neo)lycopersici* (Xiao *et al.*, 2003b). In our analysis, however, *RPW8*-mediated resistance in accessions Sha, Do-0 and possibly Sorbo (though final experimental proof for RPW8 function in Sorbo is lacking; Fig. 5c, Table 1) seems to be nonfunctional against the Wageningen isolate of *O. neolycopersici.* Instead, in case of Bay-0, at least two major loci residing on the upper arms of chromosomes II and IV appear to mediate resistance against the employed race of the latter fungal pathogen. There are at least four possible explanations for this apparent discrepancy to the results reported by Xiao *et al.* (2001). First, the above-mentioned ecotypes could lack factors that are required for *RPW8* resistance against *O. neolycopersici*, but not for resistance against

 © The Authors (2008). Journal compilation © *New Phytologist* (2008)

other powdery mildew species such as *G. orontii*. Second, given the fact that the experiments were conducted with two distinct isolates and owing to the likely existence of different *O. neolycopersici* pathovars ('*formae specialis*'; Lebeda & Mieslerová, 2000), *RPW8*-conditioned resistance could be race- or pathotype-specific in case of the *Arabidopsis*/*O. neolycopersici* interaction. Third, environmental conditions that may affect *RPW8* expression (Xiao *et al*., 2003a) could have influenced the outcome of the experiments. Finally, the most trivial explanation would be that one of the two fungi used is not a bona fide *O. neolycopersici* isolate. At least for our experiments we can rule out this possibility since the Wageningen isolate was virulent on tomato in sets of parallel experiments, and validated by analysis of the ribosomal internal transcribed spacer (ITS) sequences (Kiss *et al*., 2001; data not shown).

RPW8 inheritance – dominant or semidominant?

In this study, monogenic resistance segregated in either a semidominant or recessive manner for all accessions tested including the ecotypes for which dsRNAi analysis showed that *RPW8* is responsible for resistance. *RPW8* was originally identified in the Ms-0 ecotype and quantitative analysis of resistance to *G. cichoracearum* indicated a dominant mode of inheritance in this accession (Xiao *et al*., 1997). A subsequent study revealed that transgenic Col-0 plants harbouring *RPW8.1* and *RPW8.2* genomic sequences inherit resistance in a semidominant fashion (Xiao *et al*., 2005). The authors suggested that *RPW8* acts in a gene dosage-dependent manner to stimulate basal defence mechanisms. They further speculated that different genetic backgrounds might result in different levels of *RPW8* transcript accumulation and thus *RPW8*-mediated resistance. Consequently, the semidominant mode of inheritance observed in the present study could also be caused by a gene-dosage effect in the respective accessions. Genetic backgrounds, copy number of *RPW8* paralogues, natural variation in amino acid sequence of RPW8 polypeptides as well as the timing and level of *RPW8* expression may all contribute to quantitative differences in powdery mildew resistance, which may possibly not only become evident in a distinct mode of inheritance but also in the variation of expression of histological defence markers (Figs 2–4; see also above).

Intriguingly, segregation ratios in F_2 progeny of some accessions which subsequently showed successful *RPW8* gene silencing (e.g. Do-0 and Nok-3) or location of the resistance locus at the lower arm of chromosome III (indicative of *RPW8*; e.g. La-1 and Ob-0) was compatible with a recessive mode of inheritance for the resistance trait (Table 2). This could be simply an erroneous assessment based on the comparatively low number of F_2 individuals analysed, variation in inoculation density, biological experiment-to-experiment variation or the semiquantitative disease resistance score used in this and other studies (Adam *et al*., 1999). Alternatively, one or more additional genes with an epistatic effect on *RPW8*-mediated resistance could segregate in the crossings of these accessions and mask the true segregation pattern of *RPW8*.

Contradictions between phenotypes and genotypes

During the fine mapping process on the basis of the Col-0 × Sorbo F_2 progeny, a considerable number of plants were observed that were expected to be resistant based on their genotype in the target gene region, but that were indeed susceptible and vice versa. In addition, it was not possible to obtain full phenotype–genotype linkage when testing the Bay-0 × Sha RILs for presence/absence of the candidate locus *RPW8*. Finally, crossings between two resistant accessions with the same target gene region unexpectedly yielded a substantial proportion of susceptible F_2 plants.

