GENETIC VARIATION OF POWDERY MILDEW RESISTANCE IN *ARABIDOPSIS THALIANA*

Inaugural-Dissertation

zur

Erlangung des Doktorgrades

der Mathematisch-Naturwissenschaftlichen Fakultät

der Universität zu Köln

vorgelegt von

Katharina Göllner

aus Berlin

Köln

2006

Berichterstatter: Prof. Dr. Paul Schulze-Lefert

Prof. Dr. Martin Hülskamp

Tag der mündlichen Prüfung: 30.10.2006

I. TABLE OF CONTENTS

II. ABBREVIATIONS

°C	degrees Celsius
µ	micro-
µm	micrometer
ABRC	*Arabidopsis* Biological Resource Center
AFLP	amplified length polymorphism
Avr	avirulence gene
Bgh	*Blumeria graminis* f.sp. *hordei*
bp	base pairs
CAPS	cleaved amplified polymorphic stretch
CC	coiled-coil
cDNA	copy-DNA
CIM	composite interval mapping
cm	centimetre
DAB	diaminobenzidine
dCAPS	derived CAPS marker
DNA	desoxy-ribonucleic acid
dNTP	desoxy-nucleotide-triphosphate
dpi	days post inoculation
EMS	ethylmethane sulfonate
EFR	EF-Tu receptor
ET	ethylene
f.sp.	forma specialis
F_1	first filial generation
F_2	second filial generation
F_3	third filial generation
flg	flagellin
FLS2	Flagellin-sensitive 2
g	gramms
hpi	hours post inoculation
HR	hypersensitive response
JA	jasmonic acid
kb	kilobases
L	litre
LPS	lipopolysaccharide
LRR	leucin-rich repeat
m	milli-
M	molar
min	minutes

MLO	mildew-resistance locus o
mRNA	messenger-RNA
NASC	Nottingham *Arabidopsis* Stock Center
NBS	nucleotide binding site
P	probability
PAMPs	pathogen-associated molecular pattern
PCR	polymerase chain reaction
PR	pathogenesis-related
pv.	pathovar
R	resistance (gene)
RFLP	restriction fragment length polymorphism
RNA	ribonucleic acid
rpm	rounds per minute
RT	reverse transcription
SA	salicylic acid
SAR	systemic acquired resistance
sec	seconds
SNP	single nucleotide polymorphism
SSLP	short sequence length polymorphism
T_1	first filial generation post transformation
T_2	second filial generation post transformation
T_3	third filial generation post transformation
TAIR	The *Arabidopsis* Information Resource
T-DNA	transfer DNA
TIR	drosophila Toll/human interleukine il-1 like receptor
TLR	Toll-like receptor
TM	transmembrane
UV	ultra violet
V	Volt
WT	wildtype

III. SUMMARY

In interactions between beneficial or pathogenic microbes and host organisms, particular host proteins, referred to as compatibility factors, are considered to be essential for the establishment of compatibility. Induced resistance of the dicot plant *Arabidopsis thaliana* to powdery mildew fungi based on the lack of compatibility factors has been previously shown in the *pmr* mutants in the Col-0 ecotype. With respect to natural resistance of *Arabidopsis* to powdery mildews, only resistance mediated by the unusual resistance (*R*) gene *RPW8* was identified in different accessions.

In this study, an approach based on natural variation was conducted to analyze powdery mildew resistance in *A. thaliana* with an emphasis on the identification of new compatibility factors. To this end, loci mediating resistance were mapped in accessions, in which resistance to the compatible powdery mildew *Golovinomyces orontii* was likely due to a defect in a compatibility factor (i.e. these accessions showing monogenic and recessively or semi-dominantly inherited resistance).

In six accessions, the resistance locus was mapped to the lower arm of chromosome III. In other ecotypes analyzed, resistance was likely to be of polygenic origin. The occurrence of only few accessions selected for putative dominantly inherited resistance mediated by prototypical *R*-genes indicated that this type of resistance to powdery mildews is probably very rare if not non-existent in *A. thaliana*.

The results of allelism tests between resistant accessions yielded an unexpectedly high percentage of susceptible plants in the F_2 progeny, a contradiction to the expected segregation of either two either identical or closely

linked genes. It was suggested that this phenomenon could either reflect epistatic effects or be the consequence of pairing between two homologous epialleles (paramutation).

A comparative microscopic analysis of resistance in selected accessions revealed differences in timing and strength of defense responses in the plant; either due to different loci responsible for resistance or owing to different genetic backgrounds. In general, resistance to *G. orontii* was characterized by a reduced production of fungal conidiophores on the leaf surface, pronounced cell death, callose deposition and hydrogen peroxide accumulation, but only in some accessions by reduced host cell entry and retarded hyphal growth.

Fine mapping of resistance in segregating F_2 populations via mapping populations and analysis of recombinant inbred lines allowed a restriction of the putative target gene region, within which several candidate genes were analyzed. One of these genes was *RPW8*, an atypical *R*-gene. Based on results from dsRNAi-mediated *RPW8* transcript depletion, *RPW8* was proposed to be responsible for resistance to *G. orontii* in accessions Bu-0, Co-3, Do-0, Ei-4, Ei-5, Kas-1, Nok-3, Ob-0 and Sha. The observed recessive or semi-dominant inheritance of resistance indicated a dosage-dependent effect of *RPW8*-mediated resistance. Sequence analysis of the conceptual RPW8 protein in several accessions revealed a region of high variability between the predicted transmembrane and coiled-coil domains. It is suggested that powdery mildew resistance in *A. thaliana* appears to be either of polygenic origin or due to *RPW8*.

IV. ZUSAMMENFASSUNG

In Interaktionen zwischen nützlichen und pathogenen Mikroben und deren Wirtsorganismen sind spezielle Wirtsproteine, sogenannte Kompatibilitäts-faktoren, essenziell für die Entstehung von Kompatibilität. Mehltau-Resistenz durch defekte Kompatibilitätsfaktoren in der dikotylen Pflanze *Arabidopsis thaliana* wurde bereits in den *pmr* Mutanten induziert. Früheren Studien zeigen, dass natürlich auftretende Mehltau-Resistenz in mehreren Ökotypen durch den *RPW8* Locus vermittelt wird. Dabei handelt es sich um atypische Resistenz (*R*)-Gene für Breitsprektrumresistenz, die bereits in aus verschiedenen *Arabidopsis*-Ökotypen isoliert wurden.

Dieses Projekt beschäftigt sich mit der Identifizierung neuer Kompatibilitätsfaktoren mit Hilfe der natürlichen Variation von Mehltau-Resistenz in *Arabidopsis*. Es wurden Ökotypen selektiert, in denen die Resistenz monogen und rezessiv bzw. semi-dominant vererbt wird. Mit Hilfe von Kreuzungen und einer RIL Population konnten die Resistenz-vermittelnden Loci aus sechs Ökotypen auf dem unteren Arm von Chromosom III lokalisiert werden. In anderen Ökotypen ist diese Eigenschaft wahrscheinlich polygen vererbt. Parallel wurde eine Selektion für dominante Resistenz, vermittelt durch prototypische *R*-Gene, durchgeführt. Diese ergab jedoch, dass dieser Typ von Resistenz in *Arabidopsis* entweder sehr selten oder nicht vorhanden ist.

Es wurden Allelismus-Tests durchgeführt um zu bestimmen, ob es sich in den oben erwähnten sechs Ökotypen um denselben Resistenz-vermittelnden Locus handelt. In F_2-Populationen dieser Kreuzungen erhielt man eine unerwartet hohe Anzahl an anfälligen Individuen, was im Widerspruch zur Erwartung für zwei identische oder eng gekoppelte Loci steht. Es wurde angenommen, dass

dieses Phänomen entweder durch epistatische Effekte oder durch die Folgen der Paarung homologer Epiallele (Paramutation) entstanden ist.

Weiterhin wurde die Resistenz in ausgewählten Ökotypen mikroskopisch charakterisiert. Dabei wurden Unterschiede in der Verteidigungsantwort beobachtet, die entweder dafür sprechen, dass die Resistenz durch verschiedene Loci hervorgerufen wird oder dass die verschiedenen genetischen Hintergründe für die unterschiedliche Ausprägung verantwortlich sind. Im Allgemeinen war die Resistenz durch eine reduzierte Produktion von Konidiophoren des Pathogens, sowie von ausgeprägtem Zelltod, Einlagerung von Kallose und Ansammlung von Wasserstoffperoxid in der Pflanze gekennzeichnet.

Feinkartierung der Resistenz-vermittelnden Loci ergab eine Zielregion, aus der mehrere Kandidatengene analysiert wurden. Der bereits erwähnte *RPW8*-Locus war eines davon. Für dieses Kandidatengen wurde in den Ökotypen Bu-0, Co-3, Do-0, Ei-4, Ei-5, Kas-1, Nok-3, Ob-0 und Sha gezeigt, dass die Reduktion von *RPW8*-Transkripten mittels dsRNAi in Anfälligkeit resultiert. Die unterschiedlichen Ausprägungen von Resistenz in diesen Ökotypen bestätigen den Dosis-abhängigen Mechanismus, der für *RPW8* vermutet wurde. Weiterhin konnte durch Sequenzanalyse der angenommenen RPW8 Proteinsequenzen von verschiedenen Ökotypen ein Bereich von hoher Variabilität zwischen der Transmembran- und der Coiled-Coil-Domäne identifiziert werden.

Insgesamt scheint Mehltau-Resistenz in *Arabidopsis* entweder von polygener Natur zu sein, oder sie wird, wie hier ebenfalls gezeigt, durch die *RPW8*-Region vermittelt.

1. INTRODUCTION

Plants and animals are continuously exposed to a range of pathogens with different timing and modes of infection (Hammond-Kosack and Parker 2003; Mysore and Ryu 2004). Therefore, they have evolved a range of different defense mechanisms against intruders, leading to co-evolution of pathogen attack and plant defense mechanisms (Holub 2001; Allen et al. 2004). During the last few years, similarities between pathogen virulence and host resistance against both plant and animal diseases have been revealed (reviewed in Cohn et al. 2001; Staskawicz et al. 2001; Nürnberger and Brunner 2002). Both plants and animals utilize an innate immune system, which recognizes a broad spectrum of pathogens using a set of somatically invariant receptors (reviewed in Underhill and Ozinsky 2002). Animals possess in addition an acquired immune system based on receptors generated by somatic mechanisms during the embryonic development of each individual organism (Medzhitov and Janeway 1997), while plants lack the ability to somatically generate new resistance specificities. The only exception might be RNAi-mediated antiviral defense, which displays some features of adaptive recognition (reviewed in Lecellier and Voinnet 2004). However, plants generally rely on preformed receptors to detect pathogens and to trigger defense responses (reviewed in Holt et al., 2003). They lack a circulating immune system with specialized cell types, but they can recognize pathogens and trigger defense responses at the level of each single cell (cell-autonomous resistance; Nürnberger et al. 2004).

Plant resistance against pathogens occurs at several stages during pathogen development and involves several mechanisms and factors. Plant cells react to pathogen attack with preformed and induced antimicrobial compounds, e.g. phenols and phenolic glycosides, unsaturated lactones, sulphur compounds, saponins, cyanogenic glycosides, and glucosinolates as preformed compounds

(reviewed in Osbourn 1996) and the phytoalexin camalexin as well as pathogenesis-related (PR) proteins as induced factors (Dixon 2001; Loon et al. 2006). As a frequent reaction upon attempted pathogen invasion, plants produce cell wall appositions (papillae) in close proximity to the invading pathogen. Papillae consist of callose, cross-linked phenolics, hydroxyproline-rich glycoproteins, reactive oxygen species and hydrolases, and are thought to reinforce the cell wall to prevent infection (Ebrahim-Nesbat et al. 1986; Hippe-Sanwald et al. 1992; Kunoh et al. 1996; Belanger and Bushnell 2002). However, recent studies on the callose synthase GSL5/PMR4 indicated that papillary callose is not required for penetration resistance to powdery mildew but may either facilitate nutrient uptake by haustoria or serves as a pathogen-induced protection barrier that prevents the recognition of pathogen-derived molecules by the host (Jacobs et al. 2003; Nishimura et al. 2003).

With regard to induced defence, the plant hormones salicylic acid (SA), jasmonic acid (JA) and ethylene (ET) are the main players in the regulation of signaling networks involved (Reymond and Farmer 1998; Pieterse and Van Loon 1999; Feys and Parker 2000; Glazebrook 2001; Thomma et al. 2001; Kessler and Baldwin, 2002). In general it can be stated that SA-dependent responses are mounted during defense against pathogens with a biotrophic lifestyle, leading to host cell death to restrict fungal growth (reviewed in Greenberg and Yao 2004, see below), whereas resistance signaling in response to necrotrophic pathogens and herbivorous insects is mediated by JA/ET-signaling (Thomma et al. 2001; Glazebrook 2005).

1.1. PLANT DEFENSE MECHANISMS

1.1.1. Basal defense: Mechanical barriers and PAMPs

The first barriers a pathogen has to overcome are preformed and constitutively present on the plant surface and can prevent the pathogen from entering the plant (Nürnberger et al. 2004). These include wax layers, rigid cell walls, anti-microbial enzymes and secondary metabolites. During this first phase of attack, it is likely that the plant might already recognize the pathogen at the level of the plasma membrane: The capability to discriminate between non-self and self is the basis for the activation of innate immune responses both in plants and animals. This recognition is achieved by the detection of microbe-associated products, in general referred to as general elicitors or pathogen-associated-molecular patterns (PAMPs).

Intriguingly, these elicitors of plant defense responses cannot only be exogenous and derive from the micro-organism (e.g. soluble components of the pathogen surface; Schweizer et al. 2002), but can also be endogenous, plant-derived structures produced by microbe-associated hydrolytic enzyme activities (Esquerré-Tugayé et al. 2000). PAMPs are highly conserved structures unique to microbes, are not produced by (potential) hosts and appear to be indispensable for microbial fitness (Aderem and Ulevitch 2000; Underhill and Ozinsky 2002; McGuinness and Dehal 2003; Medzhitov and Janeway 2002). Plants recognize multiple signals derived from individual microbial species. Known plant PAMP receptors are FLS2 (FLAGELLIN-SENSITIVE 2) and EFR (EF-Tu RECEPTOR), recognizing bacterial flagellin and EF-Tu, respectively. However, not all plants may respond to and recognize all PAMPs harboured by a pathogen (Nürnberger et al. 2004). At this level, plants do not distinguish between different pathogen types but rather react to a general danger or injury. This was suggested by the observation that the PAMP receptors FLS2 and EFR are transcriptionally activated by additional PAMPs and induce the same signaling cascade (Zipfel et

al. 2006).In animals PAMP recognition is mediated by cell surface Drosophila Toll-like receptors (TLR) and mammalian TLRs through an extra-cellular leucine-rich (LRR) domain. Transduction of the signal is achieved via the cytoplasmatic Drosophila Toll-like and human IL-1 receptor (TIR) domain and a mitogen-activated protein (MAP) kinase cascade (Underhill and Ozinsky 2002; McGuinness et al. 2003).

A well-studied plant PAMP is flg22, a highly conserved N-terminal fragment of flagellin which is the main building block of eubacterial flagellae, and triggers defense responses in *Arabidopsis* and tomato (Felix et al. 1999) independent of salicylic acid, jasmonic acid and ethylene signaling (Zipfel et al. 2004). In both the monocotyledonous rice and in animals flagellin epitopes different from flg22 in *Arabidopsis* are able to trigger defense responses (Felix et al. 1999; Donnelly and Steiner 2002; Che et al. 2000). This indicates that the recognition systems for flagellin may have arisen independently from each other, probably as a result of convergent evolution. Furthermore, the LRR domains of FLS2 and the flagellin receptor in animal systems TLR5 do not share a high sequence similarity (Gomez-Gomez and Boller 2000; Hayashi et al. 2001), further emphasizing the idea of convergent evolution (Nürnberger et al. 2004).

1.1.2. *R*-gene mediated resistance

Pathogens have evolved strategies to overcome PAMP-mediated defense. In a strategy of avoidance, pathogens try to mask their PAMPs and hide them from the recognition system of the plant. This has been observed for flagellin of bacterial pathogens of animals (Ramos et al. 2004) Sequence variation in flagellin of some plant-associated bacteria might also reflect selection pressure for a non-detectable flg22 domain (Felix et al. 1999; Pfund et al. 2004).

Another strategy by the pathogen to overcome host defenses is the suppression of host defenses. For this purpose, phytopathogens acquired virulence factors

(*AVR* factors), which, in the case of bacteria, are often delivered into the host cells with the so-called type III secretion system (Espinosa and Alfano 2004; Dodds et al. 2004; Allen and Bittner-Eddy 2004). Plant pathogens such as *Pseudomonas syringae* can secrete up to 20 to 30 effectors during infection (Chang et al. 2005). Some effectors need to be activated in the plants cell; e.g. by chaperone-mediated unfolding (Akeda and Galan 2005), or in the case of AvrRpt2, by cyclophilins such as ROC1 (Coaker et al. 2005).

The biochemical function of most AVR factors is still unknown, although some bacterial effectors have been implicated in transcriptional activation (Zhu et al. 1998; Yang et al. 2000). Some effectors from *P. syringae* suppress cell wall-based defenses by inhibiting papillae formation (AvrPto) or callose deposition during the infection (AvrE and HopPtoM; DebRoy et al. 2004; Hauck et al. 2003). Others are able to suppress plant cell death responses (Jamir et al. 2004; Nomura et al. 2005) or target plant proteins for destruction via the host proteasome (Nomura et al. 2006). In general, bacterial effectors seem to be important for interaction with the host; e.g. to invade host cells, form colonies, avoid host immune responses or adjust to new nutrient resources (Van der Biezen and Jones 1998).

According to the concept of co-evolution in an arms race process, plants have evolved resistance genes (*R*-genes) to specifically detect these effectors (gene-for-gene hypothesis; Flor 1971) or, as the guard hypothesis suggests, to monitor their modifying activities (Van Der Biezen and Jones, 1998; Dangl and Jones 2001; Nimchuk and Eulgem 2003; Jones and Takemoto 2004). Resistance due to Avr/R recognition is called race-specific or *R*-gene-mediated resistance.

There are five main classes of R proteins based on different combinations of structural motifs present within them (reviewed in Martin et al. 2003; Hammond-Kosack and Parker 2003). The most prevalent class of functionally defined R proteins is characterized by a nucleotide-binding site (NBS) and a

leucine-rich repeat (LRR) domain. This group of NBS-LRR proteins can be further divided in into coiled-coil (CC) NBS-LRR and Toll-interleukin-1 (TIR) NBS-LRR, according to their N-terminal domain. The TIR domain is named such as it shows homology to the intracellular effector domains of the *Drosophila* Toll-like and human interleukin-1 receptors. LRRs appear to be involved in the formation of protein-protein interactions and influence the requirement for downstream defense-response components (Feys and Parker 2000). The CC domain is implicated in protein-protein interactions and involved in signaling during the defense reaction (Martin et al. 2003).

In addition, there are examples of R proteins with different domain architecture, like RRS1-R from tomato against *Ralstonia solanacearum*, a TIR-NB-LRR protein with a C-terminal nuclear localisation site (NLS) and a WRKY transcriptional activation domain (Deslandes 2003). Furthermore, some other do not show any homology with other characterized R proteins, like Xa27 from rice (Gu et al. 2005).

The *Arabidopsis* genomic sequence contains 149 NBS-LRR encoding genes and 58 shorter related genes (Meyers et al. 2003). Clustering of *R*-genes is a well-known phenomenon observed at many *R* gene loci (Hulbert et al. 2001). Previous studies have demonstrated that this clustering usually results from tandem duplications of paralogous sequences (Meyers et al. 2003; Michelmore and B.C. Meyers 1998; Richly et al. 2002). Tandem and segmental gene duplications, recombination, unequal crossing-over, point mutations, and diversifying selection contribute to the diversity of *R*-genes (Meyers et al. 2003).

Comparative sequence analysis of several *R*-genes indicates the presence of balancing selection, which contributes to the maintenance of polymorphism at many *R*-gene loci (Tian et al. 2002; Mauricio et al. 2003; Allen et al. 2004; Caicedo et al. 2004; Rose et al. 2004; Bakker et al. 2006), which is in contrast to the arms

race hypothesis. However, these findings are in accordance to the observation of diversity and dynamics of the natural host and pathogen populations and their interactions, which makes it unlikely that any single *R*-gene or allele will be driven to fixation (Meyers et al. 2003).

However, rather than developing receptors for every possible effector, it appears that host plants have evolved mechanisms to monitor common host targets for perturbation. In this way they are supposed to indirectly detect the enzymatic activity of multiple effectors (Van der Biezen and Jones, 1998). One example of a host factor that is monitored for bacterial effector activity is the *Arabidopsis* RIN4 protein. It is monitored by at least two R proteins, RPM1 and RPS2, which recognize the avirulence factors AvrRmp1 and AvrB (RPM1; Bisgrove et al. 1994) and AvrRpt2 (RPS2; Yu et al. 1993, Kunkel et al. 1993), respectively. The delivery of AvrRpm1 or AvrB into the host cell results in hyperphosphorylation of RIN4, leading to the activation of RPM1-mediated resistance. It has been shown that AvrRpm1 inhibits PAMP-triggered defense responses, possibly through modification of RIN4 and other host targets (Kim et al. 2005). On the other hand, AvrRpt2 has protease activity and directly cleaves RIN4 during infection (Axtell and Staskawicz 2003; Mackey et al. 2003; Coaker et al. 2005), which is then recognized by RPS2 (Axtell et al. 2003, Day et al. 2003). RIN4 is therefore a point of convergence for at least two resistance signaling pathways.

It has been suggested that R proteins generally co-localize intracellularly with the respective pathogen effectors (Martin et al. 2003). *R*-gene-mediated resistance is commonly associated with rapid necrosis of plant cells at the site of invasion, the so-called hypersensitive response (HR), resulting in efficient containment of the pathogen (Van Der Biezen and Jones 1998; reviewed in Greenberg and Yao 2004). Cell death in race-specific resistance is preceded by the accumulation of ROIs, such as H_2O_2, $^{\bullet}O_2^-$ and $OH^{\bullet}$, of which only H_2O_2 is

relatively stable in solution. H_2O_2 can function as a signaling molecule at low concentrations and directly kill the pathogen in high concentrations (Levine et al. 1994; reviewed in Lamb and Dixon 1997).

Interestingly, different classes of R proteins require different signaling components. R proteins of the TIR-NB-LRR-type require EDS1 (ENHANCED DISEASE SUSCEPTIBILITY 1) for disease resistance signaling to biotrophic and hemi-biotrophic pathogens (Glazebrook et al. 1996; Parker et al. 1996; Falk et al. 1999). In contrast, in the signal transduction mediated by CC-NB-LRR proteins, NDR1 seems to be the key component (Aarts et al. 1998). One exception to this is RPP8, a CC-NB-LRR protein, which mediates resistance to the oomycete pathogen *Peronospora parasitica* independently of EDS1 and NDR1. Based on these results, the presence of an additional signaling path was suggested (McDowell et al. 2000).

Both lipase-like proteins EDS1 and PAD4 (PHYTOALEXIN DEFICIENT 4; Jirage et al. 1999; Falk et al. 1999) are required for SA accumulation and for the potentiation of defense involving the processing of ROI-derived signals around infection foci (Feys et al. 2001; Mateo et al. 2004). This local response also serves to prime uninfected tissues against subsequent attack in a process called systemic acquired resistance (Durrant and Dong 2004; Dong 2004). Furthermore, SA contributes to the expression of both *EDS1* and *PAD4* as part of a positive feedback loop that appears to be important in the amplification of defense (Wiermer et al. 2005).

The EDS1/PAD4-dependent pathway is further regulated by other components such as *LESIONS SIMULTATING DISEASE 1* (*LSD1*), a ROI modulator (Mateo et al. 2004) and MAP kinase 4 (MPK4). These proteins appear to constitute a node in the inhibitory cross-talk between the SA and JA signaling networks (Wiermer et al. 2005; Petersen et al. 2000; Mateo et al. 2004). The JA- or ET-

activating functions of MPK4 are repressed by EDS1 and, to a lesser extent, by PAD4. These results show that EDS1 and PAD4 are involved in controlling signal antagonism between SA and JA/ET defenses (Clarke et al. 2000; Gupta et al. 2001; Wiermer 2005). Moreover, no major differences have been observed between PAMP- and *R*-gene-mediated signal transduction (Nürnberger and Scheel 2001; Yang et al. 1997). Commonly reported signals in both plants and in animals are changes in cytoplasmic Ca^{2+} levels, the production of reactive oxygen species (ROS) and nitric oxide (NO) together with the post-translational activation of MAPK cascades (Nürnberger and Scheel 2001; Jonak et al. 2002; Barton and Medzhitov 2003).

1.2 POWDERY MILDEWS

Powdery mildews are parasitic ascomycete fungi, which cause widespread plant diseases resulting in loss of plant growth and yield (Agrios 1988). The powdery mildew species *Golovinomyces orontii*, which was used in this study, belongs to the order *Erysiphales*, which contains one family (*Erysiphaceae*) with 28 genera and approximately 100 species. *G. cichoracearum* is a close relative of *G. orontii*, as determined by analysis of internal transcribed spacer (ITS) sequences (Saenz and Taylor 1999). Powdery mildews are obligate biotrophs, meaning that they require living host cells to grow and reproduce. They grow on the surface of the plant host, infecting only cells in the epidermal layer of the plant.

The mycelia (vegetative structures) as well as the conidia (asexual spores) formed on the host plant are responsible for the name powdery mildew (Bélanger et al. 2002; Braun 1987, 1995). The typical lifecycle of a powdery mildew species is shown in the example of *G. orontii* on *A. thaliana* (Figure 1). In a compatible interaction, *G. orontii* spores have formed an appressorial germ tube at 24 hours post inoculation (hpi). At this stage fungal appressoria are produced and the plant cell wall is penetrated. Following successful

establishment of a so-called haustorium, a specialized feeding structure of the pathogen, the fungal colony spreads on the leaf surface by branching and production of additional hyphae and haustoria. At approximately seven days post inoculation (dpi) asexual reproduction occurs: Conidiophores with four to five conidiospores (conidia) emerge. The sexual state includes the production of cleistothecia (ascocarps) which contain asci with ascospores, usually important in perennation (Bélanger et al. 2002; Braun 1987, 1995).

An important feature of biotrophic organisms are haustoria (see above). They are not truly intracellular, but localized in an invagination of the host cell and separated from the host cytoplasm by a specific derivative of the host plasma membrane, the extra-haustorial membrane. The space in between the two membrane layers, the extra-haustorial matrix, is enriched in carbohydrates. The host cytoplasm is separated from the extrahaustorial matrix by a haustorial neckband, which has a collar-like structure (Bélanger et al. 2002; Szabo and Bushnell 2001).

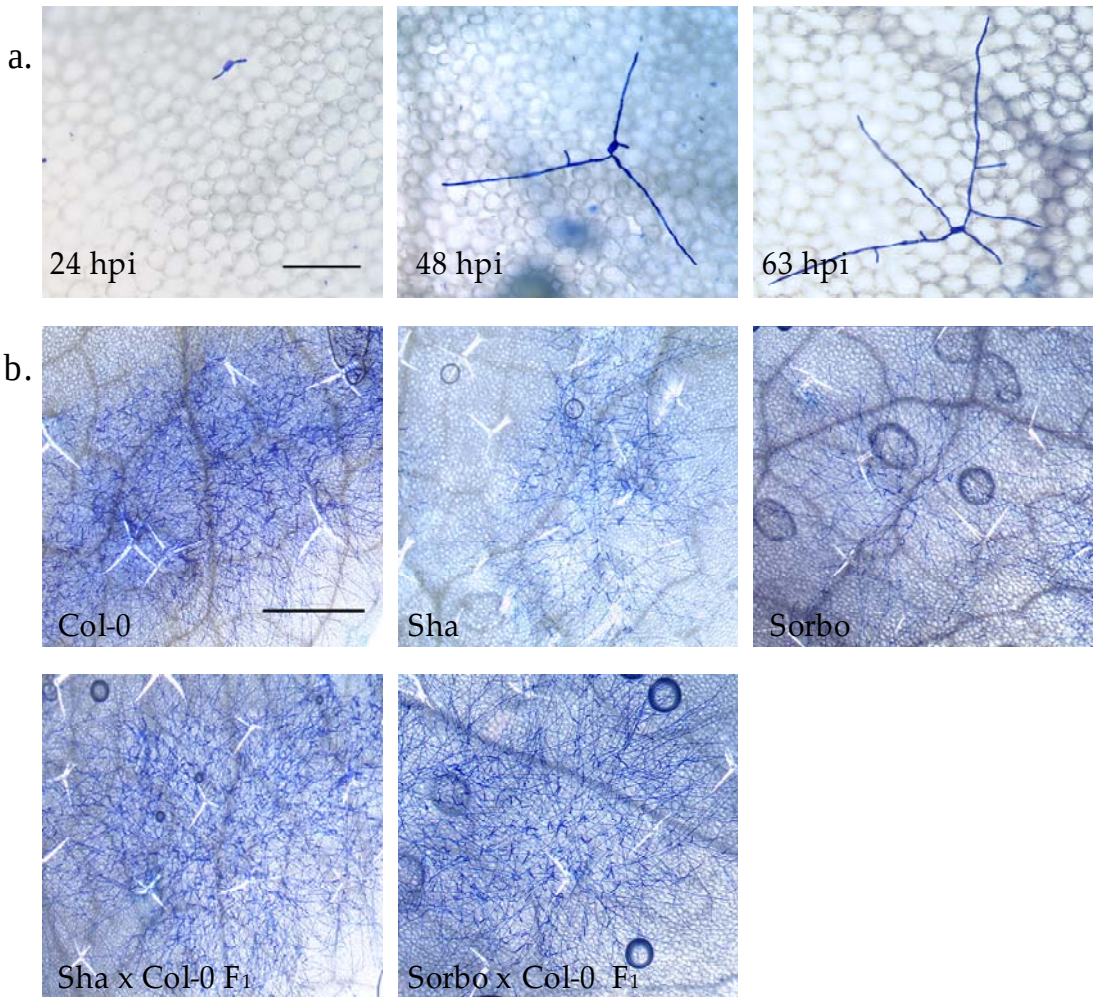

Figure 1: Development of *G. orontii* on *A. thaliana*. a. Micrographs of *G. orontii* on Col-0 at 24, 48 and 63 hpi. Size bar indicates 200 µm. **b**. Micrographs of *G. orontii* colonies at 7 dpi. Size bar corresponds to 500 µm. Fungal structures were stained with Coomassie Blue.

1.3 RECESSIVELY INHERITED RESISTANCE TO POWDERY MILDEWS

Due to their biotrophic lifestyle powdery mildews have to evade or suppress host defenses to complete their life cycle and to reproduce on the plant (Mendgen and Hahn 2002; Schulze-Lefert and Panstruga 2003). In addition, plant gene products are likely to exist that are necessary for nutrient export from the plant cells and for the establishment of the infection site as a metabolic sink. As a consequence, specific host genes and/or proteins defined as compatibility

or susceptibility factors may be essential for successful pathogenesis and a lack of these factors might result in resistance to an otherwise virulent pathogen (Vogel and Somerville 2000; Panstruga 2003).

This type of resistance is thought to be more stable in comparison to *R*-gene mediated resistance, which has a narrow spectrum and thought to be rather ephemeral, providing limited agronomic value. In contrast, recessive resistance based on non-functional compatibility factors is thought to be more durable, as it has been shown for a recessive allele of *mlo* (mildew-resistance locus o), which has been successfully used in barley lines in European agriculture for about three decades (Jørgensen 1992; Büschges et al. 1997; Piffanelli et al. 2004). Several mutants with enhanced resistance to powdery mildews were identified in *A. thaliana* in genetic screens and the analysis of other plants and pathogens revealed additional loci responsible for resistance in these pathosystems (see below, 1.3.2).

Arabidopsis constitutive immunity (*cim*) mutants express host defenses constitutively, also in the absence of pathogens (Maleck et al. 2002). In the *enhanced disease resistance 1* mutant (*edr1)* resistance is correlated with induction of several defense responses, including host cell death, which confers resistance to the powdery mildew *Golovinomyces cichoracearum* and the bacterial pathogen *P. syringae*. *EDR1* encodes a mitogen-activated kinase kinase kinase (MAPKKK), which negatively regulates SA-inducible defense responses (Frye and Innes 1998; Frye et al. 2001).

The powdery mildew resistance (*pmr*) mutants are unable to support growth of the pathogen and were suggested to represent true compatibility factors. *PMR1* and *PMR3* are not cloned yet. *PMR4* is encodes the callose synthase GSL5. The knockout of this gene leads to reduction of callose production in response to pathogen attack and resistance to the powdery mildew species *G. cichoracearum*

and *G. orontii*, as well as to the oomycete *Peronospora parasitica* (Jacobs et al.2003; Nishimura et al. 2003). *PMR6* encodes a pectate lyase and the corresponding mutant shows and increased pectin and uronic acid content in cell wall. This probably leads to constitutive activation of non-SA or JA/ET defense response not requiring the hypersensitive response (Vogel et al. 2002). In *pmr5*, similar changes have been reported, but it was suggested that these two genes act synergistically in parallel pathways (Vogel et al. 2004). The fact that *pmr5* and *pmr6* are resistant to *G. cichoracearum* and *G. orontii*, but fully susceptible to unrelated pathogens such as virulent strains of either *P. syringae* or *P. parasitica* suggests that these two proteins may in fact be true compatibility factors (Vogel and Somerville 2000; Vogel et al. 2002 and 2004).

PMR2 was found to be allelic to *AtMLO2* (Consonni et al. 2006). In *Arabidopsis AtMLO2* has a predominant role in the establishment of compatibility with two powdery mildew species together with the two additional co-orthologs *AtMLO6* and *AtMLO12*, which act in partial functional redundancy. Resistance mediated by a recessive *mlo* allele in barley is effective against all known isolates of the virulent powdery mildew fungus *Blumeria graminis* f. sp. *hordei* (*Bgh*; Jørgensen 1977). The barley MLO protein is thought to modulate defense responses to *Bgh* via a vesicle-associated and SNARE protein–dependent mechanism (Panstruga 2005). Naturally occurring recessively inherited resistance to powdery mildews is also conferred in pea by *er1* and *er2*, and by *ol-2* in tomato, which confer resistance to pea and tomato powdery mildews, respectively, when non-functional (Heringa et al. 1969; Kumar et al. 1981; Tiwari et al. 1997; Ciccarese et al. 1998 and 2000).

1.3.1 Recessively inherited resistance of *Arabidopsis* to other pathogens

With regard to other pathogens, recessive *RRS1-R* alleles have been identified that provide resistance of *Arabidopsis* to the bacterial wilt pathogen *Ralstonia solanacearum*. Although genetically defined as a recessive allele, it behaves as a dominant resistance gene in transgenic plants. RSSR1-R was identified as an R protein of the TIR-NB-LRR type which also contains a nuclear localization signal (NLS) and a WRKY domain, predicted to function in a direct *R*-gene-*avr*-gene interaction in the nucleus (Deslandes et al. 2002). Recessive resistance against bacteria also occurs naturally in rice, where *xa13* confers resistance to *Xanthomonas oryzae* pv. *oryzae* by a so far unknown mechanism (Chu et al. 2006).

Another recessive locus mediating resistance when mutated is *elf(iso)4E*, which confers resistance against potyvirus infection in *Arabidopsis*, tomato and pepper (Lellis et al. 2002; Robaglia and Caranta 2006, Ruffel et al. 2002 and 2004). It has CAP-binding activity and was suggested to function via interaction with the viral genome-linked protein (VPg) of potyvirusses and induction of viral genome expression and replication. In a mutant screen for compatibility factors in the downy mildew-*Arabidopsis* interaction, *dmr1* to *dmr6,* were identified; cloning of these genes will provide further insight in interactions with these fungal pathogens (Van Damme et al. 2005). With the exception of *RRS1-R*, all recessive resistance genes found to date in *Arabidopsis* in the studies mentioned above were obtained by mutant screening. Natural occurring recessive resistance has been found only in other plant species.

1.3.2 Dominantly inherited powdery mildew resistance in *A. thaliana*

Natural resistance to powdery mildews observed in several *Arabidopsis* accessions was found to be mediated by the dominantly inherited *RPW8* locus, identified in Kas-1, Wa-1 and Ms-0. The *RPW8* locus of Ms-0 comprises two naturally polymorphic and dominant R-genes, *RPW8.1* and *RPW8.2*, which control resistance to a broad range of powdery mildew pathogens (Xiao et al. 2001), as well as the genes *HR1, HR2* and *HR3*, which do not contribute to resistance. *RPW8.1* and *RPW8.2* encode proteins, which are structurally different from other R proteins identified, since they only possess a predicted CC and a assumed TM domain. Resistance mediated by these genes is induced by SA-dependent defense responses. In Kas-1, three independent resistance loci were identified. The strongest of them, *RPW10*, is presumably identical to *RPW8* (Wilson et al. 2001). In Wa-1, two loci were identified, again the strongest (*RPW13*) probably identical to *RPW8*. In this accession, resistance is not correlated with an HR, which was explained with different genetic backgrounds between Wa-1 and Ms-0 (Schiff et al. 2001).

Analysis of *RPW8* syntenic loci in a range of *A. thaliana* relatives suggested that this locus evolved in the *Brassicaceae* from an *HR3*-like ancestor via gene duplication and functional diversification through positive selection several million years ago (Xiao et al. 2004). It was claimed that, based on sequence analysis, *RPW8* represents the main source of broad sprectrum resistance to powdery mildews (Xiao et al. 2004).

1.4 NATURAL VARIATION

Recent studies in *Arabidopsis thaliana* exploiting 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 and Schmitt 2006). *A. thaliana* is native to Eurasia and North Africa and has been naturalized in North America (Price et al. 2004; O'Kane and Al-Shebaz 1997). It occupies disturbed environments early in succession and is consequently often found in agricultural fields and other disturbed sites associated with human activity (Bergelson *et al*. 1998; Mauricio 1998). Across its geographic range, it is exposed to a range of diverse (micro-) climates and habitats (Hoffmann 2002). This contributes to different selection pressures and diversity among different populations (Koornneef and Alonso-Blanco 2004; Mitchell-Olds and Schmitt 2006). A range of different *Arabidopsis* accessions with a diverse range of origins can be obtained at public stock centres NASC or ABRC (accessible through www.arabidopsis.org). *A. thaliana* reproduces mainly by selfing (Redei 1975; Abbot and Gomes 1989), therefore individuals are assumed to be homozygous at most loci.

Several analyses of amplified length polymorphisms (AFLP) or single nucleotide polymorphism (SNP) markers in a range of different *Arabidopsis* accessions have indicated that much of its native range was colonized from several glacial refugia, and admixture occurred in zones, which were colonized from more than one source. However, recent human disturbance tends to homogenize variation among populations, especially in agricultural regions of Europe and within the introduced populations in North America (Schmid et al 2006; Bakker et al. 2006; Jorgensen et al. 2004; Sharbel et al. 2000). Consequently, the high variability within Western European populations probably reflects those

admixture events that are a consequence of human disturbance (Le Corre 2005). Such admixture complicates the ability to understand the evolutionary forces shaping genetic variation within *A. thaliana* populations (Schmid et al. 2006; Mitchell-Olds and Schmitt 2006).

However, the polymorphisms among different *Arabidopsis* accessions can be important resources for the identification of gene function and genetic pathway structure. In this context the adoption of Recombinant Inbred Lines (RILs) between divergent parental ecotypes has been of great importance for mapping complex traits (Koornneef et al. 2004).

1.5 MAPPING IN *A. THALIANA*

Genetic variation in *A. thaliana* is mostly due to traits with a quantitative and continuous nature, known as quantitative trait loci (QTL). This is in contrast to mutants providing discrete variation (Alonso-Blanco and Koornneef 2000). The definition of single loci responsible for the trait of interest and of chromosomal regions containing QTLs for a specific trait are obtained by mapping. During this process, individuals of a segregating population are phenotyped for the trait of interest and genotyped at markers across the genome. Regions containing genes of interest are identified by a statistical association between marker genotype and trait values (reviewed in Doerge et al. 2002; Abiola et al. 2002). The genetic resolution is limited by the accuracy of trait measurement, the size of the mapping population, the genome coverage by markers and the number of recombination events in the segregating populations (Maloof 2003; Lukowitz et al. 2000). As mentioned above, RIL populations are of great importance in this context. The development of RIL populations from a diverse panel of *A. thaliana* ecotypes is currently being carried out by several research groups (www.dpw.wau.nl/natural/).

The molecular markers mainly used in mapping experiments are simple sequence polymorphisms (SSLPs), cleaved amplified polymorphic sequences (CAPS) and derived CAPS (dCAPS) and RFLPs (restriction fragment length polymorphisms). SSLPs and CAPS are co-dominant, meaning that the genotype of both chromosomes is analyzed, which allows gathering of the maximum amount of information from a mapping population. In addition, they are PCR-based and can be analyzed on agarose gels, which makes them easy to use and also cost-efficient (Lukowitz et al. 2000). Furthermore, known SSLP and CAPS markers for *Arabidopsis* can be accessed via The *Arabidopsis* Information Resource (TAIR; www.arabidopsis.org). For designing new markers of this type, several programs provide assistance (see Materials and Methods). In addition, more than 50 000 single nucleotide polymorphisms (SNPs) and several insertion/deletion markers are known between the accessions Landsberg erecta (Ler) and Col-0 (Jander et al. 2002) as well as polymorphisms between additional accessions (Schmid et al. 2003, 2006). However, most techniques for SNP analysis are still laborious, although new methods are under development (Kwok 2001; Tsuchihashi and Dracopoli 2002).

As mentioned above, the size of a mapping population has a large impact on mapping resolution. Several mapping experiments showed that resolutions between 10 and 40 kb (corresponding to between two and ten genes) can be reached with a population of 1000 plants (Lukowitz et al. 2000). When the target gene region is identified, recombinants in the vicinity of the target gene can be identified and collected for fine mapping. The identification of recombination events is possible by analyzing two markers known to be closely linked and to flank the mutation on both sides.

The ratio between the physical and genetic distance varies with respect to the location on the chromosome and the inherent recombination frequency. In general, these variations are small and a genetic distance of 1% recombination

corresponds to a physical distance of 100 to 400 kb with an average of 250 kb in *Arabidopsis*. Known exceptions are the centromeric regions and a short segment on chromosome II, where this distance is much higher (Lukowitz et al. 2000).

Once a region of ten to twenty candidate genes is identified, these genes can be tested by several approaches. Either the annotation of gene sequences provides enough information to choose a candidate gene and to determine the mutation or variation by sequencing or the target gene has to be identified by either complementation analysis (in case of a recessive mutation) or by copying the mutant phenotype after transfer of a dominant mutant allele into wildtype plants. In addition, the analysis of T-DNA insertion lines, which are available for most genes, can help to rapidly identify the gene of interest (Lukowitz et al. 2000). In case of a QTL, generation of near isogenic lines (NILs) is necessary to separate the QTL from the rest of the segregating loci. NILs ideally differ only for the alleles in a small genomic region around the QTL of interest. (Alonso-Blanco and Koornneef 2000; Maloof 2003). These lines can be obtained by performing several rounds of backcrossing to the parental accession that did not carry the trait of interest.

The mapping process can be complicated by the fact that a trait is not simply monogenically inherited, but due to several QTLs, or influenced by second site modifiers. This situation creates the necessity for NILs or RILs. Epigenetic mutations can produce further complication in a mapping project. This phenomenon leads to heritable changes in expression or gene function not due to changes in the DNA sequence (reviewed in Wolffe and Matzke 1999; Grant-Downtown and Dickinson 2005 and 2006).

1.6 PROJECT OBJECTIVES

The aim of this project was the identification of novel compatibility factors for the interaction between *Arabidopsis* and powdery mildews by exploiting natural variation. Based on this analysis I selected accessions which were either resistant to one or to both of the tested powdery mildew species *Golovinomyces cichoracearum* or *G. cruciferarum* in order to pre-select for putative compatibility factors, which are known to often confer resistance to closely related species or isolates (Vogel et al. 200, 2002, 2004; Van Damme et al. 2005). Infection phenotypes of the selected accessions were determined macroscopically with a third powdery mildew species, *Golovinomyces orontii*.

Accessions resistant to *G. orontii* and at least one further compatible powdery mildew species were crossed to the susceptible ecotype Col-0 to determine inheritance of resistance in F_1 and F_2 progeny. To select for putative compatibility factors, accessions that contained monogenic and recessively or semi-dominantly inherited resistance, i.e. having susceptible F_1 plants and F_2 progeny segregating 3 : 1 (susceptible : resistant) or 1 : 2 : 1 (susceptible : intermediate : resistant), were chosen. Resistance in these accessions was characterized microscopically and mapped with either CAPS and SSLP markers or with a population of recombinant inbred lines. Candidate genes were analyzed, which led to the identification of *RPW8* and the confirmation that *RPW8* represents the major natural source of resistance in *Arabidopsis* to several powdery mildew isolates.

2. MATERIALS AND METHODS

2.1 MATERIALS

2.1.1 Antibiotics

Ampicillin (1000 x): 50 mg/mL in H_2O

Kanamycin (200 x): 50 mg/mL in H_2O

Rifampicin (1000 x): 100 mg/mL in ethanol

Stock solutions stored at –20° C.

2.1.2 Bacterial strains

E. coli strain DH5α

Genotype: F^- *sup*E44 Δ*lac*U169 *hsd*R17 *rec*A1 *end*A1 *gyr*A96 *thi*-1 *rel*A1

Agrobacterium strain

GV3101 (pMP90RK, Gm^R, Km^R), Rif^R (Koncz and Schell 1986)

2.1.3 Pathogens

The *Arabidopsis thaliana* powdery mildew *Golovinomyces orontii* was propagated on *A. thaliana NahG* or *eds16* plants cultivated at 22°C and 10 h light and 20 °C at night with 80% humidity in a protected environment.

2.1.4 Plant material

Seeds of *A. thaliana* accessions were obtained from NASC (Nottingham *Arabidopsis* seed stock centre). Ecotypes and mutants used in this study are listed in Tables SD 1 and SD 2, Supplementary Data.

2.1.5 Vectors

pDONR201 by INVITROGEN, Heidelberg was used for GATEWAY-based cloning.

pJawohl8-Gateway (Dr. Bekir Ülker, Dr. Christina Neu) was used for construction of the *RPW8* silencing vector, which contained two copies of *RPW8.1* or *RPW8.2* arranged in inverted repeats.

pAM-PAT-Gateway (Dr. B. Ülker)

2.1.6 Oligonucleotides

Primers used in the present study are synthesized by SIGMA, INVITROGEN or PROMEGA and are listed in Supplementary Data, Table SD 3.

2.1.7 Enzymes

Nucleic acid modifying enzymes

Standard PCR reactions were performed using homemade *Taq* DNA polymerase while for the cloning of PCR products, *Pfu* polymerase was used.
Modifying enzymes were listed below and purchased from various sources:

Taq-DNA Polymerase Homemade
Pfu DNA Polymerase STRATAGENE (Heidelberg)
T4 DNA ligase ROCHE (Mannheim)
Superscript II RT INVITROGEN (Heidelberg)

GATEWAY ® -Technology INVITROGEN (Heidelberg)

BP-Clonase INVITROGEN (Heidelberg)

LR-Clonase INVITROGEN (Heidelberg)

Lysozyme ROCHE (Mannheim)

2.1.8 Chemicals

Laboratory grade chemicals and reagents were purchased from ROTH (Karlsruhe), SERVA (Heidelberg), BOEHRINGER (Mannheim), MERCK (Darmstadt), BECKMANN (München), GIBCO BRL (Neu Isenburg) and SIGMA (Deisenhofen) unless otherwise stated.

2.1.9 Media

Unless otherwise indicated all media were sterilized by autoclaving at 121°C for 20 minutes. Heat labile solutions were sterilized using filter sterilisation units prior to addition of autoclaved components. For the addition of antibiotics and other heat liable components the solution or media were cooled down to 55°C.

E.coli Media: LB (Lauria Bertani) Broth

tryptone peptone	1 %
yeast extract	0,5 %
NaCl	0,5 %
in H_2O	
For selection	Kanamycin 50 µg/mL or
	Ampicillin 100 µg/mL

Agar plates

1,5 - 2 % agar was added to the above broth.

Agrobacterium Media: YEB

Beef extract	0,5 %
Yeast extract	0,1 %
Tryptone	0,5 %
Sucrose	0,5 %

in H_2O

adjust pH to 7,2 with 0,5 M NaOH.

For selection	50 µg/mL Carbenicillin,
	100 µg/mL Rifampicin and
	25 µg/mL Kanamycin.

2.1.10 Buffers and solutions

Agarose gel, 1 % and 3,5 %

Agarose	1 g or 3,5 g
TAE buffer (10x)	100 mL
Ethidium bromide stock (10 mg/mL)	2 µL

Aniline Blue staining solution

KH_2PO_4	150 mM
Aniline Blue	0,01 %

in H_2O,

adjust pH to 9,5 with ~25 KOH pellets per 500 mL.

Buffer A for fast DNA preparation (prepare fresh)

10 M NaOH	1 %
Tween 20	2 %

in H_2O.

Buffer B for fast DNA preparation

Tris HCl	100 mM
EDTA	2 mM
in H_2O,	
adjust pH to ~ 2,0 with HCl.	

Coomassie Blue staining solution

Coomassie Blue	0,25%
in ethanol.	

Crystal Red solution for staining of PCR reactions

Crystal Red	0,2 g
in H_2O.	
Sucrose	60 g
adjust pH to 7,8,	
ad 100 mL with in H_2O.	

DAB (3,3'-Diaminobenzidine) staining solution

DAB	1 mg/mL
in H_2O.	
adjust pH to 3,8 with HCl.	

DAB destaining solution

Lactic acid	1
Glycerol	1
Ethanol	1

EDTA (ethylenediaminetetraacetic acid)-stock (0.5 M, pH 8.0)

Na_2EDTA	186,1 g
H_2O	1000 mL

Dissolve 186,1 g Na_2EDTA in 700 mL water, adjust pH to 8,0 with 10 M NaOH (~50 mL; add slowly), add water up to 1 L. Filter sterilize.

Edwards buffer for DNA isolation

Tris-HCl pH 7,5	200 mM
NaCl	250 mM
EDTA	25 mM
SDS	0,5 %

in H_2O.

Ethidium bromide stock (10 mg/mL)

Ethidium bromide	0,2 g
H_2O	20 mL

Stored at 4° C in dark bottle. Do not sterilize.

Lactophenol stock solution

Phenol	100 mL
Lactic acid	100 mL
Glycerol	100 mL
H_2O	100 mL

Dilute 1 : 2 with ethanol before usage.