These contradictions could be explained by a genetic modifier which segregates in the populations and which may interfere with the expression of resistance in particular genomic constellations. An alternative explanation could be an extraordinarily high recombination frequency in the target gene region, mimicking independent inheritance. The multigenic *RPW8* locus, which harbours multiple sequence-related paralogues, could indeed represent such a hotspot of recombination, possibly involving illegitimate crossing-over and gene conversion events. An additional reason, especially with regard to the susceptible plants from crossings between resistant accessions, could be transgression or other (epi-)genetic phenomena (e.g. paramutation) which might interfere with the establishment of resistance despite the presence of nominally functional *RPW8* gene copies.

The contribution of prototypical *R* genes to powdery mildew resistance in *Arabidopsis*

In many plant–microbe interactions, dominantly inherited *R* genes providing isolate-specific protection against a given pathogen represent a major source of plant immunity. Previous studies of the interaction of *Arabidopsis* accessions with powdery mildews identified no dominantly inherited powdery mildew resistance gene except the atypical *RPW8* genes which confer broad-spectrum immunity (Adam & Somerville, 1996; Adam *et al*., 1999; Schiff *et al*., 2001; Wilson *et al*., 2001; Xiao *et al*., 2001). Similarly, in the present study we found no evidence for a dominantly inherited resistance locus, with the possible exception of Pla-4 (Table 1). This might be not particularly surprising given the fact that we originally selected accessions that are resistant to at least two powdery mildew species (Table 1), thereby likely excluding isolate-specific resistance determinants. To elucidate the possible contribution of typical *R* gene-mediated powdery mildew immunity in natural *Arabidopsis* populations, we recently started analysing accessions showing resistance specific to *G. orontii*. Preliminary results indicate that the majority of the inspected accessions show intermediate susceptible F_1 progeny after crossing with the

© The Authors (2008). Journal compilation © *New Phytologist* (2008)

susceptible Col-0 ecotype, suggesting that likely semidominantly inherited loci or polygenic resistance but no dominant *R* genes confer immunity in these accessions (data not shown). This situation clearly differs from the frequency of *R* gene-mediated resistance to some other pathogens in *Arabidopsis*. For example, several dominantly inherited *R* genes encoding prototypical NB-LRR proteins and displaying allelic diversity have been described to confer isolate-specific resistance to the bacterial pathogen *Pseudomonas syringae* or the oomycete *Hyaloperonospora parasitica* (Stahl *et al.*, 1999; Mauricio *et al.*, 2003; Rose *et al.*, 2004).

As outlined in the Introduction, in other plant species dominantly inherited *R* genes conferring powdery mildew resistance are common. The presence of these powdery mildew-specific *R* genes likely reflects an ongoing arms race and extensive co-evolution between host and pathogen in the context of evolutionary ancient plant–microbe interactions (Maor & Shirasu, 2005). By contrast, resistance to powdery mildews in *Arabidopsis* seems to be either polygenic and/or mediated by *RPW8* genes, but not by typical *R* genes with a NB-LRR structure. This observation could indicate that either *Arabidopsis* is not the primary host for powdery mildews, as previously speculated by Schulze-Lefert & Vogel (2000), or that this plant–microbe interaction is evolutionary rather young. In case of the latter scenario, there might have been not enough time to evolve resistance that is based either on lack or on nonfunctionality of host compatibility factors or even resulting from coevolution of matching *R*/*Avr* gene pairs. However, only a subset of resistant accessions has been analysed to date, precluding any final statement.

In the light of the facts discussed earlier it is tempting to speculate that *RPW8* possibly adopted the role of conventional *R* genes in the context of *Arabidopsis*–powdery mildew interactions. This would explain why no *R* gene that mediates isolate-specific powdery mildew resistance has been identified in *Arabidopsis* to date. It is intriguing that *RPW8*-mediated resistance engages the same pathway components as prototypical *R* genes of the TIR-NB-LRR class, namely *EDS1*, *PAD4*, *EDS5*, *NPR1* and *SGT1b* (Xiao *et al.*, 2005). This has been interpreted such that RPW8 possibly engages a feedback amplification circuit that potentiates basal defence responses (Xiao *et al.*, 2005). Alternatively, RPW8 could represent a host protein ('guardee') targeted by a conserved fungal effector that is guarded by one or several as yet unidentified R protein(s) of the TIR-NB-LRR class. This situation would be similar to resistance in tomato (*Solanum lycopersicum*) against isolates of the bacterial pathogen, *Pseudomonas syringae*, expressing effectors AvrPto or AvrPtoB. In this plant–microbe interaction, the serine–threonine kinase Pto represents the polymorphic determinant which requires presence of and interaction with the NB-LRR protein Prf for execution of resistance (Mucyn *et al.*, 2006). Although such a scenario would explain the observed dependencies on various components of TIR-NB-LRR protein-mediated resistance pathways, it seems less likely since genetic analyses employing both test crosses and RILs revealed a single locus responsible for resistance. Thus, if an R protein guarding RPW8 exists, the respective gene must be genetically closely linked to *RPW8*. Additionally, respective *R* genes must be present in the ecotype Col-0 since transformation of Col-0 with *RPW8* suffices to confer resistance (Xiao *et al.*, 2001). Finally, unlike in other plant–microbe interactions, one would have to assume a highly conserved fungal effector protein, present in multiple powdery mildew species and unable to evade R protein-mediated recognition in the course of evolution. Further functional analysis of RPW8 proteins (e.g. the identification of interacting proteins from the host or the fungal invader) and/or the discovery of proteins that are needed for RPW8 function will be required to resolve this conundrum.