Loading buffer (DNA)

Glycerin	50 %
Xylene cyanol	0,1 %
Bromphenolblue	0,1%
in H_2O.	

PCR buffer

Tris-HCL pH 8,4	100 mM
KCL	500 mM
$MgCl_2$	20 mM
in H_2O.	

STET buffer for *E. coli* boiling preparations

Glucose	8 %
Triton X 100	5 %
EDTA	50 mM
Tris pH 8,0	50 mM

TAE (Tris/acetate/EDTA) buffer (10x) for gel electrophoresis

Tris base	24,2 g
glacial acetic acid	5,71 mL
$Na_2EDTA{\cdot}2H_2$	3,72 g
H_2O	to 1 L

TE (Tris/EDTA) buffer

Tris/HCl (pH 8,0, 7,5)	10 mM
EDTA (pH 8,0)	1 mM
Tris/HCl	1 M
Tris-Base	121 g

Ad 1000 mL with H_2O.

Dissolve 121 g Tris base in 800 mL, adjust to desired pH with concentrated HCl, adjust volume to 1 L with H_2O, filter sterilize if necessary. The solution can be stored up to 6 months at 4° C or at room temperature.

Trypan Blue staining solution

Trypan Blue 250 g/L

in lactophenol stock solution,

dilute 1 : 1 prior use.

Trypan Blue destaining solution

Chloral hydrate 2,5g/mL

in H_2O.

2.1.11 Software, databases, and other internet resources

Sequence alignment

MegAlign (Lasergene)

ClustalW

Translation of DNA into protein sequences

EditSeq (Lasergene)

MegAlign (Lasergene)

http://www.expasy.org/tools/dna.html

Analysis and alignment of sequencing chromatograms

SeqMan (Lasergene)

Sequence analysis and comparison

http://www.ncbi.nlm.nih.gov/BlAST/

Databases for genomic sequences of *Arabidopsis thaliana*

http://www.arabidopsis.org

http://www.tigr.org/tdb/e2k1/ath1/ath1.shtml

http://www.ncbi.nlm.nih.gov/gquery/gquery.fcgi

Searching for SSLP and CAPS markers

http://tandem.bu.edu/trf/trf.html (Benson 1999)

http://helix.wustl.edu/dcaps/dcaps.html (Neff et al. 2002)

http://www.arabidopsis.org

http://www2.mpiz-koeln.mpg.de/masc/ (Schmid et al. 2003; Törjék et al. 2003)

http://www.arabidopsis.org/Cereon/ (Jander et al. 2002)

Primer design

http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi (Rozen and Skaletsky 2000)

Databases for expression analysis (microarrays) in *Arabidopsis thaliana*

https://www.genevestigator.ethz.ch/

http://www.weigelworld.org/resources/microarray/AtGenExpress/

MicroRNA databases of *Arabidopsis thaliana*

http://sundarlab.ucdavis.edu/mirna/ (Adai et al. 2005)

http://asrp.cgrb.oregonstate.edu/ (Gustafson et al 2005)

Software for automatic imaging analysis of hyphal growth

HyphArea (Seiffert and Schweizer 2005)

Software for (microscopic) imaging

WCIF Image J (http://www.uhnresearch.ca/facilities/wcif/imagej/)

Software for RIL mapping

WinQTLCartographer (http://statgen.ncsu.edu/qtlcart/WQTLCart.htm)

2.2 METHODS

2.2.1 Growth conditions of *Arabidopsis* plants and inoculation procedures

Plants were sown on soil substrate and stratified for two days at 4°C in darkness to allow an even germination. Germination was induced by transfer of the plants to a light chamber with 22 °C during the day, 20°C during the night 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. All plants were grown for four to five weeks at a day/night cycle of 10 and 14 hours, respectively. Subsequently they were transferred to another light chamber for inoculation with *G. orontii*.

G. orontii was cultivated on *NahG* or *eds16* mutant *A. thaliana* plants (similar day/night conditions as above and 80 % humidity). Around ten to eleven days post inoculation (dpi), these plants were used to inoculate new plants by brush inoculation from a height of ~ 20 cm. With regard to the respective experiment, different inoculation densities were applied: For determination of host cell entry rates, analysis of hyphal growth with HyphArea and conidiophore production, lower inoculation densities were used to avoid overlapping of colonies.

2.2.2 Determination of infection phenotypes

Between 7 and 9 dpi, infection phenotypes were scored macroscopically at two or three time points. Therefore, plants were given disease reaction (DR) scores ranging from 3 to 0. DR 3 describes fully resistant plants, indicating that strong pathogen growth was observed similar to Col-0 wildtype and DR 0 refers to fully resistant plants, in which no fungal structures could be detected macroscopically. DR scores of 2 refer to intermediate susceptible plants, which are slightly less susceptible than Col-0, and DR 1 to intermediate resistant plants, which show only little fungal structures on the leaf surface.

During the screening of *A. thaliana* ecotypes, at least 5 plants per ecotype were inoculated with *G. orontii*. Accessions and RILs scored resistant in the first round were analyzed in a second to confirm the observed phenotype. Inoculations were performed together with the Col-0 ecotype as a reference and control. Resistant accessions were also analyzed microscopically.

2.2.3. Transformation of *E. coli*

2.2.3.1. Preparation of electro- and heatshock-competent cells

Electro-competent cells:

1. 10 mL of an overnight culture of E. coli strain DH5α was added to 1 L of LB broth and shaken at 37°C until the bacterial growth reached an OD_{600} of 0,5-0,6.
2. The bacteria were pelleted at 5000 x g for 20 minutes at 4° C and the pellet gently resuspended in ice-cold sterile water.
3. The cells were pelleted as before and again resuspended in ice-cold water. The process was repeated twice.

4. Finally the cells were gently resuspended in a 1/100 volume of the initial culture in 10% sterile glycerol, pelleted once more and then resuspended in 5 mL 10% glycerol.
5. 50 µL aliquots of cells were frozen in liquid nitrogen and stored at –80° C until use.

Heat-shock-competent cells:

1. 100 mL of an overnight culture of *E. coli* strain DH5*α* was added to 1 L of LB broth and shaken at 37°C until the bacterial growth reached an OD_{600} of 0,2
2. The bacteria were pelleted at 5000 x g for 10 minutes at 4° C and gently resuspended in 250 mL ice-cold sterile 100 mM $MgCl_2$.
3. The cells were incubated for 5 minutes on ice, then pelleted as before and again resuspended in 50 mL ice-cold 100 mM $MgCl_2$.
4. The cells were incubated for 20 minutes on ice, and then pelleted as before. Finally the cells were gently resuspended in 10 mL of a solution prepared with 85% 100 mM $CaCl_2$ and 15% glycerol.
5. 50 µL aliquots of cells were stored at –80° C until use.

2.2.3.2 Transformation of electro- and heatshock-competent cells

Heat-shock transformation:

1. 100-250 ng of plasmid DNA was mixed with 50 µL of heat-shock-competent *E. coli* cells in a 1,5 mL reaction tube and incubated on ice for 10 minutes.
2. Subsequently, the cells were transferred in a water bath at 42° C for 1,5 minutes and incubated on ice for 10 minutes.

3. 1 mL of LB medium was added to the cells before incubating them for 1 hour at 37° C. A fraction (~150-300 μL) of the transformation mixture was plated onto selection media plates.

Electro-transformation:

1. 1 μg DNA (salt-free ligated plasmid DNA) or ~1 μL of ligated mix from 10 μL ligation reaction was mixed with 50 μL of electro-competent *Agrobacterium* cells, and transferred to a cold BioRad electroporation cuvette (2 mm electrode distance).
2. The BioRad gene pulse apparatus was set to 25 μF capacitance, 2,5 kV voltage and the pulse controller to 400 Ω (capacitance 125 μF).
3. After pulsing once at the above settings for a few seconds, 1 mL of YEB medium was immediately added to the cuvette, the cells were quickly resuspended and incubated at 28°C for 2 hours in a fresh reaction tube.
4. The cells were pelleted at 2 min, 13000 rpm.
5. The pellet was resuspended in 100 μL medium, then plated onto YEB selection media plates and incubated for 2 days at 28°C.

2.2.4 Transformation of *Arabidopsis* plants

2.2.4.1 Transformation

The production of stable transgenic *A. thaliana* plants was achieved by *Agrobacterium*-mediated transformation. A binary vector carrying the sequence of interested was used to transform the *Agrobacterium* strain GV3101 (see 2.1.2). In this study, selected *Arabidopsis* accessions were transformed with the pAM-PAT-*PMR6*-cDNA-GW complementation vector and the pJawohl8 vector containing cDNA of either *RPW8.1* or *RPW8.2* in an inverted repeat. Flowering plants were transformed according to the following protocol (modified from Clough and Bent 1998):

1. A starter culture of the desired *Agrobacterium* strain was made in 3-5 mL YEB media provided with selection antibiotics with 2-3 days incubation at 28°C with shaking.
2. 200 mL YEB media with selection antibiotics were inoculated with the starter culture and incubated for another 2 to 3 days at 28°C with shaking.
3. The bacteria were spun down at 4800 rpm for 20 min at room temperature.
4. The pellet was resuspended in 50 mL 5% sucrose solution and mixed with additional sucrose solution until an OD_{600} of ~0,8 was reached.
5. 100 µL Silwet-700 was added to 300 mL bacteria solution.
6. The flowering inflorescences were dipped for 30 sec to 1 min into the solution.
7. The plants were covered with foil to ensure a high humidity for 2 days.
8. Plants were allowed to set seeds. For seed collection five plants per line were bulked together.

2.2.4.2 Selection of transformed *Arabidopsis* plants

The bulked seeds were sown on soil and the transformed T_1 seedlings were selected after 1-2 weeks by spraying with the herbicide BASTA. Only those plants equipped with the vector, which also contains a resistance gene against the herbicide, survive this treatment. Spraying was repeated after 3 days. For the transformation with the *PMR6*-cDNA-GW vector, plants were analyzed by PCR with the corresponding primers (*PMR6*-GW_F and *PRM6*-GW_R) for presence of the construct.

2.2.5 Nucleic acid extraction

2.2.5.1 DNA extraction

The extraction of DNA from most *Arabidopsis* leaf material was performed after the method of Edwards et al. (1991), modified:

1. Leaf material (at least 10 x 10 mm^2 leaf surface) was harvested and the samples were frozen immediately in liquid nitrogen.
2. The samples were ground and immediately 300 µL of Edwards buffer was added.
3. The samples were incubated for 10 min at 65°C followed by another 10 min incubation on ice.
4. 200 µL cold chloroform was added and mixed, followed by
5. Centrifugation for 5 min at 13 000 rpm.
6. 200 µL of the upper phase were transferred into a new 1,5 mL reaction tube.
7. The DNA was precipitated by addition of 200 µL cold isopropanol.
8. The DNA was pelleted by centrifugation for 5 min at 13 000 rpm.
9. The pellet was washed with 500 µL 70% ethanol, dried and resuspended in 100 µL sterile water.

For analysis of F_2 progeny of crossings a protocol for fast high-throughput DNA extraction without grinding or washing and adaptable for 96-well formats was employed (Xin et al. 2003):

1. Small leafs of F_2 plants growing in 96 well trays on soil were harvested into 96 well PCR-plates on ice.
2. 50 µL of fresh prepared buffer A was added and the plate was incubated for 10 min at 95°C in the PCR cycler and put immediately on ice.

3. Buffer B was added, the solutions were mixed and the debris was spun down for 2 min at 4000 rpm and 4°C.
4. An aliquot of each sample was transferred to a new plate and stored at 4°C. The original plate was stored at -20°C.
5. 1 µL of DNA solution was used in a 25 µL PCR mix.

2.2.5.2 RNA extraction

RNA extraction was performed with a combination of two methods, from step 2 to 7 with Tri-Reagent (SIGMA) according to the manufactures instructions, afterwards continued with the RNeasy Kit (QIAGEN):

1. Circonia beads (1 mm; BIOSPEC PRODUCTS) were added to each collection tube.
2. Leaf material was harvested and immediately frozen in liquid nitrogen.
3. The leaf material was ground for 20 sec in a Mini-Beadbeater-8 (BIOSPEC PRODUCTS) and immediately refrozen in liquid nitrogen.
4. 1 mL Tri-reagent was added and mixed.
5. 200 µL chloroform was added and the samples were vortexed for 15 sec.
6. The samples were centrifuged for 15 min at 13 000 rpm and 4°C.
7. 350 µL supernatant was transferred into a new reaction tube and an equal amount of 100 % ethanol was added. This mixture was then applied to a column of the RNeasy Plant RNA mini kit and processed according to the manufacturer's instructions.
8. The RNA was eluted in 30 µL elution buffer and stored at -20°C.

The concentration and purity of the RNA was determined by absorbance measurements at A_{260} and with the ratio A_{260}/A_{280}, respectively. For semi-quantitative RT-PCR equal amounts of RNA were used for reverse transcription into cDNA.

2.2.5.3 Plasmid preparation

Plasmid DNA was isolated by alkaline lysis method (Birnboim and Doly 1979). High quality DNA for transformation or sequencing was isolated using QIAGEN or MACHEREY-NAGEL(MN) Mini-prep kits.

2.2.6 PCR and RT-PCR and Real-Time PCR

2.2.6.1 PCR

Reaction mix:

10x PCR buffer *	2,5 µL
10x Crystal Red	2,5 µL (optional)
10 mM dNTPs	0,5 µL
1 mM Primer	1,25 µL each
Taq-DNA-Polymerase	0,5 µL
DNA template	1 µL

ad 25 µL with H_2O.

* with 2 mM $MgCl_2$

PCR-cycler conditions:

94°C 3 min

(94°C 30 sec, 52-60°C 30 sec, 72 °C 1 – 2 min) 39x

72°C 10 min.

12,5 µL of the sample were loaded onto a 3,5 % agarose gel in case of mapping PCR with SSLP markers, otherwise on 1 % agarose gel at a maximum of 150 Volt.

2.2.6.2 RT-PCR

The Superscript II Kit from INVITROGEN was used to transcribe 400 to 800 ng RNA to cDNA. PCR conditions were as described in 2.2.6.1.

2.2.6.3 Real-Time PCR

SybrGreen (BRILLIANT) was used to visualize DNA during the PCR run in ABI Prism 7700. Each sample was represented by four replicates and conducted mostly two times from the same cDNA preparation. Calculations of relative transcript ratios were normalized to the reference gene actin as suggested by Pfaffl (2001). Primers for *RPW8* were 5UTR-R81a and 3UTR-R81a and 5UTR-R82a and 3UTR-R82a, for *PMR6* pmr6i2_s and pmr6_ex3_as and for actin Actin1_F and Actin1_R (see Supplementary Data, Table SD 3).

Reaction mix:

10x PCR buffer *	5 µL
10 mM dNTPs	1 µL
SybrGreen (1 : 3000)	2,5 µL
Glycerol, 50 %	8 µL
DMSO, 100 %	1,5 µL
1 mM Primer	1 µL each
Taq-DNA-Polymerase	0,5 µL
DNA template	1 µL

ad 50 µL with H_2O.

* with 2 mM $MgCl_2$

PCR-cycler conditions:

95°C 2 min

(95°C 30 sec, 58°C 30 sec, 72 °C 1 – 2 min) 49x

72°C 3 min.

2.2.7 DNA sequencing

DNA sequences were determined by the Automatische DNA-Isolierung und Sequenzierung (ADIS-Unit) at the MPIZ on Applied Biosystems (Weiterstadt, Germany) Abi Prism 377 and 3700 sequencers using Big Dye-terminator chemistry (Sanger et al. 1977). PCR products were purified with the Nucleospin Extract-Kit (MACHEREY-NAGEL) or QIAGEN Extract Kit, ensuring sufficient amount at appropriate concentration to be directly sequenced.

2.2.8 Sequence alignment and analysis

Primers used for sequencing of *RPW8.1* and *RPW8.2* were 5UTR-R81a and 3UTR-R81a and 5UTR-R82a and 3UTR-R82a, respectively (see Supplementary Data, Table SD 3). Trace files of sequence chromatograms obtained with forward and reverse primer were aligned for each accession and analyzed with DNASTAR program of the Lasergene software. The manually revised sequences of all accessions were aligned and translated with the MegAlign module of the Lasergene software based on ClustalV alignment algorithms.

2.2.9 Microsopical analysis

2.2.9.1 Coomassie Blue staining for fungal structures

Leaves were cleared in lactophenol solution for a minimum of 4 days. After washing in 50 % ethanol and H_2O, they were incubated for ~ 1 min in Coomassie Blue solution followed by washing twice in H_2O. For microscopic analysis, they were mounted in 50 % glycerol and observed under bright light conditions.

2.2.9.2 Host cell entry

Five to seven leaves of three four to five week old plants per line were inoculated with *G. orontii* and harvested at 48 hours post inoculation. Leaves were destained in lactophenol solution and fungal structures were stained with Coomassie Blue and mounted (see 2.2.9.1). Between ~340 and ~1200 germinated spores were counted per line in the first and between ~150 and ~900 in the second experiment.

2.2.9.3 Analysis of fungal growth with HyphArea

In this experiment, a very low inoculation density was necessary to avoid overlapping of different colonies. Leaves of 3 different plants per line were harvested at three different time points: 24, 48 and 63 hours post inoculation. After destaining of leaves with lactophenol, fungal structures were stained with Coomassie Blue (see 2.2.9.1) and leaves were analyzed with light microscopy. 25 to 30 images of single colonies per line and time point were taken and analyzed with HyphArea (in cooperation with Patrick Schweizer, Gatersleben).

2.2.9.4 Quantification of conidiophores per colony

For this experiment, three four to five week old plants per line were inoculated with *G. orontii* using a low inoculation density to avoid overlapping colonies. At seven days post inoculation, four leaves per plant were harvested and destained in lactophenol solution. For analysis, fungal structures were stained with Coomassie Blue and leaves were analyzed with light microscopy at low magnification and with WCIF Image J Software using the cell counter plug-in (plug in written by Kurt De Vos, University of Sheffield). On average 59 different colonies per line were counted.

2.2.9.5 DAB staining for hydrogen peroxide accumulation

At 48 hpi three leaves of each plant (two plants per line) were harvested and stained with DAB (Thordal-Christensen et al. 1997, modified): Leafs were allowed to take up DAB solution through their petiole for eight hours under high humidity conditions and darkness, followed by another eight hours of incubation in water and darkness. Then the leaves were cleared by boiling in 70 % ethanol for 5 min. Fungal structures were stained with Coomassie Blue (see 2.2.9.1) and the leaves were analyzed with light microscopy at high magnification. Between 54 and 214 colonies were analyzed per line.

2.2.9.6 Trypan Blue staining of cell death

At seven days post inoculation four leaves per plant were harvested and boiled shortly in Trypan Blue solution (modified from Peterhänsel et al. 1997). Subsequently the leaves were destained in chloral hydrate and analyzed with light microscopy at 100 times magnification. Dead cells as well as fungal structures are now visualized by their blue staining.

2.2.9.7 Aniline Blue staining of callose

Three leaves per plant of three plants per line were destained in lactophenol solution and then, after washing in 50 % ethanol and water, incubated for two days in Aniline Blue solution (modified from Vogel and Somerville 1996). Fungal structures were stained with Coomassie Blue (see 2.2.9.1). Leaves were analyzed microscopically with UV-light excitation at 100 times magnification.

2.2.10 Mapping

2.2.10.1 Mapping with PCR-based markers

For a first localization of the target gene in the genome, resistant F_2 plants of a mapping population were analyzed with the SSLP-marker set of Lukowitz et al. (2000). In case of a putative association with markers on a chromosome, additional F_2 plants were screened with the respective markers. For further restriction of the target gene region, new markers were designed (see 2.1.11). When the target gene region was restricted to ~ 5 Mb, fine mapping was initiated. Genomic DNA from ~ 1000 F_2 plants was analyzed with two flanking markers to identify recombinant plants. These were inoculated with *G. orontii* to determine the infection phenotype.

2.2.10.2 Mapping with RI lines

The RI lines were inoculated with *G. orontii* to determine infection phenotypes. Results were analyzed with WinQTLcartographer software (Composite Interval Mapping, CIM) and marker data for the RIL population (Loudet et al. 2002). The resulting target gene region was analyzed by performing PCR with additional markers.

2.2.11 Complementation of *pmr6*

PMR6 was amplified by PCR from cDNA of the Col-0 ecotype with GATEWAY-compatible primers *PMR6*-GW_F and *PRM6*-GW_R markers (Supplementary Data, Table SD 3) and Pfu-polymerase. For construction of the GATEWAY-vector, the kit from INVITROGEN was used to insert the PCR product into the BP-vector pDONR201 and the LR-vector *35S::pAM-PAT*-GW. Positive clones were

sequenced. The plants were transformed with the constructs as described in 2.2.4.

2.2.12 DsRNAi-mediated depletion of *RPW8* transcript accumulation

RPW8.1 and *RPW8.2* cDNA was amplified with the two GATEWAY-compatible versions of primer pairs 5UTR-R81a and 3UTR-R81a and 5UTR-R82a and 3UTR-R82a, respectively (see Supplementary Data, Table SD 3). The GATEWAY-Kit from INVITROGEN was used to introduce the sequence into the binary vector pJawohl8 and plants were transformed according to 2.2.4.

3. RESULTS

3.1 SELECTION OF ACCESSIONS

Although *Arabidopsis thaliana* was described as a host for the powdery mildew species *Golovinomyces orontii* (Plotnikova 1998), not all collected *Arabidopsis* ecotypes are susceptible to powdery mildews. In an earlier study, 360 different accessions of *Arabidopsis* were inoculated with two different powdery mildew species, *Golovinomyces cruciferarum* and *G. cichoracearum*, both former *Erysiphe sp.* (Adam et al. 1999). The majority of accessions were susceptible to both isolates, but some accessions showed varying degrees of resistance to either or both pathogen species. To pre-select for ecotypes which might possibly miss putative compatibility factors, I selected accessions which were resistant to either one or to both of the tested species based on this analysis. 64 accessions from different parts of the world fulfilled the selection criteria and were analyzed for their infection phenotypes with *G. orontii*. Therefore, four to five week old plants were inoculated with *G. orontii.* At seven and nine days post inoculation (dpi), plants were assigned disease resistance (DR) scores from 0 to 3, with 0 being fully resistant and 3 fully susceptible (for further details, see 2. Materials and Methods).

Of the 64 selected accessions, 26 showed resistance to *G. orontii* (DR 0 or 0-1) and were chosen for further analysis. Of these, 16 accessions (62 %) were resistant to all three powdery mildew species, three accessions (12 %) to *G. orontii* and *G. cichoracearum,* and seven (27 %) to *G. orontii* and *G. cruciferarum* (Table 1). As already shown by Vogel et al. (1999), infection phenotypes were not correlated with geographical origin, since different accessions originating from the same area show a diverse range of DR scores. This was for example the case in the

collection of ecotypes from Burghaun/Rhön (Bu), for which infection phenotypes vary between DR 0 (fully resistant) and 3 (fully susceptible) in different accessions (see Table 1).

The selected accessions originate from different parts of the world with a strong emphasis on Europe. With three ecotypes from Spain (Pla-3, Pla-4 and Ts-7), two from Portugal (C24/Co-1, Co-4) and two from Central Asia (Sha and Sorbo from Tajikistan), accessions originating from regions representing the suggested refugia of *Arabidopsis thaliana* during the last ice age (Sharbel et al. 2000) were included in the set.

To determine the inheritance of resistance, the selected resistant accessions were crossed with the susceptible Col-0 ecotype. The infection phenotypes of the F_1 progeny of at least four plants per crossing were determined with *G. orontii* (see Table 2). Kas-1, Ei-5 and Wa-1 were not crossed for various reasons: Resistance in Kas-1 was previously identified to be caused by *RPW10*, which is suspected to be allelic to *RPW8* (Wilson et al. 2001), Ei-5 had very small flowers and failed to produce F_1 seeds, and Wa-1 was not crossed because it was known to be tetraploid (Henry et al. 2005).

All but one F_1 populations were susceptible, although to different degrees: Only few accessions showed full susceptibility comparable to Col-0 (DR 3; Do-0, La-1, Nok-3, Ob-0, Ove-0 Petergof, Pla-3 and Wt-2), while most of them were intermediate susceptible (DR 2 or DR 1-2 for Ang-0, Bu-0, Bu-3, Bu-15, C24, Co-1, Ei-4, Sha, Sorbo, Ts-7, Wt-3; see Figure 2 for F_1 plants of selected accessions). Only one resistant F_1 population (Pla-4) was identified in this approach. Since Co-1 and C24 are regarded to be different names for the same ecotype (Schmid et al. 2006), they were in future regarded as one accession.