Acknowledgements

We thank Udo Seiffert and Christian Schulze for help in the quantitative analysis of hyphal dimensions via the HYPHAREA software and acknowledge Bekir Ülker for providing gene silencing vector pJawohl8. Oliver Loudet kindly provided seeds of the Bay-0 × Sha RIL. We are grateful to Zheng Zheng and Daidi Che for their assistance in disease tests with *O. neolycopersici*. Matt Humphry is acknowledged for critical proofreading. This work was funded by generous financial support of the Max-Planck Society, in particular in form of a PhD fellowship in the context of the International Max-Planck Research School (IMPRS; to K. G.).

References

Adam L, Ellwood S, Wilson I, Saenz G, Xiao S, Oliver RP, Turner JG, Somerville S. 1999. Comparison of *Erysiphe cichoracearum* and *E. cruciferarum* and a survey of 360 *Arabidopsis thaliana* accessions for resistance to these two powdery mildew pathogens. *Molecular Plant–Microbe Interactions* **12**: 1031–1043.

Adam L, Somerville, SC. 1996. Genetic characterization of five powdery mildew disease resistance loci in *Arabidopsis thaliana*. *Plant Journal* **9**: 341–356.

Agrios GN (ed). 1988. *Plant pathology*, 3rd edn. New York, NY, USA: Academic Press Inc.

Apel K, Hirt H. 2004. Reactive oxygen species: Metabolism, oxidative stress, and signal transduction. *Annual Review of Plant Biology* **55**: 373–399.

Bai YL, van der Hulst R, Bonnema G, Marcel BC, Meijer-Dekens F, Niks RE, Lindhout P. 2005. Tomato defense to *Oidium neolycopersici*: Dominant Ol genes confer isolate-dependent resistance via a different mechanism than recessive *ol-2*. *Molecular Plant–Microbe Interactions* **18**: 354–362.

Barker CL, Donald T, Pauquet J, Ratnaparkhe MB, Bouquet A, Adam-Blondon AF, Thomas MB, Dry I. 2005. Genetic and physical mapping of the grapevine powdery mildew resistance gene, *Run1*, using a bacterial artificial chromosome library. *Theoretical & Applied Genetics* **111**: 370–377.

van der Biezen EA, Jones JDG. 1998. Plant disease-resistance proteins and the gene-for-gene concept. *Trends in Biochemical Science* **23**: 454–456.

Clough SJ, Bent AF. 1998. Floral dip: a simplified method for *Agrobacterium*-mediated transformation of *Arabidopsis thaliana*. *Plant Journal* **16**: 735–743.

 © The Authors (2008). Journal compilation © *New Phytologist* (2008)

Consonni C, Humphry ME, Hartmann HA, Livaja M, Durner J, Westphal L, Vogel J, Lipka V, Kemmerling B, Schulze-Lefert P *et al.* 2006. Conserved requirement for a plant host cell protein in powdery mildew pathogenesis. *Nature Genetics* **38**: 716–720.

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

Donald TM, Pellerone F, Adam-Blondon, AF, Bouquet A, Thomas MR, Dry IB. 2002. Identification of resistance gene analogs linked to a powdery mildew resistance locus in grapevine. *Theoretical & Applied Genetics* **104**: 610–618.

Edwards K, Johnstone C, Thompson C. 1991. A simple and rapid method for the preparation of plant genomic DNA for PCR analysis. *Nucleic Acids Research* **19**: 1349.