Table 1: Selected genotypes of *Arabidopsis thaliana* and their infection phenotypes with *G. orontii*

	origin		DR score*		
Accession	city	country	*Go***	*Gcr***	*Gci***
Ag-0	Argentat	France	2	2	0
Ang-0	Angleur	Belgium	0	2	0
Bla-4	Blanes/Gerona	Spain	2	0	2
Bla-10	Blanes/Gerona	Spain	2	2	0
Bu-0	Burghaun/Rhon	Germany	0-1	0	3
Bu-3	Burghaun/Rhon	Germany	0	2	0
Bu-11	Burghaun/Rhon	Germany	3	0	3
Bu-15	Burghaun/Rhon	Germany	0	0-1	2
Bu-18	Burghaun/Rhon	Germany	2	0	0
Bu-21	Burghaun/Rhon	Germany	2	0	2
Bu-23	Burghaun/Rhon	Germany	3	3	0
C24	Coimbra	Portugal	0	0	0
Cit-0	Citou/Aude	France	3	0	0
Ct-1	Catania	Italy	2	0	3
Co-1	Coimbra	Portugal	0	0	0
Co-4	Coimbra	Portugal	1	0	0
Di-2	Dijon	France	2	0	2
Do-0	Donsbach/Westerwald	Germany	0	0	3
Dra-0	Drahonin	Czech Republic	1	3	0
Dra-1	Drahonin	Czech Republic	2	0	0
Dra-2	Drahonin	Czech Republic	3	0	0
Ei-4	Eifel	Germany	0	0	0
Ei-5	Eifel	Germany	0	0	0
Es-0	Espoo	Finland	2	3	0
Et-0	Etragyes	France	2	2	0
Fl-1	Finland	Finland	1	0	0
Ga-0	Gabelstein	Germany	3	0	0
Gr-3	Graz	Austria	1-2	0	2
Gr-5	Graz	Austria	3	0	3
Gy-0	La Minière	France	3	0	0
Je54	Relichova	Czech Republic	2	0	2
Jl-2	Vranov u Brno	Czech Republic	2	3	0
Jl-5	Vranov u Brno	Czech Republic	2	2	0
Jm-2	Jamolice	Czech Republic	2	0	2
Kä-0	Kärnten	Austria	2	0	3
Kas-1	Kashmir	India	0	0	0
Ksk-1	Keswick	United Kingdom	2	0	0
La-1	Landsberg-Warthe	Germany	0	0	0
Li-1	Limburg	Germany	2	0	2
Mir-0	Miramare/Trieste	Italy	2	0	2
Nok-0	Noordwijk	Netherlands	2	0	0
Nok-1	Noordwijk	Netherlands	1	0	0
Nok-3	Noordwijk	Netherlands	0	0	0
Nw-0	Neuweilnau	Germany	0	0	0
Nw-3	Neuweilnau	Germany	1	3	0

* Disease reaction score: 0 fully, and 1 intermediate resistant; 2 intermediate, and 3 fully susceptible. See Materials and Methods for complete description.

** *Gcr* stands for *G. cruciferarum* , *Gci* for *G. cichoracearum* and *Go* for *G. orontii*

Table 1 Continued

	origin		DR score*		
Accession	**city**	**country**	***Go*** **	***Gcr*** **	***Gci*** **
Ob-0	Oberursel/Hasen	Germany	0-1	3	0
Ove-0	Ovelgoenne	Germany	0-1	0	2
Oy-1	Oystese (N)	Norway	2	0	3
Per-1	Perm	Russia	2	0	3
Petergof	Petergof	Russia	0	0	0
Pla-2	Playa de Aro	Spain	2	0	0
Pla-3	Playa de Aro	Spain	0	0	0
Pla-4	Playa de Aro	Spain	0	0	2
Rak-2	Raksice	Czech Republic	0	0	2
Sg-1	St. Georgen	Germany	2	0	3
Sha	Palmiro-Alay	Tajikistan	0	0	0
Sorbo	Tajikistan	Tajikistan	0	0	0
Ta-0	Tabor	Czech Republic	2	0	2
Ts-7	Tossa de Mar	Spain	0	0	0
Uk-1	Umkirch	Germany	0	0	0
Wa-1	Warsaw	Poland	0	0	0
Wt-2	Wietze	Germany	0	0	0
Wt-3	Wietze	Germany	0-1	0	3
Wt-5	Wietze	Germany	2	0	3

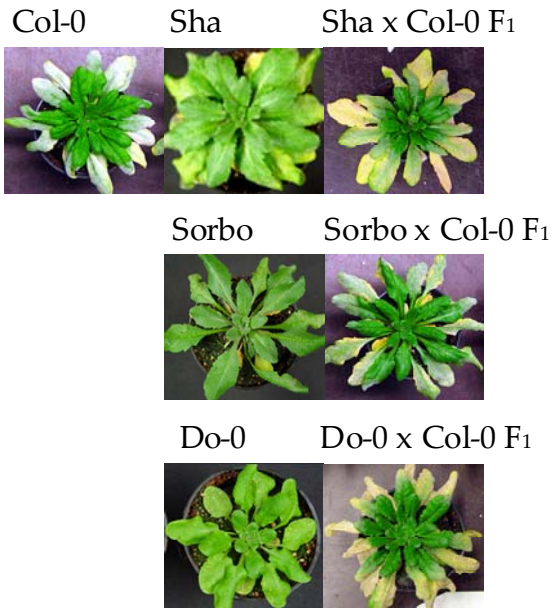

Figure 2: Infection phenotypes of F1 plants of crossings between candidate accessions and the Col-0 ecotype. Macroscopic infection phenotypes of F1 plants of Sha, Sorbo and Do-0 crossed with the susceptible Col-0 ecotype at 11 days post inoculation with *G. orontii*.

Only accessions with F_1 progeny showing infection phenotypes scored as DR 3 or DR 2 were analyzed in the F_2 progeny, since resistance in accessions with weakly susceptible F_1 phenotypes is probably not caused by a recessively inherited gene. Segregation of resistance was tracked in the F_2 generation to assess whether the resistance trait is monogenic or determined by more than one gene. At least 92 F_2 plants per accession of 16 accessions (except for Sha and Uk-1 with ~ 50 F_2 plants) were analyzed upon inoculation with *G. orontii* (Table 3). In twelve accessions segregation of resistance was compatible with a 3 : 1 (susceptible : resistant) segregation pattern with $P > 0,01$ for two and $P > 0,05$ for ten accessions based on χ^2 analysis (Ang-0, C24, Co-1, Do-0, La-1, Nok-3, Ove-0, Pla-3, Sha, Sorbo, Uk-1 and Wt-2). For five accessions resistance segregated 1 : 2 : 1 (susceptible : intermediate susceptible : resistant) with $P > 0,01$ for one and $P > 0,05$ for four accessions (Ang-0, Pla-3, Sha, Sorbo and Uk-1). All five accessions showed also significant χ^2 test results for a 3 : 1 segregation.

With regard to the aim of finding new compatibility factors by map based cloning, accessions showing either 3 : 1 or 1 : 2 : 1 segregation of resistance with a P-value of at least 0,05 were selected for further analysis. In six accessions (Bu-0, Bu-3, Ob-0, Petergof, Uk-1 and Wt-2) none of these segregation patterns was supported by χ^2 analysis. For this reason they were not considered for further analysis, since resistance in these accessions is probably multigenic.

To determine the number of genes involved in mediating resistance as well as their inheritance mode, the accessions were tested with $\chi 2$ test for selected additional scenarios with regard to two and three genes conferring resistance either acting independently or dependently from each other (see Table 4). For accessions Ob-0 and Petergof, no significant scenario with two or three genes was identified; probably the inheritance of resistance is even more complex in these accessions. Interestingly, several accessions were significant for different scenarios.

Table 2: Selected genotypes of *Arabidopsis thaliana* resistant to *G. orontii* and their infection phenotypes for three powdery mildew species

	Origin		DR score*			
accession	**City**	**Country**	***Go*****	***Gci*****	***Gcr*****	**F_1*****
Ang-0	Angleur	Belgium	0	0	2	2
Bu-0	Burghaun/Rhön	Germany	0-1	3	0	2
Bu-15	Burghaun/Rhön	Germany	0	2	0-1	1-2
Bu-3	Burghaun/Rhön	Germany	0	0	2	2
C24	Coimbra	Portugal	0	0	0	2
Co-1	Coimbra	Portugal	0	0	0	3
Do-0	Donsbach/Westerwald	Germany	0	3	0	3
Ei-4	Eifel	Germany	0	0	0	1-2
Ei-5	Eifel	Germany	0	0	0	n.p.
La-1	Landsberg-Warthe	Germany	0	0	0	2-3
Nok-3	Noordwijk	Netherlands	0	0	0	3
Nw-0	Neuweilnau	Germany	0	0	0	1-2
Ob-0	Oberursel/Hasen	Germany	0-1	0	3	3
Ove-0	Ovelgoenne	Germany	0-1	2	0	3
Petergof	Petergof	Russia	0	0	0	3
Pla-3	Playa de Aro	Spain	0	0	0	3
Pla-4	Playa de Aro	Spain	0	2	0	0
Rak-2	Raksice	Czech Republic	0	2	0	(1-)2
Sha	Palmiro-Alay	Tadjikistan	0	0	0	2
Sorbo	Tadjikistan	Tadjikistan	0	0	0	2
Ts-7	Tossa de Mar	Spain	0	0	0	1-2
Uk-1	Umkirch	Germany	0	0	0	(1-)2
Wa-1	Warsaw	Poland	0	0	0	n.p.
Wt-2	Wietze	Germany	0	0	0	3
Wt-3	Wietze	Germany	0-1	3	0	2

* Disease reaction score: 0 fully, and 1 intermediate resistant; 2 intermediate, and 3 fully susceptible.
** *Go* stands for *G. orontii, G.ci* for *G. cichoracearum* and *Gcr* stands for *G. cruciferarum.*
*** F_1 progeny of a crossing with the susceptible Col-0 ecotpye.
n.p. not performed.

Table 3: Segregation of resistance in F_2 progeny of selected genotypes of *Arabidopsis thaliana* resistant to *G. orontii*

							Segregation 3 : 1						Segregation 1 : 2 : 1								
	infection	# F_2 plants in each group					actual		expected				actual			expected					
accession	phenotype*	DR** 3	DR 2	DR 1	DR 0	Total	S	R	S	R	χ^2 test	P	S	I	R	S	I	R	χ^2 test	P	mapping trial
Ang-0	*Go, Gci*	13	17	29	32	91	59	32	68	22,75	0,025	0,01	13	46	32	23	46	23	0,019	0,01	no success
Bu-0	*Go, Gcr*	4	12	34	40	90	50	40	68	22,5	2E-05	n.s.	4	46	40	23	45	23	5E-07	n.s.	n.p.
Bu-3	*Go, Gci*	13	5	21	53	92	39	53	69	23	5E-13	n.s.	13	26	53	23	46	23	5E-12	n.s.	n.p.
C24	*Go, Gci, Gcr*	7	39	19	15	80	65	15	60	20	0,197	0,05	7	58	15	20	40	20	1E-04	n.s.	no success
Co-1	*Go, Gci, Gcr*	8	35	24	23	90	67	23	68	22,5	0,903	0,05	8	59	23	23	45	23	0,001	n.s.	Chromosome III
Do-0	*Go, Gcr*	5	38	32	16	91	75	16	68	22,75	0,102	0,05	5	70	16	23	46	23	5E-07	n.s.	Chromosome III
La-1***	*Go, Gci, Gcr*	140	n.d.	n.d.	44	184	140	44	138	46	0,733	0,05	140	0	44	n.d.	n.d.	n.d.	n.d.	n.d.	Chromosome III
Nok-3	*Go, Gci, Gcr*	44	29	1	18	92	74	18	69	23	0,229	0,05	44	30	18	23	46	23	2E-06	n.s.	Chromosome III
Ob-0	*Go, Gci*	60	17	10	5	92	87	5	69	23	1E-05	n.s.	60	27	5	23	46	23	2E-18	n.s.	n.p.
Ove-0***	*Go, Gcr*	73	n.d.	n.d.	19	92	73	19	69	23	0,336	0,05	73	0	19	n.d.	n.d.	n.d.	n.d.	n.d.	no success
Petergof***	*Go, Gci, Gcr*	88	n.d.	n.d.	5	93	88	5	70	23,25	1E-05	n.s.	88	0	5	n.d.	n.d.	n.d.	n.d.	n.d.	n.p.
Pla-3	*Go, Gci, Gcr*	31	25	18	17	91	74	17	68	22,75	0,164	0,05	31	43	17	23	46	23	0,101	0,05	n.p.
Sha	*Go, Gci, Gcr*	13	28	0	13	54	41	13	41	13,5	0,875	0,05	13	28	13	14	27	14	0,964	0,05	Chromosome III
Sorbo	*Go, Gci, Gcr*	18	15	4	12	49	37	12	37	12,25	0,934	0,05	18	19	12	12	25	12	0,14	0,05	Chromosome III
Uk-1	*Go, Gci, Gcr*	11	17	14	7	49	42	7	37	12,25	0,083	0,05	11	31	7	12	25	12	0,129	0,05	n.p.
Wt-2	*Go, Gci, Gcr*	60	n.d.	n.d.	32	92	60	32	69	23	0,03	0,01	60	0	32	n.d.	n.d.	n.d.	n.d.	n.d.	no success

* *Go* stands for resistance to *G. orontii* , *Gci* for resistance to *G. cichoracearum* and *Gcr* for resistance to *G. cruciferarum* .

** DR: Disease reaction score: 0 fully, and 1 intermediate resistant; 2 intermediate, and 3 fully susceptible.

******* intermediate and fully susceptible plants were grouped together in the F_2 progeny of these accessions.

R: resistant, S: susceptible, I: intermediate.

n.d. not determined, n.p. not performed, n.s. not significant.

This was the case for Ang-0, in which besides the already described significance for a monogenic recessive or semi-dominant inheritance (P > 0,01 each) the segregation pattern was significant for two independently segregating recessively inherited genes (P > 0,05) and for two dependent genes, one recessive, one semi-dominant (P > 0,01). Also Bu-0 was significant for two and three independent recessively inherited genes conferring resistance (P > 0,05 and P > 0,01, respectively).

For Bu-3, significance was obtained for the scenario of one recessive, one semi-dominant gene (independent, P > 0,01) as well as for two recessive and one semi-dominant gene (P > 0,05). La-1 and Ove-0 did not show significance for other segregation ratios than previously reported. Nevertheless, the situation of La-1, Ove-0, Petergof and Wt-2 is not fully informative, since intermediate phenotypes were not scored in the corresponding mapping populations. In Wt-2 resistance seemed to be mediated by two recessive and independent genes (P > 0,05). The analysis of the remaining crossings in Table 4 will follow in the corresponding paragraphs 5, 7 and 8.

The results of this analysis suggest that the significance of an expected segregation ratio for a monogenic inheritance should be handled with care, since other scenarios involving two or even three genes affecting the same trait, dependent or independent of each other, could as well be significant. This indicates that polygenic inheritance of a trait could mask the segregation ratios to appear as monogenic and vice versa.

Taken together, twelve accessions were selected, which showed resistance to at least two different powdery mildew species, one of them being *G. orontii*. In addition, the F_1 progeny of a cross with the susceptible Col-0 ecotype was susceptible and the F_2 progeny segregated according to Mendel's laws for a monogenic trait in either a recessively or intermediate hereditary path.

Table 4: Significance of segregation ratios of selected scenarios for two and three genes conferring resistance to *G. orontii*

accession	1 recessive	1 semidominant	2 recessive (ind.)	2 recessive (dep.)	2 semidominant (ind.)	2 semidominant (dep.)	1 recessive, 1 semidominant (ind.)	1 recessive, 1 semidominant (dep.)	3 recessive (ind.)	3 recessive (dep.)	3 semidominant (ind.)	1 recessive, 2 semidominant (ind.)	1 recessive, 2 dominant (ind.)	2 recessive, 1 semidominant (ind.)	2 recessive, 1 dominant (ind.)
Ang-0	0,01	0,01	0,05	n.s.	n.s.	n.s.	0,01	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Bu-0	n.s.	n.s.	0,05	n.s.	n.s.	n.s.	n.s.	n.s.	0,01	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Bu-3	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	0,01	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	0,05	n.s.
C24	0,05	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	0,05	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Co-1	0,05	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	0,05	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Do-0	0,05	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	0,05	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
La-1	0,05	n.d.	n.s.	n.d.	n.d.	n.d.	n.d.	n.d.	n.s.	n.s.	n.d.	n.d.	n.s.	n.d.	n.s.
Nok-3	0,05	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	0,05	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Ob-0	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Ove-0	0,05	n.d.	n.s.	n.d.	n.d.	n.d.	n.d.	n.d.	n.s.	n.s.	n.d.	n.d.	n.s.	n.d.	n.s.
Petergof	n.s.	n.d.	n.s.	n.d.	n.d.	n.d.	n.d.	n.d.	n.s.	n.s.	n.d.	n.d.	n.s.	n.d.	n.s.
Pla-3	0,05	0,05	n.s.	n.s.	n.s.	n.s.	n.s.	0,05	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Sha	0,05	0,05	n.s.	n.s.	n.s.	n.s.	0,01	0,05	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Sorbo	0,05	0,05	n.s.	n.s.	n.s.	n.s.	n.s.	0,05	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Uk-1	0,05	0,05	n.s.	n.s.	n.s.	n.s.	n.s.	0,05	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Wt-2	0,01	n.d.	0,05	n.d.	n.d.	n.d.	n.d.	n.d.	n.s.	n.s.	n.d.	n.d.	n.s.	n.d.	n.s.
Fr-5	0,01	0,01	n.s.	n.s.	n.s.	n.s.	n.s.	0,01	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Is-0	0,01	n.s.	n.s.	0,01	n.s.	n.s.	n.s.	0,05	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Pla-4	0,01	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	0,05	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Sorbo x Sha	n.s.	n.s.	n.s.	n.s.	0,01	n.s.	n.s.	n.s.	n.s.	n.s.	0,05	0,05	n.s.	n.s.	n.s.
Do-0 xSha	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	0,05	n.s.	n.s.	n.s.	0,05
Sorbo x *pmr6*	n.s.	n.d.	0,05	n.s.	n.d.	n.d.	n.d.	n.s.	0,05	n.s.	n.d.	n.d.	n.s.	n.d.	n.s.
Do-0 x *pmr6*	n.s.	n.s.	0,05	n.s.	0,05	n.s.	0,05	n.s.	0,05	n.s.	n.s.	0,01	n.s.	0,05	n.s.

Crossings between Do-0 and Sorbo and C24 and Co-1 were not included, since all plants were resistant.
ind. independent
dep. dependent
* 0,05 indicates P > 0,05; 0,01 indicates P > 0,01 and n.s. indicates not significant
n.d. not determined

3.2 COMPARATIVE ANALYSIS OF RESISTANCE TO *G. ORONTII*

The selected accessions differ in the constellation of infection phenotypes; some are resistant against three powdery mildew species, some only against two (Table 2). In addition, they differ in inheritance of resistance, since in some accessions, a fully susceptible F_1 progeny suggested a dominant-recessive heredity path (Co-1, Do-0, Nok-3, Ob-0, Ove-0, Petergof, Pla-3, Wt-2 and La-1) while in others resistance seems to be inherited in a more intermediate manner (Ang-0, Bu-0, Bu-3, C24, Sha, Sorbo, Wt-3, Bu-15, Ei-4, Nw-0, Ts-7, Rak-2 and Uk-1). To determine whether resistance occurs at the same stage of fungal development in these accessions or whether different levels of resistance can be observed, a selected subset of accessions was microscopically analyzed with regard to different stages of the pathogen's infection cycle (for macroscopic images of selected resistant accessions see Figure 3).

Host cell entry rates served as an early marker of successful fungal pathogenesis while intermediate stages were tracked by quantifying growth of fungal hyphae during the first three days post inoculation. Finally, reproductive success was analyzed by quantification of conidiophore production. Regarding defense reactions in the plant, early production of reactive oxygen species was quantified considering H_2O_2 accumulation in infected cells as a representative example. At later stages of fungal development, callose deposition and cell death in infected plant tissue were assessed around seven days post inoculation.

3.2.1 Host cell entry

When a powdery mildew spore has penetrated successfully the plant cell wall, it starts to build up a haustorium in an epidermal cell and probably avoids or suppresses plant defense responses to ensure supply with nutrients from the plant. If this has been successful, the spore can produce secondary hyphae which themselves try to establish haustoria in other epidermal cells.

By those means, the fungal colony can grow and spread on the leaf surface by branching of fungal hyphae. Should the plant's defense reaction not allow establishment of haustoria, then fungal growth is arrested and the spore cannot produce secondary hyphae. Therefore, quantification of secondary hyphal growth is a suitable indicator of successful penetration and haustoria development and can thus serve as an approximation of host cell entry rates. *Arabidopsis* mutants with a penetration resistance phenotype like *Atmlo2* show drastically reduced levels of host cell entry after inoculation with several powdery mildew species (Consonni et al. 2006).

The percentage of host cell entry was determined for a subset of the seven selected macroscopically resistant accessions (Ang-0, Co-1, Do-0, La-1, Nok-3, Sha, Sorbo, Sha F_1 progeny, Sorbo F_1 progeny) in comparison with the susceptible ecotype Col-0 and the resistant *Atmlo2 Atmlo6 Atmlo12* triple mutant (*Atmlo2/6/12*) as well as the resistant *pmr6-3* mutant, for which resistance to powdery mildew was reported to occur mainly at later stages of fungal development (Vogel et al. 2002). One further *Arabidopsis* accession named Ms-0, which shows *R*-gene mediated resistance to powdery mildew based on *RPW8*, was included as an additional control. For two accessions, Sha and Sorbo, also plants of F_1 progeny were analyzed in this approach.

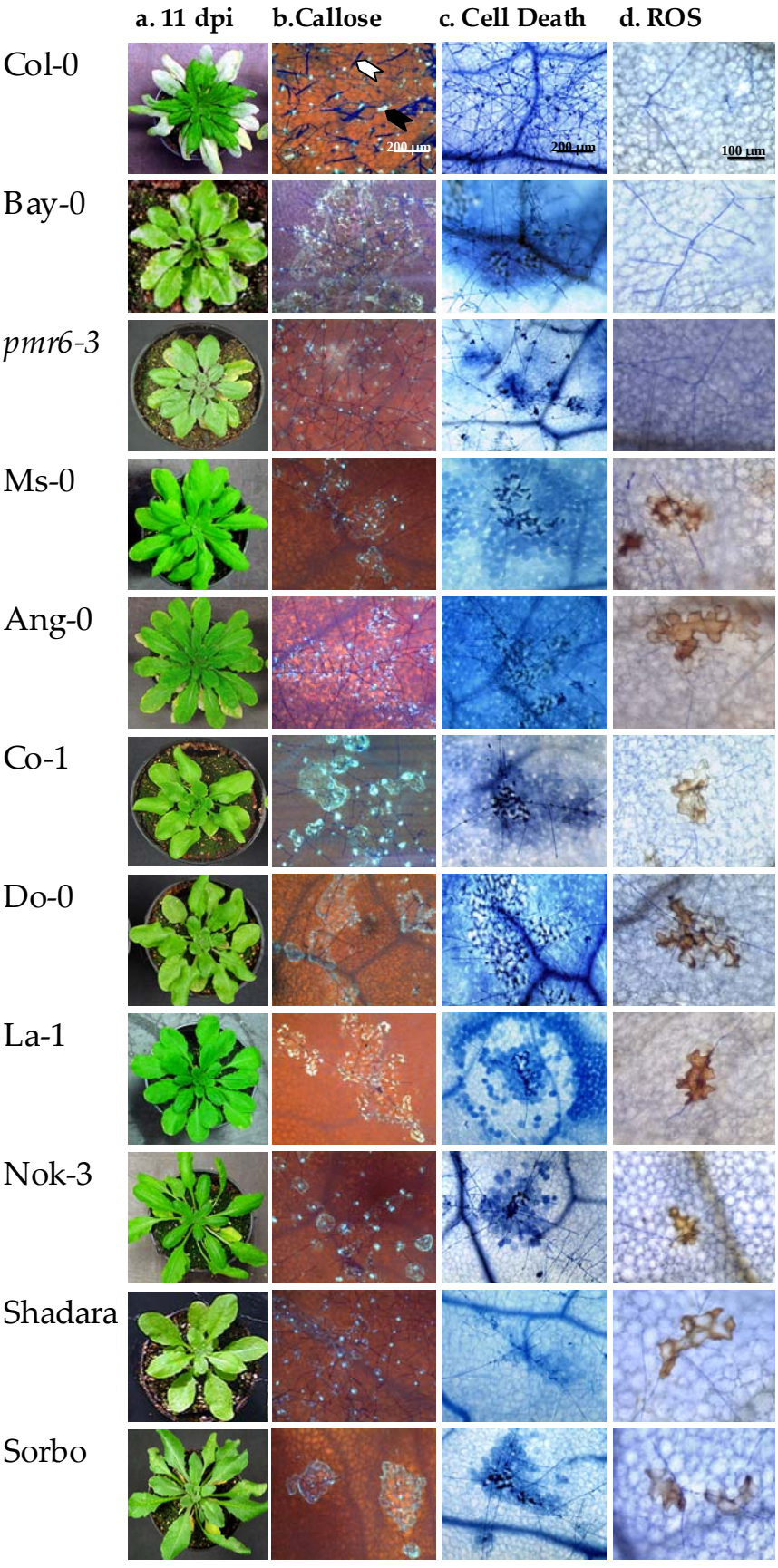
a. 11 dpi
b.Callose
c. Cell Death
d. ROS
Col-0
Bay-0
pmr6-3
Ms-0
Ang-0
Co-1
Do-0
La-1
Nok-3
Shadara
Sorbo
200 µm
200 µm
100 µm

Figure 3: Comparative analysis of infection phenotypes of selected accessions resistant to *G. orontii*. Five-week-old plants are shown a. as macrographs at 11 dpi with *G. orontii*; and as micrographs showing b. callose deposition at seven dpi stained with Aniline Blue; c. cell death stained with Trypan Blue at seven dpi; d. accumulation of H_2O_2 stained with DAB at 48 hpi. Fungal structures are stained with Coomassie Blue. Conidiophores are indicated by a white arrow, positive callose staining by a black arrow.