Ellis C, Turner JG. 2001. The *Arabidopsis* mutant *cev1* has constitutively active jasmonate and ethylene signal pathways and enhanced resistance to pathogens. *Plant Cell* **13**: 1025–1033.

Frye CA, Innes RW. 1998. An *Arabidopsis* mutant with enhanced resistance to powdery mildew. *Plant Cell* **10**: 947–956.

Halterman D, Zhou FS, Wei FS, Wise RP, Schulze-Lefert P. 2001. The MLA6 coiled-coil, NBS-LRR protein confers *AvrMla6*-dependent resistance specificity to *Blumeria graminis* f. sp. *hordei* in barley and wheat. *Plant Journal* **25**: 335–348.

Huang CC, Hoefs-van de Putte PM, Haanstra-van der Meer JG, Meijer-Dekens F, Lindhout P. 2000. Characterization and mapping of resistance to *Oidium lycopersicum* in two *Lycopersicon hirsutum* accessions: evidence for close linkage of two Ol-genes on chromosome 6 of tomato. *Heredity* **85**: 511–520.

Jacobs AK, Lipka V, Burton RA, Panstruga R, Strizhov N, Schulze-Lefert P, Fincher GB. 2003. An *Arabidopsis* callose synthase, GSL5, is required for wound and papillary callose formation. *Plant Cell* **15**: 2503–2513.

Jones H, Whipps JM, Gurr SJ. 2001. The tomato powdery mildew fungus *Oidium neolycopersici*. *Molecular Plant Pathology* **2**: 303–309.

Jørgensen JH. 1994. Genetics of powdery mildew resistance in barley. *Critical Reviews in Plant Science* **13**: 97–119.

Kiss L, Cook RTA, Saenz GS, Cunnington JH, Takamatsu S, Pascoe I, Bardin M, Nicot PC, Sato Y, Rossman Y. 2001. Identification of two powdery mildew fungi, *Oidium neolycopersici* sp. nov. and *O-lycopersici*, infecting tomato in different parts of the world. *Mycological Research* **105**: 684–697.

Koch E, Slusarenko AJ. 1990. Fungal pathogens of *Arabidopsis thaliana* (L.) Heynh. *Botanica Helvetica* **100**: 257–268.

Koornneef M, Alonso-Blanco C, Vreugdenhil D. 2004. Naturally occurring genetic variation in *Arabidopsis thaliana*. *Annual Review of Plant Biology* **55**: 141–172.

Lebeda A, Mieslerová B. 2000. Case study of host–pathogen interaction: tomato (*Lycopersicon* spp.)-tomato powdery mildew (*Oidium lycopersici*). *Plant Protection Science* **36**: 156–162.

Linde M, Debener T. 2003. Isolation and identification of eight races of powdery mildew of roses (*Podosphaera pannosa*) (Wallr.:Fr.) de Bary and the genetic analysis of the resistance gene *Rpp1*. *Theoretical & Applied Genetics* **107**: 256–262.

Linde M, Mattiesch M, 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. *Theoretical & Applied Genetics* **109**: 1261–1266.

Loudet O, Chaillou S, Camilleri C, Bouchez D, Daniel-Vedele F. 2002. Bay-0 × Shahdara recombinant inbred line population: a powerful tool for the genetic dissection of complex traits in *Arabidopsis*. *Theoretical & Applied Genetics* **104**: 1173–1184.

Lukowitz W, Gillmor CS, Scheible WR. 2000. Positional cloning in *Arabidopsis*. Why it feels good to have a genome initiative working for you. *Plant Physiology* **123**: 795–805.

Maor R, Shirasu K. 2005. The arms race continues: battle strategies between plants and fungal pathogens. *Current Opinion in Microbiology* **8**: 399–404.

Mauricio R, Stahl EA, Korves T, Tian D, Kreitman M, Bergelson J. 2003. Natural selection for polymorphism in the disease resistance gene *Rps2* of *Arabidopsis thaliana*. *Genetics* **163**: 735–746.

Mitchell-Olds T, Schmitt J. 2006. Genetic mechanisms and evolutionary significance of natural variation in *Arabidopsis*. *Nature* **441**: 947–952.

Mucyn TS, Clemente A, Andriotis VME, Balmuth AL, Oldroyd GED, Staskawicz BJ, Rathjen JP. 2006. The tomato NBARC-LRR protein Prf interacts with Pto kinase in vivo to regulate specific plant immunity. *Plant Cell* **18**: 2792–2806.

Nawrath C, Metraux JP. 1999. Salicylic acid induction-deficient mutants of *Arabidopsis* express PR-2 and PR-5 and accumulate high levels of camalexin after pathogen inoculation. *Plant Cell* **11**: 1393–404.

Nishimura MT, Stein M, Hou BH, Vogel JP, Edwards H, Somerville SC. 2003. Loss of a callose synthase results in salicylic acid-dependent disease resistance. *Science* **301**: 969–972.

Peterhänsel C, Freialdenhoven A, Kurth J, Kolsch R, Schulze-Lefert P. 1997. Interaction analyses of genes required for resistance responses to powdery mildew in barley reveal distinct pathways leading to leaf cell death. *Plant Cell* **9**: 1397–1409.

Pfaffl, M W 2001. A new mathematical model for relative quantification in real-time RT-PCR. *Nucleic Acids Research* **29**: e45.

Plotnikova JM, Reuber TL, Ausubel FM. 1998. Powdery mildew pathogenesis of *Arabidopsis thaliana*. *Mycologia* **90**: 1009–1016.

Rose LE, Bittner-Eddy PD, Langley CH, Holub EB, Michelmore RW, Beynon JL. 2004. The maintenance of extreme amino acid diversity at the disease resistance gene, RPP13, in *Arabidopsis thaliana*. *Genetics* **166**: 1517–1527.

Schiff CL, Wilson IW, Somerville SC. 2001. Polygenic powdery mildew disease resistance in *Arabidopsis thaliana*: quantitative trait analysis of the accession Warschau-1. *Plant Pathology* **50**: 690–701.

Schmid K, Torjek O, Meyer R, Schmuths H, Hoffmann MH, Altmann T. 2006. Evidence for a large-scale population structure of *Arabidopsis thaliana* from genome-wide single nucleotide polymorphism markers. *Theoretical and Applied Genetics* **112**: 1104–1114.

Schulze-Lefert P, Vogel J. 2000. Closing the ranks to attack by powdery mildew. *Trends in Plant Science* **5**: 343–348.

Seiffert U, Schweizer P. 2005. A pattern recognition tool for quantitative analysis of *in planta* hyphal growth of powdery mildew fungi. *Molecular Plant–Microbe Interactions* **18**: 906–912.

Sharbel TF, Haubold B, Mitchell-Olds T. 2000. Genetic isolation by distance in *Arabidopsis thaliana*: biogeography and postglacial colonization of Europe. *Molecular Ecology* **9**: 2109–2118.

Stahl EA, Dwyer G, Mauricio R, Kreitman M, Bergelson J. 1999. Dynamics of disease resistance polymorphism at the *Rpm1* locus of *Arabidopsis*. *Nature* **400**: 667–671.

Stone BA, Clarke AE. 1992. Chemistry and physiology of higher plant 1,3-ß-glucans (callose). In: Stone BA, Clarke AE, eds. *Chemistry and biology of (1,3)-ß-glucans*. Bundoora, Australia: La Trobe University Press, 365–429.

Thordal-Christensen H, Zhang Z, Wei Y, Collinge DB. 1997. Subcellular localization of H_2O_2 in plants: H_2O_2 accumulation in papillae and hypersensitive response during the barley-powdery mildew interaction. *Plant Journal* **11**: 1187–1194.

Vogel J, Somerville S. 2000. Isolation and characterization of powdery mildew-resistant *Arabidopsis* mutants. *Proceedings of the National Academy of Sciences, USA* **97**: 1897–1902.

Vogel JP, Raab TK, Schiff C, Somerville SC. 2002. *PMR6*, a pectate lyase-like gene required for powdery mildew susceptibility in *Arabidopsis*. *Plant Cell* **14**: 2095–2106.

Vogel JP, Raab TK, Somerville CR, Somerville SC. 2004. Mutations in *PMR5* result in powdery mildew resistance and altered cell wall composition. *Plant Journal* **40**: 968–978.

Wang S, Basten CJ, Zeng ZB. 2006. *Windows QTL cartographer 2.5*. Raleigh, NC, USA: Department of Statistics, North Carolina State University, http://statgen.ncsu.edu/qtlcart/WQTLCart.htm

© The Authors (2008). Journal compilation © *New Phytologist* (2008)

Wildermuth MC, Dewdney J, Wu G, Ausubel FM. 2001. Isochorismate synthase is required to synthesize salicylic acid for plant defence. *Nature* 414: 562–565.