During the microscopical analysis all spores that germinated were grouped into three different categories: Spores that produce branched secondary hyphae, or where more than one secondary hypha emerges from the spore; spores producing only one very short and not branched secondary hypha and spores without secondary hyphae. For a subset of the tested lines (Col-0, Ms-0, Sha, Sorbo, Sha x Col-0 F_1, Sorbo x Col-0 F_1 and Do-0), mean results of two independent experiments are presented, the others were analyzed once.

Concerning the total percentage of host cell entry, that is spores producing secondary hyphae regardless of branching or not, in the susceptible Col-0 ecotype a host cell entry rate of 77 % was observed (see Figure 4 and Supplementary Data, Table SD 4). In contrast, in the highly resistant *Atmlo2/6/12* triple mutant 0 % of spores succeeded in producing secondary hyphae. This finding is in accordance with results from Consonni et al. (2006). The *pmr6-3* mutant showed with 64 % a slight reduction compared to Col-0 as previously reported (Vogel et al. 2002). In Ms-0, this reduction seems to be more severe (32 %). This early resistance phenotype has not been reported before for this accession.

Regarding the selected resistant accessions, most of them showed only a slight reduction of host cell entry rates compared to the Col-0 ecotype: rates of accessions Ang-0, Nok-3, Sha and Sorbo all range between 60 % and 70 %. These values are comparable to the *pmr6-3* mutant (64 %, see above). The strongest

reduction occurred in Co-1, Do-0 and La-1, where values between 44 % and 56 % respectively were observed. F_1 plants of Sha and Sorbo (70 and 71 %, respectively) showed only a marginal increase compared to the resistant parents and did not reach Col-0 level (77 %).

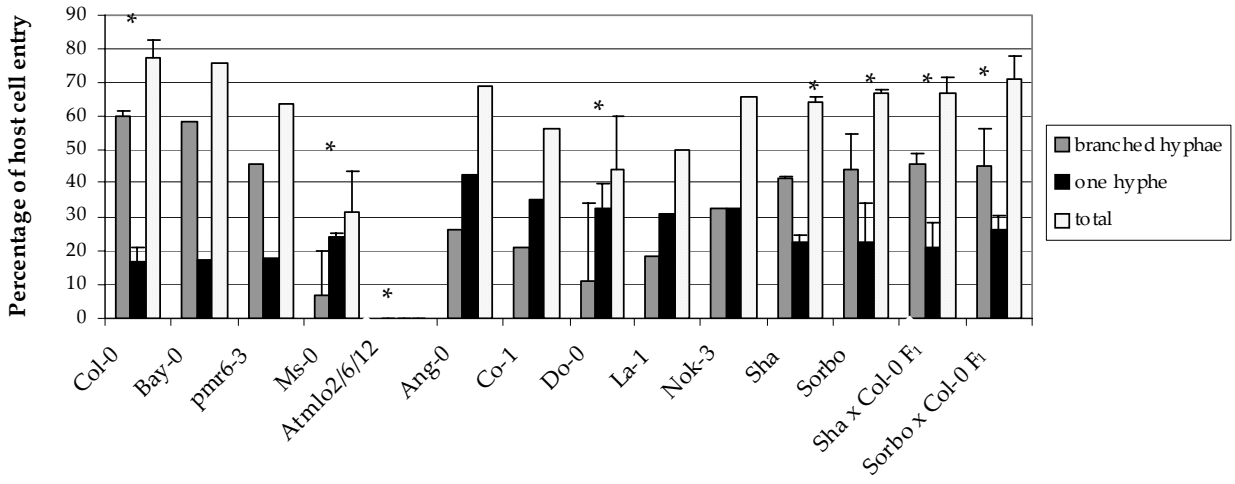

Figure 4: Quantitative assessment of host cell entry at 48 hpi of *A. thaliana* with *G. orontii*. Colonies were grouped into two categories: either having branched secondary hyphae (medium grey bars) or only one secondary hypha (black bars). The light grey bars indicate the total sum of host cell entry observed in a line. Asterisks * indicate that results are presented as mean ± s.d of two independent experiments. Results for other lines are based on data from one experiment.

Regarding the more detailed analysis of spores producing either branched or more than one secondary hypha in comparison to spores with only one non-branched secondary hypha, in accessions Ang-0, Co-1, Do-0, La-1 as well as Ms-0 the latter situation exceeds the level of the first category and differs therefore from the Col-0 ecotype. In Nok-3 proportions of these two categories were equal. In the accessions Sha, Sorbo, and in the mutant *pmr6-3*, the second category had an equal level as in Col-0.

In summary, it seems that in the accessions Ang-0, Nok-3, Sha and Sorbo resistance was not effective at this early stage of fungal development, since the pathogen was only marginally impaired in host cell entry. In the accessions Co-1, Do-0 and La-1, the pathogen was clearly less efficient in host cell entry. In these accessions, even the percentage of spores with only one non-branched secondary hypha was higher in comparison to other resistant accessions, emphasizing that an early mechanism of restricting fungal growth is contributing to resistance in these lines. Nevertheless, the effect is less drastic compared to *Atmlo2/6/12* and the *Atmlo2* single mutant. Taken together, these data suggest that the early response to powdery mildew attack differs between the analyzed resistant accessions.

3.2.2 Analysis of hyphal growth with HyphArea

After establishment of the first haustorium, the pathogen produces secondary hyphae which themselves attempt to penetrate plant cells and to establish further haustoria to allow pathogen spread and colony growth. To comparatively track hyphal growth of the pathogen on various lines, it is possible to measure the average hyphal length of fungal macro-colonies at various time points. To avoid tedious measuring of hyphae by hand, a more automated approach was applied based on the HyphArea software developed by Seiffert and Schweizer (2005).

This software allows automatic measurement of pixels per image which correspond to epiphytic fungal structures. These are stained with a dye, e.g. Coomassie Blue, and are therefore significantly darker than the plant cells in the background. So far, the program was successfully tested with the barley powdery mildew species *Blumeria graminis* f. sp. *hordei* (*Bgh*) on barley. The following analysis showed that it can be applied as well to determine fungal growth of *G. orontii* on *Arabidopsis.* Tested lines comprise the selected resistant

accessions Do-0, Sha and Sorbo, plants of the respective F_1 progeny of Sha x Col-0 and Sorbo x Col-0 as well as the controls Col-0 and Ms-0. Samples of five week old plants were taken at 24, 48 and 63 hpi.

The HyphArea software was able to identify fungal structures in most of the images. At 63 hpi however, some images were not successfully processed, probably due to the size of the colony, which may cause parts of it to be in another focal plain due to the natural curvature of the *Arabidopsis* leaf. Results of the HyphArea analysis indicate that between 24 and 48 hpi, there was no significant difference between the accessions. Between 48 and 63 hpi, hyphal growth increased exponentially (see Figure 5 and Supplementary Data, Table SD 5). When comparing susceptible and resistant accessions with each other, it is notable that in Ms-0 and Do-0 the increase of fungal structures was less compared to the susceptible Col-0 at 63 hpi. However, Sha, Sorbo and the respective F_1 progeny were not that strongly reduced in fungal growth when compared to Col-0: Sha did not seem to be impaired at all and Sorbo showed only a minor reduction.

The results obtained by measuring hyphal growth between 24 and 63 hpi suggest that hyphal growth in this developmental stage of the powdery mildew was impaired in Do-0 and Ms-0, while Sorbo and Sha showed only a minor or no reduction.

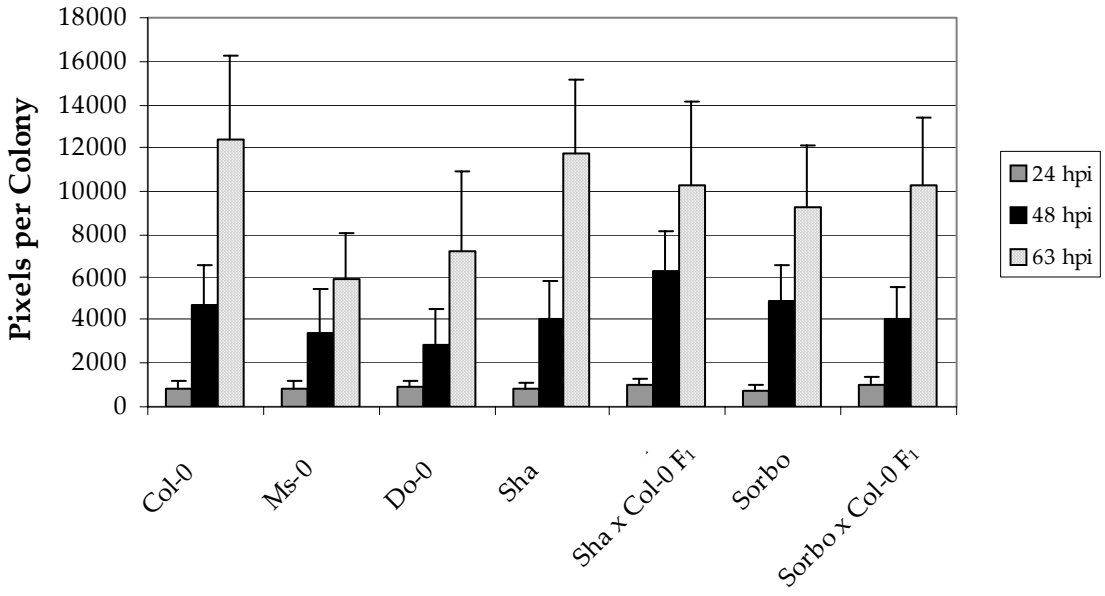

Figure 5: Quantitative analysis of hyphal growth from 24 to 63 hpi. Results are presented as mean ± s.d. of one single experiment. Microscopic images were taken at 24, 48 and 63 hpi with *G. orontii*. Three plants per line and 30 colonies per line and time point were analyzed. The amount of pixels per fungal colony was determined with the HyphArea software (see Materials and Methods).

3.2.3 Conidiophore production

Around seven dpi, *G. orontii* reproduces on susceptible plants by producing multiple conidiophores per colony, each carrying three to five conidiospores. The number of conidiophores per colony can therefore be used to quantitatively characterize successful pathogenesis at a late stage of the fungal infection cycle. Infection phenotypes of all resistant accessions analyzed seem to be characterized by a reduced number of conidiophores per colony (Figures 1 and 3).

To quantify this finding, the number of conidiophores produced per colony at 7 dpi was determined and compared between the susceptible ecotype Col-0 and the resistant accessions Ang-0, Co-1, Do-0, La-1, Nok-3, Sha, and Sorbo, together with Sha x Col-0 F_1 and Sorbo x 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, a reduced number of conidiophores was already determined upon challenge with compatible powdery mildew species (Vogel et al. 2002; Xiao et al. 1997).

Conidiophores were counted either directly or, for colonies with higher numbers of conidiophores, images of colonies were analyzed with the aid of WCIF ImageJ software (Abramoff et al. 2004). In this experiment the number of conidiophores per colony was found to be extremely variable even within an accession, ranging for example from 0 to 122 in resistant Sorbo. This leads to high error bars when the data is averaged. Nevertheless, the averaged data suggests that there was a strong reduction of conidiophore production in resistant accessions from more than 200 conidiophores per colony in Col-0 to less than 10 in *pmr6-3*, Ang-0, Co-1, Do-0, Nok-3 and Sha and to even 0 in Ms-0 and La-1 (see Figure 6 and Supplementary Data, Table SD 6).

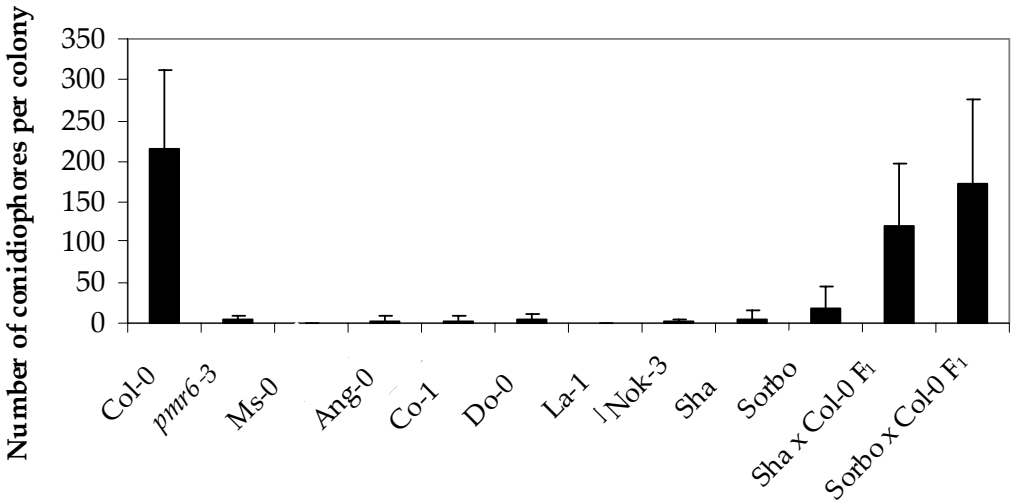

Figure 6: Production of conidiophores per colony at 7 days post inoculation with *G. orontii*. Results are presented as mean ± s.d. of one experiment.

Only Sorbo showed a slightly higher value of 17 conidiophores per colony compared to the other resistant accessions. In the analyzed F_1 plants of Sha and Sorbo, conidiophore production was elevated but did not reach more than 56 % of the Col-0 level in Sha F_1 and 81 % in Sorbo F_1 progeny, respectively. This means that in the F_1 progeny of Sha and Sorbo the pathogen can reproduce at a lower level compared to the fully susceptible Col-0; they showed a rather intermediate phenotype. Based on these results, resistance in Sha and Sorbo seems to be inherited in an intermediate hereditary path rather than a dominant-recessive one. This observation is consistent with the segregation of infection phenotypes in the F_2 generation of Sha and Sorbo, in which a pattern of 1 : 2 : 1 (susceptible : intermediate susceptible : resistant) was observed.

Recapitulating the results obtained, the data indicates that on all resistant accessions analyzed, growth of *G. orontii* was impaired with regard to production of conidiophores per colony. Results obtained for F_1 plants of Sha and Sorbo suggest that they were rather intermediate susceptible compared to fully susceptible Col-0.

3.2.4 Accumulation of hydrogen peroxide

Several accessions showed an effect of resistance already at time points as early as 48 hours post inoculation (see 3.2.1 and 3.2.2). A common early defense reaction to pathogen attack is the production of reactive oxygen species (Apel and Hirt 2004). To determine the reaction in the plant at this early stage of fungal development, colonies of *G. orontii* were examined for the production of H_2O_2 as a representative for other reactive oxygen species.

DAB staining was performed to visualize H_2O_2 accumulation (Thordal-Christensen et al. 1997). For this experiment the resistant accessions Ang-0, Co-1, Do-0, La-1, Nok-3, Sha and Sorbo together with the susceptible Col-0 and

intermediate susceptible Bay-0 ecotype as well as the *pmr6-3* mutant and Ms-0 (carrying *RPW8*) were inoculated with *G. orontii*. Based on earlier studies, Col-0 and *pmr6* are not expected to show hydrogen peroxide production (Vogel et al. 2004), while *RPW8*-mediated powdery mildew resistance was previously shown to be associated with positive DAB staining (Xiao et al. 2002). Cells in which H_2O_2 is produced are stained brown after DAB treatment. In susceptible accession Col-0 only 20 % of cells reacted with H_2O_2 production to pathogen attack (see Figures 3 and 7 and Supplementary Data, Table SD 7). This percentage was even lower (5 %) in the intermediate susceptible Bay-0 ecotype. As expected, also in *pmr6-3* only a low percentage of cells responded with H_2O_2 production at this stage of pathogen attack (14 %). The percentage of colonies inducing H_2O_2 production was drastically higher in all resistant accessions analyzed including Ms-0, ranging from 70 to 97 %. In not inoculated accessions, no spontaneous DAB production was observed (not shown).

The results of this experiment suggest that in contrast to the susceptible controls Col-0 and Bay-0 and the powdery mildew resistant mutant *pmr6-3*, all tested resistant accessions react with local H_2O_2 production at 48 hours post inoculation to powdery mildew attack. Even accessions that were not impaired in host cell entry and hyphal growth at this time point showed this reaction. This suggests that the production of H_2O_2 in the attacked cell has no direct influence on host cell entry.

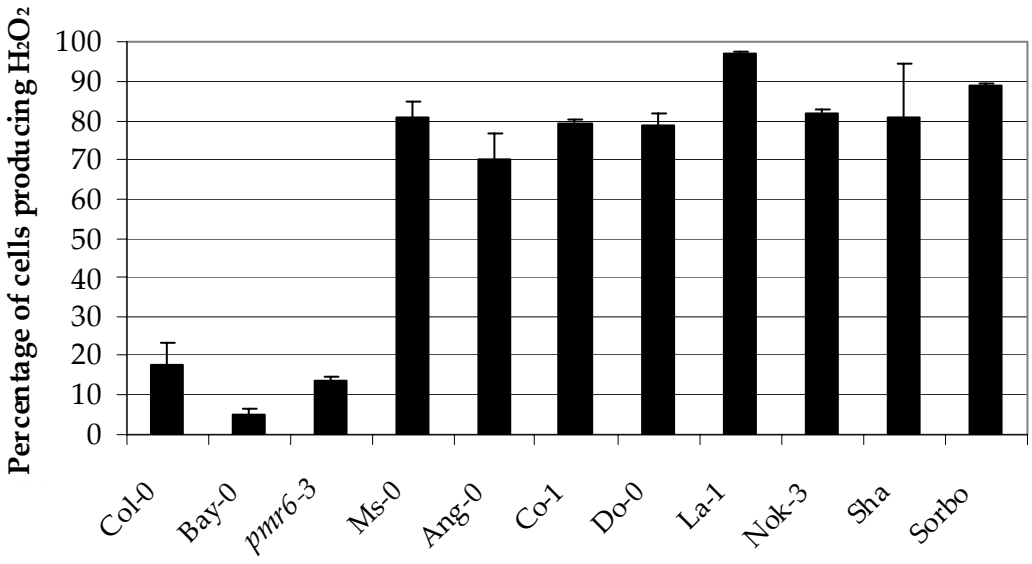

Figure 7: Quantitative analysis of H_2O_2 accumulation in response to powdery mildew attack. Leaves were harvested at 48 hpi with *G. orontii*. Following DAB staining leaves were analyzed by light microscopy. Results of one experiment are presented as mean ± s.d. of three different plants per line.

3.2.5 Quantification of cell death with Trypan Blue staining

Due to the biotrophic lifestyle of powdery mildews, cell death could be an important feature of resistance by inhibiting pathogen growth via cutting off nutrient supply (Schulze-Lefert and Panstruga 2003). To experimentally access the extent of cell death during late stages of pathogen development, inoculated plants were analyzed by Trypan Blue staining according to Keogh et al. (1980) at 7 dpi. Trypan blue staining exploits the loss of membrane integrity in dead cells. The dye is excluded by intact cell membranes, but when there is sufficient damage to the membrane, it can enter the cell and bind to intracellular protein. This results in blue staining of dead cells while living cells remain unstained. In addition, the dye can also stain fungal structures due to the presence of surface proteins (Boedjin 1956).

Four to five week old plants were inoculated with *G. orontii*. As controls the susceptible Col-0 accession, the intermediate susceptible ecotype Bay-0, the resistant *pmr6-3* mutant as well as the Ms-0 ecotype carrying *RPW8* were included. Additionally, all accessions were analyzed without pathogen inoculation.

When unchallenged, most resistant accessions and Col-0 did not show spontaneous cell death, except some patches at leaf margins and tips which are probably due to normal senescence. In Co-1 and Ang-0, small patches of light blue mesophyll cells distributed over the entire leaf blade could be observed (not shown). The previously described microlesions along veins in unchallenged *pmr6* plants (Vogel et al. 2002) were not found under these experimental conditions.

In inoculated tissue Trypan Blue staining could be observed mostly in mesophyll cells located along the hyphae or in round-shaped regions below the entire colony (see Figure 3). Two types of stained cells could be differentiated: First, mesophyll cells which stain dark blue and seem to be collapsed, indicated in Figure 8 as massive cell death (e.g. observed in Do-0), and second, cells which are less dark blue and do not seem to be collapsed yet, indicated as medium cell death. The amount of collapsed cells varied between resistant accessions. In La-1, many colonies were surrounded by a halo of dying and non-dying cells.

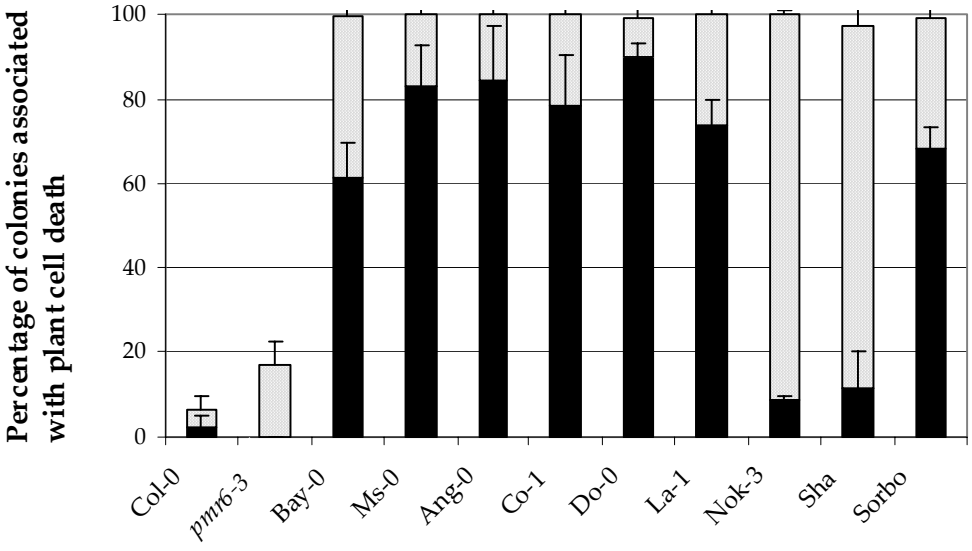

Figure 8: Quantitative analysis of cell death in accessions of *A. thaliana* inoculated with *G. orontii*. Leaves harvested at 7 dpi were stained with Trypan Blue for dead cells and fungal structures. Results are presented as mean ± s.d. of two plants from one experiment. Black bars indicate massive and grey bars medium cell death.

In all resistant accessions including Ms-0 and in the intermediate susceptible ecotype Bay-0 the percentage of colonies associated with cell death (mostly 100 %) was clearly much higher compared to less than 10 % in Col-0 and 17 % in *pmr6-3* (Figure 8, Supplementary Data, Table SD 8). While the percentage of total cell death was rather invariant in resistant accessions, the intensity of cell death differed drastically. In Nok-3 and Sha the percentage of colonies associated with dark blue-stained and collapsed cells was 9 % and 13 %. This is much lower than in other resistant accessions, in which the observed values range from 68 % in Sorbo to 90 % in Do-0. Interestingly, also in Bay-0, which is intermediate susceptible to *G. orontii*, a high percentage of colonies (62 %) was associated with cell death. Although the percentage of colonies associated with collapsed mesophyll cells was much lower in Nok-3 and Sha compared to the other resistant accessions analyzed, the production of conidiophores per colony was equally reduced in all resistant accessions (see 3.2.3). This observation

suggests that the occurrence of cell death but not the intensity seems to coincide with reduced conidiophore production.

Taken together, in all resistant accessions analyzed, dead mesophyll cells below the infected epidermal tissue could be observed. The occurrence and difference in intensity of cell death at 7 dpi was not correlated with the observed differences in conidiophore production.

3.2.6 Callose deposition

Callose deposition between the plasma membrane and the cell wall occurs in plants in response to various abiotic and biotic stresses, including wounding, desiccation, metal toxicity, and microbial attack (Stone and Clarke, 1992). Especially after microbial attack, it contributes to formation of cell wall appositions (papillae) around penetration pegs and haustoria.

Although it is thought that the papillae act as a physical barrier to impede microbial penetration, GSL5-generated callose is postulated to be important for a successful infection, since *gsl5* (=*pmr4*) mutant plants are resistant to powdery mildews (Nishimura et al. 2001). Besides the role of callose in papillae, it is also deposited in dying cells.

Callose staining with Aniline Blue was performed as described by Dietrich et al. (1994). Callose deposition at seven dpi was analyzed for a selected subset of resistant accessions and compared to Col-0, Bay-0, *pmr6* and Ms-0. In all analyzed lines, callose was deposited close to penetration pegs and it encapsulated haustoria (see Figure 3). In Col-0, no additional deposition of callose was detected. In Bay-0, an intermediate susceptible ecotype, callose was deposited in several cells of the epidermis and mesophyll underneath fungal colonies. The same was true for the other resistant accessions, except Sha, in

which callose deposition in whole cells was rather weak. Especially La-1, Do-0 and Sorbo showed a strong callose deposition underneath fungal colonies. In Nok-3, mainly small groups of mesophyll cells directly below the penetration sites of the colony exhibited fluorescence.

In summary, all resistant accessions including Ms-0 showed callose deposition associated with fungal infection sites, with strong callose deposition in La-1, Do-0, Nok-3 and Sorbo and rather weak in Ang-0 and Sha. These results did not correspond to the comparison of cell death by Trypan Blue staining (see 3.2.5), where Ang-0 showed a high percentage of strong cell death. Only in Sha and Nok-3, cell death was not as strong as in the other accessions, comparable to weaker callose deposition in this experiment.

3.2.7 Summary of 3.2 Comparative analysis of resistance to *G. orontii*

With this diverse panel of examinations of pathogen development and plant defense reactions at several stages of the infection cycle, it was aimed to identify at what stage the resistance functions and which defense reactions occur in resistant accessions. Summarizing the comparative analysis and quantification data (see also Table 5), microscopic analysis revealed that *G. orontii* can still penetrate, grow and reproduce on the macroscopically resistant plants. The resistant accessions differ with regard to hyphal growth, which was clearly inhibited at earlier time points in some accession while others showed a marginal reduction of secondary hyphal growth rates and hyphal growth until 63 hpi.

At the stage of asexual reproduction, resistance was characterized by strongly reduced production of conidiophores in all resistant accessions. Values of F_1 plants indicate an intermediate phenotype at this stage of fungal development. On the plant side, a correlation of early hydrogen peroxide production in

attacked cells with resistance of the accession could be observed. At late stages of fungal development, cell death and callose deposition in mesophyll cells below fungal colonies was observed.

Table 5: Summary of comparative microscopic analysis of resistance

	Results differing from Col-0* with regard to					
line	**host cell entry**	**hyphal growth**	**# conidiophores**	**H_2O_2**	**cell death**	**callose**
Bay-0	**-**	n.d.	n.d.	-	**+**	**+**
pmr6-3	-	n.d.	+	-	-	-
Ms-0	+	+	++	+	+	(+)
Atmlo2/6/12	++	n.d.	n.d.	n.d.	n.d.	n.d.
Ang-0	-	n.d.	+	+	+	+
Co-1	+	n.d.	+	+	+	+
Do-0	+	+	+	+	++	+
La-1	+	n.d.	++	+	+	+
Nok-3	-	n.d.	+	+	(+)	+
Sha	-	-	+	+	(+)	(+)
Sorbo	-	(+)	+	+	+	+
Sha F_1	-	-	(+)	n.d.	n.d.	n.d.
Sorbo F_1	-	-	(+)	n.d.	n.d.	n.d.

* -: no difference; +: different from Col-0; (+): weak difference; ++: strong difference.
n.d. not determined

3.3. LINKAGE ANALYSIS OF RESISTANCE USING SSLP MARKERS AND RILS

3.3.1 Mapping populations

To identify the region which contains the locus responsible for resistance in the selected accessions, ecotypes Do-0, La-1, Nw-0, Ove-0, Sorbo, and Wt-2 were crossed with the susceptible ecotype Col-0 to establish respective mapping populations. The resulting F_2 progenies were analyzed with a set of 22 SSLP markers distributed evenly throughout the genome (Lukowitz et al. 2000) to detect linkage of one or several markers to the resistance phenotype observed in the accessions. For these aims, a subset of 10 to 20 fully resistant F_2 plants of the mapping population were chosen and analyzed by PCR with all 22 markers. In accessions C24, Nw-0, Ove-0 and Wt-2, resistance was not linked to any of the tested markers and could therefore not be localized in the genome. Nevertheless, since not all 22 tested markers designed based on polymorphisms between Col-0 and Ler ecotypes were polymorphic between Col-0 and the resistant accession, it cannot be excluded that an association with a chromosomal region was simply not detected.

However, experience with other accessions showed that normally two ore more markers of a chromosome should be associated with the target gene. Therefore, even if one marker of the chromosome was not polymorphic between the crossed accessions, a putative association should still be detected with the neighbouring marker. In accessions Do-0, La-1 and Sorbo resistance was linked to markers CIW4 and NGA6 located on the lower arm of chromosome III. For Do-0, the two markers tested showed 88 % and 90 % association, respectively (see Table 6). In La-1, these percentages were a little lower with 75 % for both markers, but for Sorbo values of 98 % were observed, suggesting a very high

association of resistance with this chromosomal region. All markers used for this association analysis are located on the lower arm of chromosome III.

Table 6: Results of first marker analysis on mapping population of candidate accessions

	associaton of phenotype with				# F_2 plants	
Accession	**left Marker*** **	**association**	**right Marker*** **	**association**	**used for mapping**	**in total**
Ang-0**	Sorb34	55%	Sorb38	39%	31	92
C24**	NGA162	54%	CIW11	60%	13	92
Co-1*	CIW4	86%	Sorb39	85%	61	92
Do-0	Do11	88%	Sorb34	90%	89	91
La-1	Sorb52	75%	Sorb55	75%	41	184
Nok-3*	Sorb41	92%	n.d.	n.d.	75	92
Sorbo*	Sorb15	98%	Sorb10	98%	84	101

* in cosegregation analysis plants with DR 1 were excluded.

** in cosegregation analysis only plants with DR 0 were tested.

***Left and right marker were not the same for all tested F_2 generations, but all were located on on the lower arm of chromosome III, between 1 and 4 Mb apart.

However, due to the low number of sampled plants in the first set of mapping populations and since different markers were chosen for the various ecotypes in this analysis, the levels of association cannot be compared directly between the different accessions.

Results of a representative subset of Sorbo F_2 plants analyzed with a selected SSLP marker located on chromosome III is shown in Figure 9. The association of resistance in Sorbo to the lower arm of chromosome III was further confirmed by analysis of the first set of the mapping population with additional SSLP markers located on chromosome III (see Figure 10). A region of 2,8 Mb between markers CIW4 (18,9 Mb) and NGA707 (21,7 Mb) was determined, in which the resistance locus probably resides. Some F_2 plants like #81 did not match to the region on chromosome III. Although being scored as resistant, it was genotyped heterozygous and is therefore expected to be susceptible.

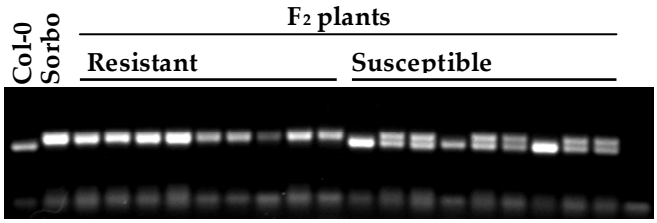

Figure 9: Selected Sorbo x Col-0 F_2 plants with SSLP Marker on chromosome III. Nine resistant, nine susceptible plants and the parental ecotypes Col-0 and Sorbo were tested with marker Sorb51.

3.3.2 Recombinant Inbred Lines

For the resistant accession Shadara (Sha) a population of Recombinant Inbred Lines (RILs) obtained from a crossing between Bay-0 and Shadara (Loudet et al. 2002) was employed to localize the resistance locus. This RIL population was supposed to be informative, since Bay-0 was scored to be intermediate susceptible (DR 2). A subset of 165 RILs assumed to represent maximal diversity (Loudet et al. 2002) as well as 38 additional lines were inoculated with *G. orontii.* The 203 RILs were given DR scores from 0 to 3, where 0 is fully resistant and 3 is fully susceptible (macroscopically).

For 104 RI lines, a DR of 0 was determined, six RILs were scored DR 1, 29 DR 2 and 64 RILs were fully susceptible (DR 3; see Figure 11). These numbers indicate that resistant and susceptible plants are distributed in the RIL sub-population analyzed in a ratio of 1 : 1 (104 resistant : 99 susceptible), as expected for a population of inbred lines. In addition to the parental phenotypes of Bay-0 and Sha with DR 2 and 0, respectively, plants with DR 1 and DR 3 were observed.

plant	phenotype	Sorb34 (18,24 Mb)	CIW4 (18,9 Mb)	Sorb15 (19,4 Mb)	Sorb39 (20,41 Mb)	nga707 (21,7 Mb)	Sorb10 (21,91 Mb)	Sorb13 (22,65 Mb)	Sorb03 (22,75 Mb)	Sorb02 (22,8 Mb)	NGA6 (23 Mb)	nga112 (23,1 Mb)	Sorb32 (23,32 Mb)
Col-0	Susceptible	C	C	C	C	C	C	C	C	C	C	C	C
Sorbo	Resistant	S	S	S	S	S	S	S	S	S	S	S	S
F_1	Susceptible	H	H	H	H	H	H	H	H	H	H	H	H
6	Resistant	S	S	S	S	S	S	S	S	S	S	S	S
16	Resistant	S	S	S	S	S	S	S	S	S	S	S	S
27	Resistant	S	S	S	S	S	S	S	S	S	S	S	S
33	Resistant	S	S	S	S	S	S	S	S	S	S	S	S
48	Resistant	S	S	S	S	S	S	S	S	S	S	S	S
51	Resistant	S	S	S	S	S	S	S	S	S	S	S	S
55	Resistant	S	S	S	S	S	S	S	S	S	n.d.	n.d.	S
60	Resistant	S	S	S	S	S	S	n.d.	n.d.	n.d.	n.d.	S	S
61	Resistant	S	S	S	S	S	S	S	S	S	S	n.d.	S
68	Resistant	S	S	S	S	S	S	S	S	S	S	n.d.	S
72	Resistant	S	S	S	S	S	S	S	S	S	S	n.d.	S
74	Resistant	S	S	S	S	S	S	S	S	S	S	n.d.	S
78	Resistant	S	S	S	S	S	S	S	S	S	S	n.d.	S
79	Resistant	S	S	S	S	S	S	n.d.	S	S	S	n.d.	S
80	Resistant	S	S	S	S	S	S	S	S	S	S	n.d.	S
81**	Resistant	H	H	H	H	H	H	H	H	H	H	H	H
85*	Resistant	S	S	S	S	H	H	H	H	H	H	H	H
98	Resistant	n.d.	S	S	S	S	S	n.d.	S	n.d.	S	n.d.	S
100	Resistant	S	S	S	S	S	S	n.d.	n.d.	n.d.	n.d.	S	S
2	Susceptible	n.d.	C	C	H	H	H	H	H	H	H	H	H
3	Susceptible	n.d.	H	H	H	H	H	H	H	H	H	H	H
4	Susceptible	H	H	H	H	H	H	H	H	H	H	H	H
5	Susceptible	n.d.	C	C	C	C	C	C	C	C	C	C	C
7	Susceptible	n.d.	H	H	H	H	H	H	H	H	C	C	C
8	Susceptible	n.d.	H	H	H	H	H	H	H	H	H	H	H
11*	Susceptible	n.d.	H	H	H	H	H	S	S	S	S	S	S
12	Susceptible	n.d.	C	C	C	C	C	C	C	C	C	C	H
13*	Susceptible	n.d.	H	H	H	H	C	H	H	H	S	S	S
14	Susceptible	n.d.	H	C	C	C	C	C	C	C	n.d.	n.d.	C
15	Susceptible	n.d.	C	C	H	H	H	H	H	H	H	H	H
17	Susceptible	n.d.	H	H	C	H	C	C	C	C	H	C	C
18	Susceptible	C	C	C	H	n.d.	H	H	H	H	H	H	H
19	Susceptible	n.d.	n.d.	H	H	n.d.	H	H	H	H	H	H	H
20	Susceptible	n.d.	C	C	C	n.d.	C	C	C	C	C	C	C
22	Susceptible	n.d.	H	H	H	n.d.	H	H	H	H	H	H	H
23	Susceptible	n.d.	n.d.	C	C	n.d.	C	C	C	C	C	n.d.	C
25	Susceptible	n.d.	C	C	C	n.d.	C	C	C	C	C	C	C
26	Susceptible	n.d.	H	H	H	H	H	H	H	H	H	H	H
29*	Susceptible	n.d.	S	H	H	n.d.	H	H	H	H	H	n.d.	C
30	Susceptible	n.d.	C	C	C	n.d.	C	C	C	C	C	C	C
31	Susceptible	n.d.	H	H	H	n.d.	H	H	H	H	n.d.	n.d.	C
35	Susceptible	n.d.	C	H	H	n.d.	H	H	H	H	H	H	H
36	Susceptible	n.d.	C	C	C	n.d.	C	C	C	C	C	C	C
37	Susceptible	n.d.	C	H	H	n.d.	C	C	C	C	C	n.d.	C
38	Susceptible	n.d.	C	C	C	n.d.	C	C	C	C	C	C	C
40	Susceptible	n.d.	C	C	C	n.d.	C	C	C	C	C	C	C

Figure 9: A subset of resistant and susceptible Sorbo F_2 plants demonstrating association of resistance markers located on the lower arm of chromosome III. *recombinant plant, **contradictory plant, n.d. not determined. C= PCR-product is Columbia-like in size, S= Sorbo-like, H = heterozygous.

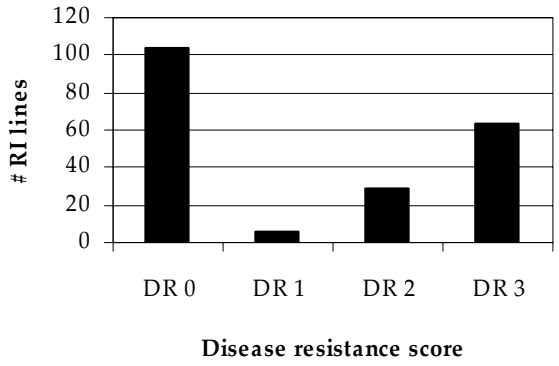

Figure 11: Distribution of infection phenotypes among 203 RI lines Bay x Sha inoculated with *G. orontii*.

This observation of transgression, that is variation among the lines that exceeds the variation between the parental accessions, was already shown for other traits studied with this RIL population (Loudet et al. 2002). The infection phenotype results were analyzed with WinQTLcartographer software (Wang et al. 2006). In this approach, resistance in Sha was likewise localized to the lower arm of chromosome III (see Figure 12). The single peak with a LOD score of ~64 indicates that resistance is likely to be a monogenic trait in this accession, although several loci in close proximity can not be excluded with this approach.

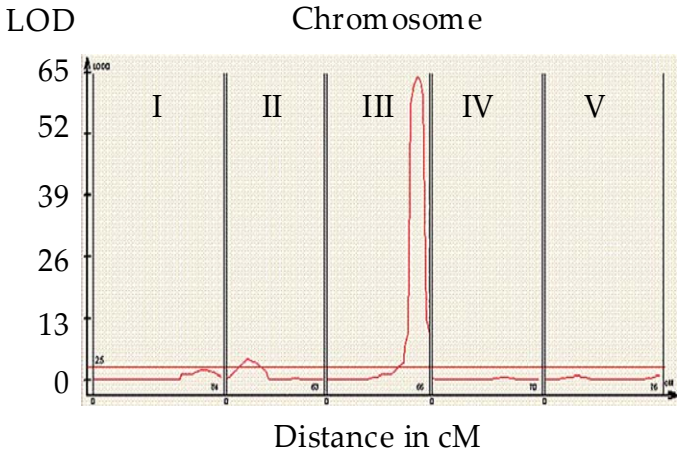

Figure 12: Mapping of resistance loci in the RI line population Bay-0 x Sha. Disease resistance scores of 191 lines were analysed with WinQTLcartographer. Chromosomes are indicated with roman numbers.

3.3.3 Targeted analysis in other accessions

Based on the results obtained with the accessions mentioned above, ecotypes Ang-0, C24, Co-1 and Nok-3 were tested with markers located on chromosome III in a targeted manner (see Table 6). Co-1 and Nok-3 showed a similar association of resistance to the lower arm of chromosome III with percentages ranging between 85 % and 92 %. In contrast, association of markers with resistance in Ang-0 and C24 was around 50 %, indicative of free segregation. Intriguingly, though Co-1 and C24 are regarded as one genotype (Schmid et al. 2006, see above), resistance in Co-1 localizes to chromosome III, while in C24, no true association could be determined. A likely explanation for this contradiction is that not enough plants were sampled in case of the C24 F_2 generation.

Summarizing the attempts of mapping the resistance locus of ten different powdery mildew resistant accessions, in four different accessions it was not

possible to identify markers linked to resistance. In the remaining six accessions, resistance was associated with markers located on the lower arm of chromosome III.

3.4 FINE MAPPING ON CHROMOSOME III

Accessions Sorbo and Sha were chosen for fine mapping of the resistance locus. A total number of 1112 F_2 plants for Sorbo and 203 RILs for Sha were analyzed in order to identify plants with at least one recombination event between two markers located on the lower arm of chromosome III. The marker Do2 was used together with Sorb39 to identify recombinant F_2 plants. Some plants were also analyzed with the marker NGA707, which is located further downstream of Sorb39. PCR reactions with these two flanking markers was performed using the genomic DNA of 1112 Sorbo F_2 plants as a template. Plants being Sorbo-like for one marker and heterozygous for the other were chosen to be analyzed with *G. orontii* to determine the infection phenotype. A total number of 58 F_2 plants were identified with at least one recombination event between the two markers (see Figure 13). The recombination events were confirmed with a second PCR on these specific markers. Although for most plants the results of the phenotypic analysis were in accordance to the genotype determined, 10 plants of 56 plants (18 %) analyzed with DR 0, 2 or 3 showed conflicting phenotypes, also with regard to the target gene region.

plant		DR*	Do2 (16,4 Mb)	Do11(16,84 Mb)	Sorb34 (18,24 Mb)	Sorb51 (18,66 Mb)	Sorb52 (18,77 Mb)	Sorb54 (18,97 Mb)	Sorb55 (19,14 Mb)	Sorb42 (19,55 Mb)	Sorb44 (19,78 Mb)	Sorb18 (20,04 Mb)	Sorb47 (20,33 Mb)	Sorb40 (20,35 Mb)	Sorb39 (20,41 Mb)	NGA707 (21,7 Mb)	F_3 with *G. orontii*
Col-0		3	C	C	C	C	C	C	C	C	C	C	C	C	C	C	
Sorbo		0	S	S	S	S	S	S	S	S	S	S	S	S	S	S	
F_1		2	H	H	H	H	H	H	H	H	H	H	H	H	H	H	
I.	85	0	H	S	S	S	S	S	S	S	S	S	S	S	S	H	all resistant
II.	D10	0	S	S	S	S	S	S	S	S	S	H	H	H	H	H	all resistant
III.	C10	0	S	S	S	S	S	S	S	H	H	H	H	H	H	H	n.d.
III.	E2	0	S	S	S	S	S	S	S	S	H	H	H	H	H	H	n.d.
III.	E3	0	S	S	S	S	S	H	H	H	H	H	S	S	S	S	segregated: H**
IV.	A5	0	S	S	S	S	S	S	S	S	S	S	S	S	S	H	segregated: H**
IV.	C12	0	H	H	S	S	S	S	S	S	H	H	H	H	H	H	segregated: H**
V.	F1	0	S	S	S	S	S	S	S	S	H	H	C	C	C	C	n.d.
V.	E2	0	S	S	S	S	S	S	S	S	S	S	S	S	S	H	all resistant
V.	A11	0	S	S	S	S	S	S	S	S	H	H	H	H	H	H	n.d.
VI.	G1	0	H	H	S	S	S	S	S	S	S	S	H	H	H	H	n.d.
VI.	B7	0	S	S	S	S	S	S	S	S	S	S	S	S	S	H	n.d.
VI.	G9	0	S	S	S	S	S	S	S	S	S	S	S	S	S	H	n.d.
VII	G10	0	S	S	S	S	S	S	H	H	H	H	H	H	H	n.d.	n.d.
VIII.	D5	0	S	S	S	S	S	H	H	H	H	H	H	H	H	n.d.	n.d.
VIII.	G6	0	H	H	H	S	S	S	S	S	S	S	S	S	S	n.d.	n.d.
VIII.	H8	0	C	C	H	S	S	S	S	S	S	S	S	S	S	n.d.	n.d.
VIII.	D11	0	S	S	S	S	S	S	S	H	H	H	H	H	H	n.d.	n.d.
X.	E3	0	H	S	S	S	S	H	H	H	H	H	H	H	H	n.d.	n.d.
XI	D9	0	S	S	S	S	S	S	S	H	H	H	H	H	H	n.d.	all resistant
[C]IV.	G7	0	H	H	H	H	H	H	H	H	H	H	S	S	S	n.d.	segregated: H
[C]V.	B2	0	H	H	H	H	H	H	H	H	H	H	H	H	H	n.d.	segregated: H
[C]V.	G2	0	H	H	H	H	H	H	H	H	H	H	H	H	H	n.d.	n.d.
[C]VI.	C7	0	S	S	S	H	H	H	H	S	S	S	S	S	S	n.d.	n.d.
[C]VI.	H4	0	C	C	H	H	H	H	H	H	S	S	S	S	S	n.d.	n.d.
[C]VII	E11	0	H	H	H	H	H	H	H	S	S	S	S	S	S	n.d.	segregated: H
[C]VIII.	G12	0	H	H	H	H	H	S	S	S	S	S	S	S	S	n.d.	n.d.
[C]VIII.	H11	0	C	C	C	C	C	S	S	S	S	S	S	S	S	n.d.	n.d.
[C]X.	D2	0	H	H	H	H	H	H	H	H	H	H	H	H	H	n.d.	n.d.
XI.	G1	0	C	H	H	H	S	S	S	S	S	S	S	S	S	n.d.	segregated: H

Figure 13: Fine mapping of resistance in Sorbo by analysis of recombinant F_2 plants. Plants were sorted according to their infection phenotype with *G. orontii* and to whether they fit the target region around *RPW8*. *Disease resistance score: 3 is fully, 2 intermediate susceptible, 1 is intermediate and 0 fully resistant (macroscopically). **contradiction in F_3 to genotype for the predicted target gene region. [C]Phenotype in contradiction to target region around *RPW8* (flanked by Sorb51 and Sorb52). Marker Sorb40 is located in the 2nd intron of *PMR6*. n.d.: not determined.

plant		DR*	Do2 (16,4 Mb)	Do11(16,84 Mb)	Sorb34 (18,24 Mb)	Sorb51 (18,66 Mb)	Sorb52 (18,77 Mb)	Sorb54 (18,97 Mb)	Sorb55 (19,14 Mb)	Sorb42 (19,55 Mb)	Sorb44 (19,78 Mb)	Sorb18 (20,04 Mb)	Sorb47 (20,33 Mb)	Sorb40 (20,35 Mb)	Sorb39 (20,41 Mb)	NGA707 (21,7 Mb)	F_3 with *G. oronti*
II.	G5	1	S	S	S	S	S	S	S	S	H	H	H	H	H	C	n.d.
VII.	A5	1	H	H	H	H	H	H	H	S	S	S	S	S	S	n.d.	segregated: H
IX.	G3	1	C	C	C	C	C	C	C	S	S	S	S	S	S	n.d.	segregated: H**
XI.	A4	1	S	S	S	S	S	S	S	S	H	H	H	H	H	n.d.	all resistant
XI.	D4	1	H	H	H	H	H	H	H	H	H	H	S	S	S	n.d.	segregated: H
X.	A3	2	H	H	H	H	H	S	S	S	S	S	S	S	S	n.d.	n.d.
II.	G1	2	H	H	H	H	H	H	H	H	S	S	S	S	S	n.d.	segregated: H
III.	D7	2	C	C	C	C	C	C	C	C	C	H	H	H	H	S	n.d.
V.	B4	2	H	H	H	H	H	H	H	H	S	S	S	S	S	S	segregated: H
VII.	H5	2	S	S	S	H	H	H	H	H	H	H	H	H	H	n.d.	segregated: H
VIII.	D10	2	H	H	H	H	H	H	H	S	S	S	S	S	S	n.d.	n.d.
VIII	F11	2	H	H	H	H	H	H	H	H	H	H	S	S	S	n.d.	n.d.
VIII.	C12	2	H	H	H	H	H	S	S	S	S	S	S	S	S	n.d.	n.d.
IX.	B2	2	S	S	S	H	H	H	H	H	H	H	H	H	S	n.d.	n.d.
IX.	H3	2	H	H	H	H	H	H	H	H	S	S	S	S	S	n.d.	segregated: H
X.	A4	2	C	H	H	H	H	H	H	S	S	S	S	S	S	n.d.	n.d.
XI.	B2	2	S	S	S	H	H	H	H	H	H	H	H	H	H	n.d.	segregated: H
XI.	D5	2	H	H	H	H	H	H	H	H	H	H	H	H	S	n.d.	segregated: H
XI.	D12	2	H	H	H	H	H	H	S	S	S	S	S	S	S	n.d.	segregated: H
I.	41	2	S	S	S	S	S	S	S	S	H	H	C	H	H	H	all resistant
^{C}II.	A9	2	S	S	S	S	S	S	S	S	S	S	H	H	H	H	segregated: H**
^{C}II.	A11	2	S	S	S	S	S	S	S	S	S	S	S	S	S	S	segregated H**
^{C}II.	C12	2	S	S	S	S	S	S	S	S	S	S	H	H	H	H	segregated H**
^{C}IV.	A12	2	S	S	S	S	S	S	S	S	S	H	H	H	H	H	only DR 1 and 0
^{C}VIII	D7	2	H	H	H	S	S	S	S	S	S	S	S	S	S	n.d.	n.d.
^{C}VIII	D9	2	H	H	S	S	S	S	S	S	S	S	S	S	S	n.d.	n.d.
IV.	A8	3	H	H	H	H	H	H	H	H	H	S	S	S	S	S	n.d.
V.	D3	3	H	H	H	H	H	H	H	H	H	H	H	H	H	S	segregated: H
V.	A7	3	H	H	H	H	H	H	H	H	H	H	S	S	S	S	n.d.
VI.	H11	3	H	H	H	H	H	H	H	H	H	S	S	S	S	S	segregated: H

Figure 13: Continued.