Wilson IW, Schiff CL, Hughes DE, Somerville SC. 2001. Quantitative trait loci analysis of powdery mildew disease resistance in the *Arabidopsis thaliana* accession Kashmir-1. *Genetics* **158**: 1301–1309.

Xiao, SY, Brown, S, Patrick, E, Brearley, C, Turner, JG. 2003a. 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 SY, Calis O, Patrick E, Zhang GG, Charoenwattana P, Muskett P, Parker JE, Turner JG. 2005. The atypical resistance gene, *RPW8*, recruits components of basal defence for powdery mildew resistance in *Arabidopsis*. *Plant Journal* **42**: 95–110.

Xiao SY, Charoenwattana P, Holcombe L, Turner JG. 2003b. The *Arabidopsis* genes *RPW8.1* and *RPW8.2* confer induced resistance to powdery mildew diseases in tobacco. *Molecular Plant–Microbe Interactions* **16**: 289–294.

Xiao SY, Ellwood S, Calis O, Patrick E, Li TX, Coleman M, Turner JG. 2001. Broad-spectrum mildew resistance in *Arabidopsis thaliana* mediated by *RPW8*. *Science* **291**: 118–120.

Xiao SY, Ellwood S, Findlay K, Oliver RP, Turner JG. 1997. Characterization of three loci controlling resistance of *Arabidopsis thaliana* accession Ms-O to two powdery mildew diseases. *Plant Journal* **12**: 757–768.

Xiao SY, Emerson B, Ratanasut K, Patrick E, O'Neill C, Bancroft I, Turner JG. 2004. Origin and maintenance of a broad-spectrum disease resistance locus in *Arabidopsis*. *Molecular Biology and Evolution* **21**: 1661–1672.

Xin Z, Velten JP, Oliver MJ, Burke JJ. 2003. High-throughput DNA extraction method suitable for PCR. *Biotechniques* **34**: 820–826.

Zhou FS, Kurth JC, Wei FS, Elliott C, Vale G, Yahiaoui N, Keller B, Somerville S, Wise R, Schulze-Lefert P. 2001. Cell-autonomous expression of barley *Mla1* confers race-specific resistance to the powdery mildew fungus via a *Rar1*-independent signaling pathway. *Plant Cell* **13**: 337–350.

Supplementary Material

The following supplementary material is available for this article online:

Table S1 Association of powdery mildew resistance with selected chromosome III markers in segregating F_2 populations

Table S2 Disease reaction scores, marker genotypes (lower arm of chromosome III) and *RPW8.1* and *RPW8.2* genotypes of selected Bay-0 × Sha recombinant inbred lines (RILs)

Table S3 Presence of *RPW8.1* and *RPW8.2* in selected resistant accessions

Table S4 Numbers and disease phenotypes of plants transformed with *RPW8* dsRNAi constructs

Table S5 Oligonucleotides used in this study

This material is available as part of the online article from: http://www.blackwell-synergy.com/doi/abs/10.1111/j.1469-8137.2007.02339.x
(This link will take you to the article abstract).

Please note: Blackwell Publishing are not responsible for the content or functionality of any supplementary materials supplied by the authors. Any queries (other than missing material) should be directed to the journal at *New Phytologist* Central Office.

About *New Phytologist*

- *New Phytologist* is owned by a non-profit-making **charitable trust** dedicated to the promotion of plant science, facilitating projects from symposia to open access for our Tansley reviews. Complete information is available at **www.newphytologist.org**.

- Regular papers, Letters, Research reviews, Rapid reports and both Modelling/Theory and Methods papers are encouraged. We are committed to rapid processing, from online submission through to publication 'as-ready' via *OnlineEarly* – our average submission to decision time is just 28 days. Online-only colour is **free**, and essential print colour costs will be met if necessary. We also provide 25 offprints as well as a PDF for each article.

- For online summaries and ToC alerts, go to the website and click on 'Journal online'. You can take out a **personal subscription** to the journal for a fraction of the institutional price. Rates start at £135 in Europe/$251 in the USA & Canada for the online edition (click on 'Subscribe' at the website).

- If you have any questions, do get in touch with Central Office (**newphytol@lancaster.ac.uk**; tel +44 1524 594691) or, for a local contact in North America, the US Office (**newphytol@ornl.gov**; tel +1 865 576 5261).

 © The Authors (2008). Journal compilation © *New Phytologist* (2008)