Since the target gene is expected to be inherited either recessively or in a semi-dominant manner, the putative target region of resistant plants should be genotypically like the Sorbo parent. As a consequence, the target gene region could be restricted to 0,74 Mb between markers Sorb34 (18,24 Mb) and Sorb54 (18,97 Mb). Six plants which were scored DR 1 in the F_2 were not included in this analysis, since it was not clear whether they were intermediate resistant due to locally high inoculation densities or due to their genotypic constellation. To confirm the phenotype observed in F_2 plants, a subset of the corresponding F_3 families were screened with *G. orontii*. In most cases, earlier conflicts between

phenotype and genotype could be resolved. Some contradictions were confirmed and some were even newly established (see Table 7).

In parallel, the population of RILs was genotyped with markers of the region identified above with the Sorbo F_2 mapping population to identify the putative target gene region in the Sha ecotype. This marker analysis and the infection phenotypes of the RILs are shown in Figure 14. A total number of 204 RILs were analyzed with seven SSLP markers over a region of 5,52 Mb on the lower arm of chromosome III. The majority of plants indicate a target gene region of 1,28 Mb between markers Sorb50 (18,49 Mb) and Sorb42 (19,77 Mb). Results for 18 % of all RILs (except those with DR 1) are in conflict with this target gene region. The percentage of contradictory plants was much higher in the group of resistant RIL plants (DR 0) compared to susceptibles (DR 2 and 3).

In summary, the genotypic and phenotypic analysis of resistant and susceptible F_2 plants with recombination events on the lower arm of chromosome III and plants from a RIL population led to the identification of new left and right borders for the target gene region of ~ 1 Mb in Sha and Sorbo, although some exceptions were observed.

Table 7: Analysis of F_3 progeny of selected F_2 plants with *G. orontii*

	F_3***: number of plants with				ratio	genotype F_2 around *RPW8**		DR** phenotype F_2		
F_2 parent	DR 3	DR 2	DR 1	DR 0	S : I : R****	expected	actual	expected	observed	contradiction?
I.11	0	0	3	11	0 : 3 : 11	S or H	H	0 or 2	3	no
I.13	0	6	7	2	0 : 13 : 2	H	H	2	3	no
I.41	0	0	0	12	0 : 0 : 12	S	S	0	0	no
I.85	0	0	0	9	0 : 0 : 9	S	S	0	0	no
II.A9	0	9	9	5	0 : 7 : 1	H	S	2	2	yes (confirmed)
II.D10	0	0	0	15	0 : 0 : 15	S	S	0	0	no
II.C12	0	5	6	3	0 : 11 : 3	H	S	2	2	yes (confirmed)
II.G1	2	7	3	1	2 : 10 : 1	H	H	2	2	no
III.E3	0	13	11	6	0 : 24 : 6	H	S	2	0	yes (new)
IV.A12	0	0	11	4	0 : 11 : 4	S	S	0	1-2	not anymore
IV.A5	0	7	7	17	0 : 14: 17	H	S	2	0	yes (new)
IV.C12	3	9	6	10	3 : 15 : 10	H	S	2	0	yes (new)
IV.G7	4	7	4	1	4 : 11 : 1	H	H	2	0	not anymore
V.B2	5	2	5	3	5 : 7 : 3	H	H	2	0	not anymore
V.B4	12	2	0	1	12 : 2 : 1	H	H	3	2	no
V.D3	2	13	7	9	2 : 20 : 9	H	H	2	3	no
V.E2	0	0	0	16	0 : 0 : 16	S	S	0	0	no
VI.H11	2	6	4	4	2 : 10 : 4	H	H	2	3	no
VII.A5	0	7	5	3	0 : 12 : 3	H	H	2	1	not anymore
VII.E11	0	2	12	1	0 : 14 : 1	H	H	2	0	not anymore
VII.H5	5	7	2	0	5 : 7 : 0	C or H	H	2	2	no
IX.G3	0	15	1	0	0 : 16 : 0	C or H	C	2	1	not anymore
IX.H3	0	8	5	2	0 : 13 : 2	H	H	2	2	no
XI.G1	0	12	3	0	0 : 15 : 0	H	H ǀ S	2	0	no
XI.A4	0	0	0	16	0 : 0 : 16	S	S	0	1	no
XI.B2	4	7	3	1	4 : 10 : 1	H	H	2	2	no
XI.D12	7	8	0	0	7 : 8 : 0	C or H	H	2 or 3	2	no
XI.D4	4	1	2	2	4 : 3 : 2	H	H	2	1	not anymore
XI.D5	0	2	4	10	0 : 6 : 10	H	H	2	2	no
XI.D9	0	0	0	16	0 : 0 : 16	S	S	0	0	no

* "S" means Sorbo-like, "H" heterozygous and "C" Col-0-like. H ǀ S indicates recombination between flanking markers.

** DR is disease resistance score.

**** susceptible : intermediate : resistant

Line	DR*	Do2 (16,39 Mb)	Sorb50 (18,49 Mb)	Sorb52 (18,77 Mb)	Sorb42 (19,55 Mb)	Sorb44 (19,77 Mb)	Sorb47 (20,33 Mb)	Sorb10 (21,91 Mb)
Bay	2	B	B	B	B	B	B	B
Sha	0	S	S	S	S	S	S	S
13	0	S	S	S	S	S	S	S
29	0	S	S	S	S	S	S	S
30	0	S	S	S	B	B	B	S
33	0	B	S	S	S	S	S	S
34	0	S	S	S	S	S	S	S
48	0	S	S	S	S	S	S	S
55	0	S	S	S	S	S	S	S
59	0	S	S	S	S	S	S	S
70	0	B	S	S	S	S	S	S
75	0	B	B	S	S	S	S	S
77	0	S	S	S	S	S	S	S
80	0	B	B	S	S	S	S	S
83	0	B	S	S	S	S	S	S
107	0	B	S	S	S	S	S	S
112	0	S	S	S	S	S	S	S
116	0	S	S	S	S	S	S	S
122	0	S	S	S	S	S	S	B
123	0	S	S	S	S	S	BS	BS
137	0	S	S	S	S	S	S	S
143	0	S	S	S	S	S	S	S
146	0	S	n.d.	S	B	S	S	B
150	0	B	S	S	B	S	S	S
170	0	S	S	S	B	B	B	B
171	0	S	S	S	S	B	B	B
173	0	S	S	S	B	S	S	S
180	0	S	S	S	B	S	S	S
183	0	S	S	S	S	S	S	S
191	0	B	S	S	S	S	B	B
194	0	n.d.	S	S	B	B	B	B
195	0	B	S	S	S	S	S	S
200	0	S	S	S	S	S	S	B
204	0	B	S	S	B	B	B	B
220	0	B	S	S	S	S	S	S
236	0	S	S	S	B	B	B	S
264	0	S	S	S	S	S	S	S
268	0	B	S	S	S	S	S	S

Figure 14: Infection phenotypes and SSLP analysis of the RIL population Bay-0 x Sha. *Part I.* Plants are sorted according to their infection phenotype with *G. orontii* and to whether they fit the target region around *RPW8*. *Disease resistance score: 3 is fully, 2 intermediate susceptible, 1 is intermediate and 0 fully resistant (macroscopically). Target region around *RPW8* is flanked by Sorb50 and Sorb52. n.d.: not determined.

Line	DR*	Do2 (16,39 Mb)	Sorb50 (18,49 Mb)	Sorb52 (18,77 Mb)	Sorb42 (19,55 Mb)	Sorb44 (19,77 Mb)	Sorb47 (20,33 Mb)	Sorb10 (21,91 Mb)
274	0	S	S	S	S	S	S	S
309	0	S	S	S	S	S	S	S
313	0	S	S	S	S	S	S	B
314	0	S	S	S	S	S	S	B
319	0	S	S	S	S	BS	BS	BS
328	0	S	S	S	S	S	S	S
334	0	S	S	S	S	S	S	S
336	0	S	S	S	S	B	B	S
349	0	S	S	S	S	S	S	S
351	0	S	S	S	S	S	S	S
365	0	S	S	S	S	S	S	S
366	0	B	B	S	S	S	S	S
376	0	S	S	S	S	S	B	S
380	0	S	S	S	B	B	S	B
381	0	S	S	S	S	S	S	B
387	0	B	S	S	S	S	S	S
390	0	S	S	S	S	S	S	S
400	0	S	S	S	S	S	S	S
409	0	S	S	S	S	S	S	S
410	0	B	S	S	S	S	S	S
412	0	S	S	S	S	S	S	S
431	0	S	S	S	S	S	S	S
103	0	S	S	S	S	S	B	B
119	0	B	B	S	S	S	S	S
211	0	S	S	S	S	S	S	S
286	0	S	S	S	S	S	S	S
359	0	S	S	S	S	S	S	S
362	0	S	S	S	S	S	B	B
15	0	B	S	S	S	S	S	S
49	0	S	S	S	S	S	S	S
65	0	S	S	S	S	S	S	S
67	0	B	S	S	S	S	S	S
102	0	B	S	S	S	S	S	S
110	0	S	S	S	S	S	S	S
129	0	S	S	S	S	S	S	S
157	0	S	S	S	S	S	S	S
179	0	S	S	S	S	S	S	S
186	0	B	S	S	S	S	S	S
197	0	B	S	S	S	S	S	S
220	0	B	S	S	S	S	S	S
232	0	S	S	S	S	S	S	S
271	0	S	S	S	S	S	S	S
18	0	B	B	B	S	S	S	S

Figure 14: Continued.

Line	DR*	Do2 (16,39 Mb)	Sorb50 (18,49 Mb)	Sorb52 (18,77 Mb)	Sorb42 (19,55 Mb)	Sorb44 (19,77 Mb)	Sorb47 (20,33 Mb)	Sorb10 (21,91 Mb)
37	0	S	B	B	B	B	B	B
106	0	B	B	B	B	S	S	S
118	0	B	B	B	B	B	B	B
125	0	S	B	B	B	B	B	B
130	0	S	B	B	B	B	B	B
133	0	B	B	B	B	B	B	B
134	0	B	B	B	B	B	B	B
175	0	B	B	B	S	B	B	S
182	0	S	B	B	B	B	B	B
214	0	B	B	B	B	B	B	B
226	0	B	B	B	B	B	B	S
252	0	B	B	B	B	B	B	B
260	0	B	B	B	B	B	S	S
325	0	S	S	B	S	B	B	S
395	0	B	B	B	B	B	S	S
398	0	B	B	B	B	B	S	S
419	0	B	B	B	B	B	S	S
421	0	S	B	B	B	B	B	B
308	0	B	B	B	B	B	B	B
368	0	B	B	B	BS	BS	B	B
62	0	B	B	B	B	B	B	B
140	0	B	B	B	B	B	B	B
176	0	B	B	B	B	B	B	B
320	0	B	B	B	B	B	B	B
397	0	BS	B	B	B	B	BS	BS
87	1	S	S	B	B	B	B	B
246	1	S	B	B	B	B	B	S
299	1	BS	BS	BS	BS	BS	BS	BS
392	1	B	B	B	B	B	B	S
265	1	B	S	S	S	S	S	S
393	1	S	S	S	S	S	S	B
7	2	B	B	B	B	B	B	B
16	2	B	B	B	S	S	S	S
25	2	B	B	B	S	S	S	S
42	2	B	B	B	B	B	B	S
81	2	B	B	B	B	B	B	B
92	2	S	B	B	B	B	B	B
93	2	B	B	B	n.d.	B	B	S
94	2	B	B	B	B	B	S	S
147	2	B	B	B	S	B	B	S
152	2	S	B	B	B	B	B	B
253	2	B	B	B	B	B	S	S
256	2	B	B	B	B	B	B	B

Figure 14: Continued.

Line	DR*	Do2 (16,39 Mb)	Sorb50 (18,49 Mb)	Sorb52 (18,77 Mb)	Sorb42 (19,55 Mb)	Sorb44 (19,77 Mb)	Sorb47 (20,33 Mb)	Sorb10 (21,91 Mb)
272	2	S	B	B	B	B	B	B
300	2	B	B	B	B	B	B	B
379	2	S	B	B	B	B	B	B
384	2	B	B	B	B	S	S	S
394	2	S	B	B	B	B	B	B
285	2	S	B	B	B	B	B	B
302	2	B	B	B	S	S	S	S
3	2	B	B	B	B	B	B	B
58	2	S	B	B	B	B	B	B
61	2	B	B	B	B	B	B	B
90	2	B	B	B	B	B	B	B
100	2	B	B	B	B	B	B	B
114	2	B	B	B	B	B	B	B
262	2	B	B	B	B	B	B	B
2	3	B	B	B	B	S	S	S
19	3	B	B	B	B	B	B	B
22	3	B	B	B	B	B	B	B
24	3	S	B	B	B	B	B	B
32	3	B	B	B	B	S	S	S
45	3	B	B	B	B	B	B	S
47	3	B	B	B	B	B	B	B
71	3	S	B	B	B	B	B	B
73	3	B	B	B	B	B	B	S
78	3	B	B	B	B	B	B	B
84	3	B	B	B	B	B	B	B
97	3	B	B	B	S	B	B	B
98	3	B	B	B	S	S	S	S
104	3	S	B	B	B	B	B	B
111	3	B	B	B	B	B	B	B
120	3	B	B	B	B	B	B	B
121	3	S	B	B	B	B	B	B
126	3	S	B	B	B	B	B	B
131	3	B	B	B	S	S	S	S
136	3	S	B	B	B	B	B	B
162	3	B	B	B	S	B	B	B
190	3	B	B	B	B	B	B	B
210	3	B	B	B	BS	BS	BS	BS
234	3	S	B	B	B	B	B	B
240	3	B	B	B	B	B	B	B
248	3	S	B	B	B	B	B	B
267	3	B	B	B	B	B	B	B
277	3	B	B	B	B	B	B	B
305	3	B	B	B	B	B	B	B

Figure 14: Continued.

Line	DR*	Dq2 (16,39 Mb)	Sorb50 (18,49 Mb)	Sorb52 (18,77 Mb)	Sorb42 (19,55 Mb)	Sorb44 (19,77 Mb)	Sorb47 (20,33 Mb)	Sorb10 (21,91 Mb)
329	3	B	B	B	B	S	S	S
343	3	S	B	B	B	B	B	B
357	3	B	B	B	B	B	B	B
358	3	B	B	B	B	B	S	S
382	3	S	B	B	B	B	B	B
402	3	B	B	B	B	B	n.d.	B
413	3	BS	B	B	n.d.	n.d.	S	n.d.
424	3	B	B	B	B	B	B	B
155	3	B	B	B	B	n.d.	S	S
199	3	B	B	B	S	S	S	S
279	3	B	B	B	S	S	S	S
363	3	B	B	B	B	B	B	B
364	3	B	B	B	B	B	B	S
17	3	S	B	B	B	B	B	B
35	3	B	B	B	B	B	B	B
44	3	B	B	B	B	B	B	B
53	3	B	B	B	B	B	B	B
98	3	B	B	B	B	S	S	S
99	3	B	B	B	B	B	B	B
127	3	B	B	B	B	B	B	B
198	3	B	B	B	B	B	B	B
273	3	B	B	B	B	B	B	B
279	3	B	B	B	S	S	S	S
289	3	B	B	B	B	B	B	B
342	3	B	B	B	B	B	B	B
202	3	S	BS	BS	B	B	B	B
187	2	S	S	S	S	S	S	S
307	2	S	S	S	S	B	S	n.d.
355	2	S	S	S	S	S	S	B
135	3	S	S	S	S	S	S	B
165	3	S	S	S	S	S	S	S
306	3	S	S	S	S	S	S	S
339	3	S	S	S	S	S	S	B
345	3	B	S	S	S	S	S	S
426	3	S	S	S	S	S	B	B
430	3	S	S	S	S	S	S	S
432	3	B	S	S	S	S	S	S
298	3	S	S	S	S	S	S	B

Figure 14: Continued.

3.5 DOMINANT RESISTANCE IN *ARABIDOPSIS THALIANA* AGAINST POWDERY MILDEW

During the analysis of resistance segregation (see 3.1 and Table 3), almost no accession showed evidence for dominantly inherited resistance, that is a resistant F_1 and a F_2 generation segregating 3 : 1 (resistant : susceptible). Only for Pla-4, a resistant F_1 progeny was observed. This bias is probably due to the preselection criteria used for this study: Accessions had to be resistant to at least two different powdery mildew species to enter the analysis. Application of these criteria will probably exclude all accessions in which resistance is caused by dominant *R*-genes, which is often highly specific for single isolates of a given pathogen species. In *Arabidopsis* several *R*-genes were identified providing resistance against bacterial and oomycete pathogens *Pseudomonas syringae* and *Peronospora parasitica* (reviewed in Martin et al. 2003).

The only *R*-genes effective against powdery mildew known so far reside at the broad-spectrum resistance-mediating *RPW8* locus. However, the gene product is not a typical NBS-LRR structured R-protein, but atypical concerning structure and specificity.

Does classical specific *R*-gene-mediated resistance of *Arabidopsis* to powdery mildews actually exist, as it has been described for other pathogens? To answer this question, accessions were selected, which are susceptible to both *G. cichoracearum and cruciferarum,* but show resistance to *G. orontii*. For these aims, the data of the study by Vogel et al. 1999 were employed again.

Of 188 accessions susceptible to both *G. cichoracearum and G. cruciferarum*, 172 germinated and were analyzed with *G. orontii*. Of these, 34 (20%) were resistant to *G. orontii.* Eight accessions have been crossed to Col-0 and analyzed in the F_1 progeny (see Table 8), but only two of them, Pa-2 and Si-0, were scored both

macroscopically and microscopically resistant in the F_1 generation. All other F_1 populations had either intermediate or susceptible phenotypes.

For three accessions, Fr-5, Is-0 and Pla-4, data about segregation of resistance in the F_2 generation is available (see Table 4). The results of Is-0 and Pla-4 indicate a 3 : 1 (susceptible : resistant; $P > 0,01$) for 92 F_2 plants analyzed. For Pla-4, the segregation pattern of one recessive, one semi-dominant gene conferring resistance in dependency of each other was in theory significant as well ($P>0,05$). For Fr-5, a 3 : 1 and a 1 : 2 : 1 segregation (susceptible : intermediate : resistant; $P > 0,01$) was significant. Interestingly, results of both accessions were also significant for the segregation of one recessive and one semi-dominant locus depending on each other, with $P > 0,01$ for Fr-0 and $P > 0,05$ for Is-0.

Another interesting candidate harbouring a putative *R*-gene could be Ep-0, which showed a strong HR reaction when inoculated, a phenomenon frequently associated with *R*-gene mediated resistance (see review of Martin et al. 2003). Unfortunately, this accession was germinating very badly and has therefore not been crossed yet.

In summary, so far no accession was identified providing strong evidence for *R*-gene-mediated resistance to *G. orontii.* Despite the fact that analysis of several candidates is in progress, it seems that *R*-gene-mediated resistance to *G. orontii* is either very rare or even non-existent in *Arabidopsis*.

Table 8: Accessions specifically resistant to *G. orontii* and analysis of resistance inheritance

	origin		DR*		DR*
Accession	City	country	*G. orontii*	crossed	F_1
An-1	Antwerpen	Belgium	0		
Bu-17	Burghaun/Rhon	Germany	0	+	
Bu-9	Burghaun/Rhon	Germany	0		
Co-3	Coimbra	Portugal	0	+	2
Ep-0	Eppenheim/Taunus	Germany	0		
Fr-5	Frankfurt	Germany	0	+	1-2
Ha-0	Hannover	Germany	0	+	
Hl-0	Holtensen	Germany	0	+	
Is-0	Isenburg/Neuwied	Germany	0	+	2
Ita-0	Ibel Tazekka	Marocco	0		
Je-0	Jena	Germany	0		
Kl-3	Koeln	Germany	0		
ko-2	Kopenhagen	Denmark	0		
Kro-0	Krotzenburg	Germany	0	+	3
Ll-1	Llagostera	Spain	0		
Ll-2	Llagostera	Spain	0		
Mh-0	Mühlen	Germany	0-1		
Nie-0	Niederlauken/Ts.	Germany	0-1		
Nw-1	Neuweilnau	Germany	0		
Ob-1	Oberursel/Friedhof	Germany	0-1		
Pa-1	Palermo	Italy	0		
Pa-2	Palermo	Italy	0	+	0
Pa-3	Palermo	Italy	0-1		
Pf-0	Pfrondorf	Germany	0		
Pla-0	Playa de Aro	Spain	0		
Po-0	Poppelsdorf	Germany	0	+	
Rou-0	Rouen	France	0		
Rsch-4	Rschew/Starize	Russia	0		
Sav-0	Slavice	Czech Republic	0		
Sei-0	Seis am Schlemm	Italy	0		
Si-0	Siegen	Germany	0	+	0-1
Ste-0	Stendal	Germany	0-1		
Sy-0	Isle of Skye	United Kingdom	0		
Ts-1	Tossa del Mar	Spain	0-1		
Tu-1	Turin	Italy	0	+	2-3
Ty-0	Taynuilt	United Kingdom	0		
Uk-3	Umkirch	Germany	0		
Van-0	Vancouver	Canada	0	+	
XX-0	unknown	unknown	0-1		
Zü-1	unknown	unknown	0	+	+

* Disease reaction score

** Segregation pattern can be accepted based on $\chi 2$.

Empty boxes indicate that the experiment was not performed yet.

3.6 MUTANT ANALYSIS

To determine which factors and pathways are important for resistance of the selected accessions to *G. orontii*, the Sorbo ecotype was crossed to selected mutants defective in salicylic acid, jasmonic acid and ethylene-mediated defense signaling. If resistance in Sorbo is dependent on a process or pathway which is impaired in any of the mutants, F_2 progeny homozygous for the resistance locus and the defective signaling component will be susceptible.

These dependencies can in addition provide further information with respect to the candidate genes. For example, SA is required for *RPW8.1*- and *RPW8.2*-mediated HR and resistance (Xiao et al. 2001), but not for resistance in the *pmr6* mutant (Vogel et al. 2002). Sorbo was crossed to mutants *Atmlo2/6/12*, *eds1-2*, *eds5-1*, *ein2-1*, *etr1-1*, *ndr1-1*, *npr1-1*, *pad2-1*, *pad3-1*, *pad4-1*, *pen1-1*, *pen2-1*, *pmr4-1*, *rar1-10* and *sid2-1* as well as to a NahG transgenic line.

So far, the majority of these crossings are still in the F_1 generation, so no results were obtained yet. Preliminary results are available for a crossing between Sorbo and Col-0 plants expressing salicylate hydroxylase which converts salicylic acid (SA) to catechol (*NahG*; Yamamoto et al. 1965) and thereby affects SA-dependent defense signaling (Gaffney et al. 1993; Delaney et al. 1994). 92 F_2 plants were analyzed with *G. orontii*. Subsequent PCR analysis with markers representing putative borders for the target gene region and with primers specific for the *NahG* transgene revealed several plants containing *NahG* and being Sorbo-like for the genomic region on the lower part of chromosome III. All individuals were susceptible to *G. orontii*, suggesting that this type of resistance is likely dependent on SA. Crossings with other mutants defective in SA-dependent signaling, like *sid2*, will confirm whether this susceptibility was due to the absence of SA in the plants or just to the accumulation of the degradation product of salicylate hydroxylase catechol (Van Wees et al. 2003).

In summary, resistance to *G. orontii* could be SA-dependent in Sorbo, since introduction of the SA-degrading salicylate hydroxylase impairs resistance. Crossings with additional mutants will provide further insight.

3.7 ALLELISM TESTS BETWEEN ACCESSIONS

Six different accessions have been identified in which the resistance gene could be located to the same region on chromosome III. Although there is the possibility that resistance could be caused by different genes located in that region, it is more probable that the same locus is responsible for resistance. To test this hypothesis, accessions were crossed to each other and allelism tests were performed by screening the F_1 and F_2 progeny of these crossings with *G. orontii*. If resistance was allelic, that is mediated by the same locus, then F_1 and F_2 progeny are both expected to be resistant.

If resistance is due to different genes located on the same chromosomal region, parental phenotypes as well as F_1-like combinations should be observed in the F_2 generation. Consequently, the majority of F_2 plants should be resistant, only some susceptible plants can be expected due to recombination events. Crossings of Sha x Sorbo, Do-0 x Sha, Do-0 x Sorbo and Co-1 x C24 were analyzed (Table 4). It is notable that F_1 plants of all crossings were resistant to *G. orontii* (Figure 15), arguing for allelism of resistance in these accessions. The data for some F_2 progeny were in clear contradiction to the expected scenario, in which only resistant plants should appear. In crossings of Sha x Sorbo and Do-0 x Sha, resistance segregated in the F_2 generation. In Sha x Sorbo F_2 progeny 18,5 % of all plants were susceptible to different degrees, while in the Do-0 x Sha crossing 13,5 % of the F_2 plants showed only intermediate susceptibility. The analysis of different scenarios for resistance segregation (Table 4) shows that in the Sha x Sorbo crossing, the observed segregation pattern is significant in theory for two

independent and semi-dominantly inherited loci (P > 0,01) as well as for three independent semi-dominant and one recessive, two semi-dominant loci (P > 0,05). For Do-0 x Sha, only the segregation ratio for three independent and semi-dominantly inherited loci was found to be significant (P > 0,05). In the F_2 progeny of crossings Do-0 x Sorbo and Co-1 x C24, all plants were resistant, arguing for allelism of the resistance locus. Especially in the latter crossing, the result corresponded well to the expected scenario, since these lines are assumed to represent an identical accession (Schmid et al. 2006).

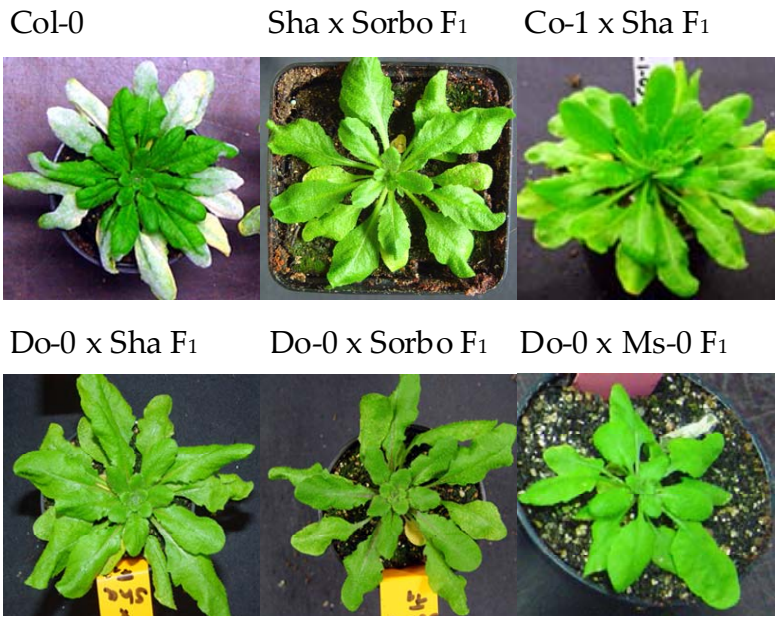

Figure 15: Allelism test between resistant accessions. Macoscopic infection phenotypes of five-week-old F_1 plants derived from crossings between selected resistant accessions at 11 dpi after inoculation with *G. orontii*.

Summarizing the results obtained with crossings between different resistant accessions in which resistance maps to the lower arm of chromosome III, segregation patterns in the F_2 generation of some crossings are incompatible with the hypothesis that resistance is caused by the same gene in the accessions.

Likewise, the data do not support the hypothesis that resistance is caused by two distinct loci residing in the same chromosomal region.

3.8 CANDIDATE GENES

In parallel to further fine mapping with additional markers and recombinant plants (see 3.4.1), the target region on chromosome III was analyzed for candidate genes known to confer resistance to pathogens or which are involved in defense reactions. A (recessively inherited) candidate gene could be defective due to different reasons: it could either not be present in the resistant accessions or it could have changes in the amino acid sequence disturbing its functionality. Additionally, changes in untranslated regions upstream of the start codon or downstream of the stop codon could interfere with transcriptional or post-transcriptional regulation.

To test for these defects, the following approaches can be followed: A PCR using genomic DNA as a template can be used to test for presence or absence of the gene. RT-PCR or Real-Time PCR are suitable techniques to test whether the gene is transcribed. Additionally, cDNAs can be sequenced to detect defects in amino acid sequence as well as mutations in untranslated regions. For a recessively inherited defect in a compatibility factor, complementation of resistance can provide the final proof. Complementation can be achieved by transforming the resistant accessions with a functional copy of the candidate gene. If the transformed plants of a resistant accession are susceptible after inoculation with *G. orontii*, the functional copy of the candidate gene has overcome resistance and restored susceptibility.

3.8.1 Candidate gene *PMR6*

The chromosomal region identified as the target gene region prior to fine mapping contains more than 700 predicted genes in Col-0, amongst others also several candidate genes. The powdery mildew susceptibility gene *PMR6* resides at ~ 20,35 Mb in the target gene region on chromosome III. This susceptibility factor, isolated in an EMS mutagenesis screen in Col-0, encodes a pectate lyase. It is required for establishing full susceptibility to *G. cichoracearum* and *G. orontii* (Vogel et al. 2002): When both copies are non-functional, plants are resistant. All resistant accessions except Do-0 show resistance to both *G. cichoracearum* and *G. orontii*. Considering a defect in *PMR6* as the cause for resistance, candidate Do-0 may either have a different allele which provides a different specificity of resistance or resistance in Do-0 could be due to another gene.

To test experimentally whether a defect in *PMR6* is responsible for resistance in the selected candidates several approaches were followed for accessions Sha and Sorbo. The ecotypes La-1, Nok-3 and Co-1 and for some experiments also Do-0 were not yet included in this analysis because their mapping data were not available at this point. With regard to the comparative analysis of resistance in a selected subset of resistant accessions (see 3.2), Sha and Sorbo showed values of host cell entry similar to *pmr6*, while Ang-0, Co-1, Do-0 and La-1 did not. In the analysis of H_2O_2 production in attacked cells, all tested accessions showed H_2O_2 production in contrast to the *pmr6* mutant. During the quantification of conidiophores per colony, all analyzed accessions showed a reduction of conidiophores similar to *pmr6*, except Sorbo, on which the pathogen was able to produce slightly more than on *pmr6*. Finally, quantification of cell death and callose deposition led to different results for *pmr6* compared to all other analyzed resistant accessions. Considering microscopical analysis, *pmr6* does not seem to be responsible for resistance in any of the selected accessions. However, the different genetic backgrounds of the selected ecotypes should be taken into

account, since they might interfere with the phenotype. Therefore, additional analyses were performed.

To test the possibilities mentioned above, the presence of *PMR6* in the genome was analyzed by PCR and transcription existence by RT-PCR in resistant accessions Sha, Sorbo and Do-0. Furthermore, *PMR6* was sequenced in Sha and Sorbo. The sequence data of *PMR6* was analyzed and compared to the functional *PMR6* allele in Col-0. Putative defects in *PMR6* were tested by complementation of *PMR6* in resistant accessions with a functional allele of Col-0. In this case resistant plants should become susceptible, when resistance is due to a defect in *PMR6*.

Results of PCR and RT-PCR showed that *PMR6* is present and transcribed in Col-0, Sha, Sorbo and Do-0. Preliminary Realtime PCR data indicate that *PMR6* might be expressed even at higher levels in Sha, Sorbo, Do-0 compared to Col-0 (data not shown). Protein levels were not analysed.

Sequencing of *PMR6* sequence revealed that the predicted PMR6 amino acid sequences in Sha and Sorbo are 100 % identical to Col-0. At the nucleotide level, there is a very low level of variation resulting in identities of 99,7 % for Sorbo and 99,9 % for Sha with regard to the Col-0 sequence (data not shown).

For complementation analysis, accessions Col-0, Do-0, Ms-0, Sha, Sorbo, as well as the *pmr6-4* mutant were transformed with a vector carrying the *PMR6* cDNA sequence from Col-0. T_1 or T_2 plants were screened with *G. orontii* (see Table 9). Between 8 and 24 T_1 plants per line were selected after spraying with Basta. For Col-0, *pmr6* and Sha, T_1 plants were analyzed by PCR for presence of the transgene. In T_1 plants of Col-0, five plants positive for the insert were detected, for two of them the T_2 progeny was analyzed with *G. orontii*. For *pmr6*, 11 T_1 plants were positive for the insert out of 24 tested. Of these, five were selected to

be analyzed with *G. orontii*. For Sha, seven out of 16 T_1 plants were positive for the insert and five were selected for T_2 analysis with *G. orontii*. In accessions Do-0, Ms-0 and Sorbo, the T_1 plants were tested with *G. orontii* after Basta-selection.

Although the 35S::*PMR6* construct complemented resistance in the *pmr6* mutant by rendering progeny of three different T_1 plants susceptible, it did not change the infection phenotype in T_1 or T_2 plants of accessions Do-0, Ms-0, Sha or Sorbo (see Figure 16), which remained resistant. T_2 plants of Col-0 remained susceptible, indicating that overexpression of *PMR6* has no effect on susceptibility. The results of the complementation analysis suggest that resistance in Do-0, Ms-0, Sha and Sorbo is not caused by a defect in *PMR6*.

Moreover, allelism tests were performed to determine if resistance in Sorbo is mediated by *pmr6*. When two recessive mutants are crossed in a standard complementation test, the phenotype of the resulting "double mutant" usually reveals whether the two mutations are allelic and the respective genes encode the same gene product. Therefore Do-0, Sha and Sorbo were crossed to the *pmr6-3* mutant and the F_1 and F_2 progeny were analyzed with *G. orontii*.

Table 9: Transformation of selected accessions and the *pmr6* mutant with *35S::PMR6* cDNA-GW: T_1 and T_2 plants analyzed with *G. orontii*

Accession	# T_1 plants	DR* T_1	positive for insert	DR T_2	# T_2 plants	DR T_2
Col-0	16	n.d.	2,5,6,8,13	T_1.2	15	3
				T_1.8	15	3
Do-0	16	0	n.d.	n.d.	n.d.	n.d.
Ms-0	16	0	n.d.	n.d.	n.d.	n.d.
pmr6	24	n.d.	2, 4, 7, 8, 9, 11, 12, 17, 18, 19, 24	T_1.2	15	3
				T_1.4	15	3
				T_1.8	15	3
				T_1.9	15	0
				T_1.11	15	0
Sha	16	n.d.	1, 5, 6 7,8,9,12	T1.11	15	0
				T_1.6	40	0
				T_1.7	40	0
				T_1.9	40	0
Sorbo	8	0	n.d.	n.d.	n.d.	n.d.

* DR: disease resistance score, 3 is fully susceptible, 0 fully resistant.
n.d. not determined

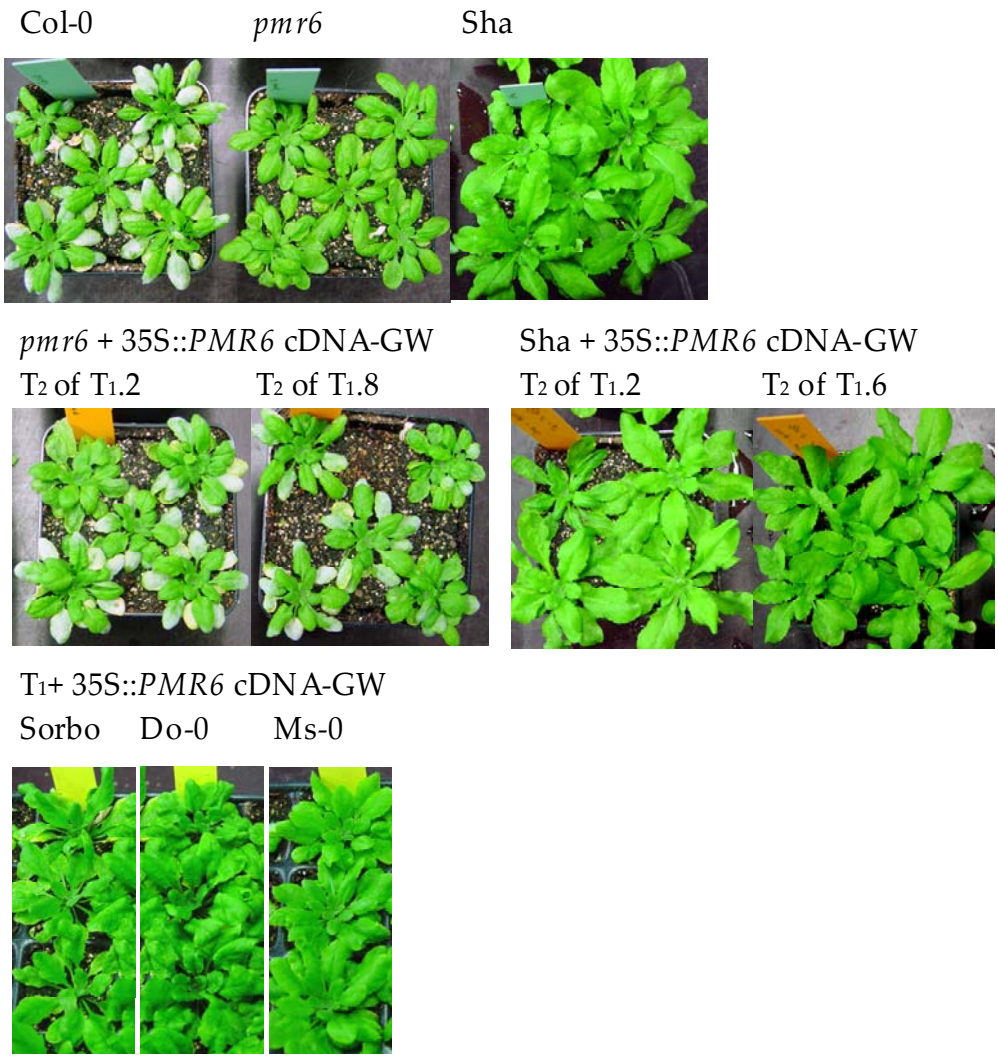

Figure 16: Complementation analysis of resistance in *pmr6* and in selected resistant accessions. Macrographs of five-week-old plants are shown at 11 days post inoculation with *G. orontii*. For the *pmr6* mutant and Sha T_2 plants, and for Sorbo, Do-0 and Ms-0 T_1 plants with 35S::*PMR6* cDNA-GW are shown.

Analysis of these crossings showed a resistant F_1 population, indicative for allelism (see Figure 17), but in the F_2 generation resistance segregated again, arguing against allelism of *pmr6* and the resistance gene in the accession. In the F_2 generation of Sorbo crossed with *pmr6-3*, the F_2 progeny segregated in 48 susceptible to 44 resistant plants. The χ^2 test for a segregation pattern of 1 : 1

was 0,677, a significant value with P > 0,05. In the F_2 generation of Do-0 crossed with *pmr6-3*, the F_2 population segregated in 3 fully resistant (DR 3), 8 intermediate susceptible (DR 2), 9 intermediate resistant (DR 1) and 24 fully resistant (DR 0) plants. Different segregation scenarios were tested for these crossings (Table 4). Results of both crossings were significant for the segregation of two independent recessive genes (P > 0,05), in case of Do-0 also for one recessive and one independent semi-dominant resistance (P > 0,05) as well as for two independent semi-dominant genes and one recessive with two semi-dominant genes.

An expected segregation pattern for an F_2 generation of a crossing between two different genes conferring the same phenotype and being closely located on the same chromosome is expected to show linkage. This implies that the F_2 generation will consist mostly of either parental phenotypes or F_1-like heterozygous plants, which is contradictory to the observation that both parents and the F_1 progeny are resistant, but approximately half of the F_2 generation is susceptible.

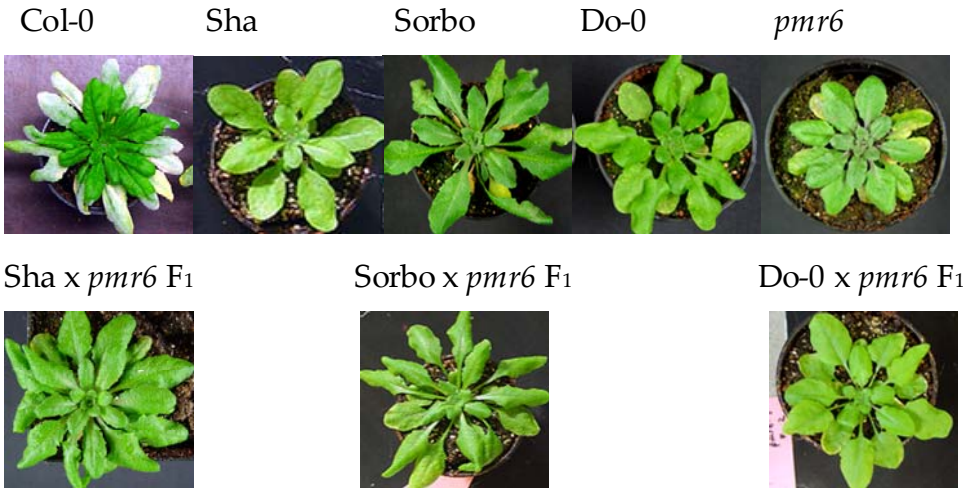

Figure 17: Test for allelism with the *pmr6-3* mutant. Macroscopic infection phenotypes of five-week-old F_1 plants, which derived from crossings of resistant accessions Sha, Sorbo and Do-0 with the resistant mutant *pmr6-3* at 11 dpi with *G. orontii.*

It is not known whether susceptibility in Sorbo x *pmr6* is full or intermediate, because the inoculation was not very strong for these plants. For this reason, they were grouped into susceptible and resistant plants without further discrimination.

In summary, amino acid sequence and transcript accumulation of *PMR6* is unchanged in resistant accessions Sha and Sorbo. Comparative microscopic and quantitative analysis of resistance do likewise not support the hypothesis that resistance is due to loss of *pmr6* function. Crossing analysis of Sorbo x *pmr6-3* and Do-0 x *pmr6-3* suggested allelism in the F_1 progeny, which was resistant, but rejected it in the F_2 generation, which in contrast showed susceptible plants. Attempted complementation analysis with a functional *PMR6* allele was not able to restore susceptibility in Sha, Sorbo or Do-0 although it did in the *pmr6* mutant. These data suggest that loss of *PMR6* function is not responsible for resistance in Sha, Sorbo or Do-0.

3.8.2 Other candidate genes

In order to find new candidate genes, a transcript-based cloning approach as suggested in Mitra et al. (2004) was employed. Analysis of gene expression data based on microarrays (accessible at www.genevestigator.com) revealed three genes which are located in the region identified in the first mapping steps and which show elevated transcript levels after powdery mildew attack (*G. cichoraceaum*). These genes encode a fructose-1,6-bisphosphatase (located at ~20,03 Mb), a phenylalanine lyase (~19,8 Mb) and a photosystem II oxygen evolving complex (~18,9 Mb). No genes in the region were identified which are differentially expressed between Sha and Col-0. The new candidate genes were sequenced in Sha and Sorbo but showed no differences to Col-0 sequence at the amino acid level (data not shown).

3.8.3 Candidate gene *RPW8*

A further locus in the target gene region conferring resistance to powdery mildews is *RPW8*, located at ~18,8 Mb on chromosome III. In earlier studies *RPW8* was described as a dominant and atypical *R*-gene being the main source of resistance against powdery mildew among *Arabidopsis thaliana* accessions (Xiao et al. 1997, 2001, 2002, 2003, 2004). Due to its dominant nature, it would normally not be regarded as a candidate for a recessively inherited compatibility factor. However, in a recent publication, *RPW8* was considered to be inherited in a semi-dominant manner (Xiao et al. 2005). Since segregation patterns of F_2 progeny of many of the candidate accessions do also fit the 1 : 2 : 1 (susceptible : intermediate : resistant) hypothesis (Table 3), *RPW8* could be responsible for resistance in the candidates.

Nevertheless, this semi-dominant phenotype was observed only for a transgenic line of Col-0 carrying the *RPW8* locus. For Ms-0, in which *RPW8* was originally identified, quantification of hyphal length and conidiophore production suggest a clear dominant inheritance of resistance (Xiao et al. 1997). In addition, *RPW8* is known to confer broad-spectrum disease resistance, while one out of six candidates, Do-0, is susceptible for one of three powdery mildew species.

Several approaches were followed to test for *RPW8*-mediated resistance in the mapping candidates. First, presence or absence of *RPW8.1* and *RPW8.2* was tested by PCR and transcript accumulation by RT-PCR. RT-PCR products of *RPW8* were sequenced and nucleotide and predicted amino acid sequences were compared to each other and to *RPW8* from Ms-0 ecotype. Furthermore, the population of recombinant inbred lines was employed to determine whether infection phenotypes of the RI lines were correlated to presence or absence of *RPW8.1* and *RPW8.2* sequences in the individual lines. In contrast to segregating F_2 populations, RI lines offer the advantage of genetic stability.

Since *RPW8* is either dominantly or semi-dominantly inherited, one gene copy should be sufficient to provide resistance leading to at least intermediate phenotypes. Therefore, a complementation approach could not be applied for this locus, because resistant accessions are not expected to have a defect in this candidate gene but rather to possess a functional copy of *RPW8*. Consequently, depletion of *RPW8.1* and *RPW8.2* transcript accumulation in resistant accessions by double-stranded RNA interference (dsRNAi) could provide further evidence whether resistance is mediated by *RPW8* in these ecotypes.

3.8.3.1 Analysis for presence and transcription of *RPW8.1* and *RPW8.2* in different accessions

Accessions with a diverse range of infection phenotypes and specificities to different powdery mildew species were selected and analyzed by PCR and RT-PCR for presence of *RPW8* genomic sequences and transcripts, respectively. A total number of 92 accessions with different infection phenotypes were screened for existence of *RPW8.1* and *8.2* (see Table 10). *RPW8.1* was identified by PCR on genomic DNA in 64 (70 %) accessions. In two accessions, Hh-0 and Jm-1, *RPW8.1* was present but not transcribed: Both were susceptible to the three tested powdery mildew species. Some accessions (Is-1, Pla-0, Ste-0, Ty-0) did not possess *RPW8.1* but were resistant to *G. orontii*. Is-1 and Ste-0 did also lack *RPW8.2* and in Pla-0 no *RPW8.2* transcript was found. In all seven mapping candidates *RPW8.1* was present and transcribed.

RPW8.2 was found in 74 % of all tested accessions. In two accessions, Jm-1 and Pla-0, no transcript was amplified. Two further accessions, Is-0 and Ste-0, did not possess *RPW8.2* but were resistant to *G. orontii*. All six mapping candidates showed presence and transcription for *RPW8.2*. Five accessions (7 %) transcribe

only *RPW8.2* alone (Gre-0, Gü-0, Hh-0, Jl-4, Ty-0) and four of them also lack the genomic *RPW8.1* sequence. However, the opposite scenario was not observed.

In summary, PCR and RT-PCR of *RPW8.1* and *RPW8.2* showed that both genes are present and transcribed in most of the tested accessions and in all mapping candidates. Not all resistant accessions possess and transcribe both *RPW8.1* and *RPW8.2*, in these instances resistance could be due to another gene. Both genes were also identified in susceptible accessions, in which a modified or defect allele or a distinct level or timing of expression could be responsible for susceptibility. Some accessions possess only *RPW8.1* or *RPW8.2* alone.

Table 10: Presence of genomic sequence and transcript of *RPW8.1* and *RPW8.2* in selected accessions

	DR*	*RPW8.1*			*RPW8.2*		
Accession	***G. orontii***	**PCR**	**RT-PCR**	**sequenced**	**PCR**	**RT-PCR**	**sequenced**
An-2	3	+	+	+	+	+	+
Ang-0	0	+	+	+	+	+	+
Bay-0	2	-	-	n.p.	-	-	n.p.
Bd-0	3	-	-	n.p.	-	-	n.p.
Be-0	3	-	-	n.p.	-	-	n.p.
Bla-11	3	+	+	+	+	+	+
Bla-2	2	+	+	+	+	+	+
Bla-3	2	+	+	+	+	+	+
Blh-2	3	+	+	+	+	+	+
Bs-2	2	+	+	+	+	+	+
Bu-0	0-1	+	+	n.p.	+	+	+
Bu-13	3	+	+	+	+	+	+
Bu-25	3	+	+	+	+	+	+
Bu-3	0	+	+	+	+	+	+
Bu-6	2	+	+	+	+	+	+
Bur-0	2	+	+	+	+	+	+
C24	0	+	+	+	+	+	+
Chi-0	2	+	+	+	+	+	+
Cnt-1	2	+	+	+	+	+	+
Co-1	0	+	+	+	+	+	+
Co-3	0	+	+	+	+	+	n.p.
Col-0	3	-	-	n.p.	-	-	n.p.
Col-1	3	-	-	n.p.	-	-	n.p.
Col-4	3	-	-	n.p.	-	-	n.p.
Col-5	3	-	-	n.p.	-	-	n.p.
Da(1)-12	2	+	+	+	+	+	n.p.
Di-0	2	+	+	+	+	+	+
Di-1	2	-	-	n.p.	-	-	n.p.
Dijon G	3	+	+	+	+	+	n.p.
Do-0	0	+	+	+	+	+	+
Ei-2	0	+	+	+	+	+	+
Ei-4	0	+	+	+	+	+	+
Ei-5	0	+	+	+	+	+	+
Ei-6	1-2	+	+	+	+	+	+
Enkheim D	2	+	+	+	+	+	+
Er-0	3	-	-	n.p.	-	-	n.p.
Fr-2	2	-	-	n.p.	-	-	n.p.
Fr-6	2	+	+	+	+	+	n.p.
Gd-1	3	-	-	n.p.	-	-	n.p.
Ge-1	2	-	-	n.p.	-	-	n.p.
Ge-2	3	-	-	n.p.	-	-	n.p.
Gr-1	3	-	-	n.p.	-	-	n.p.
Gre-0	2	-	-	n.p.	+	+	+
Gü-0	3	-	-	n.p.	+	+	+
Gü-1	2	-	-	n.p.	-	-	n.p.
H55	3	+	+	+	+	+	+
Hh-0	3	+	-	+	+	+	n.p.
Hl-2	3	-	+	+	+	+	n.p.

* DR: disease resistance score

n.p. not performed, n.d. not determined.

Table 10 Continued

	DR*	*RPW8.1*			*RPW8.2*		
Accession	***G. orontii***	**PCR**	**RT-PCR**	**sequenced**	**PCR**	**RT-PCR**	**sequenced**
In-0	2	+	+	+	+	+	n.p.
Is-0	0	+	+	+	+	+	+
Is-1	0	-	-	n.p.	-	-	n.p.
Ita-0	0	+	+	+	+	+	+
Jl-1	2	-	-	n.p.	-	-	n.p.
Jl-4	3	-	-	+	+	+	n.p.
Jm-1	2	+	-	n.p.	+	n	n.p.
Kb-0	3	+	+	+	+	+	+
Kin-0	2	+	+	+	+	+	n.p.
Kl-2	1	+	+	+	+	+	n.p.
Ko-2	1-2	+	+	+	+	+	+
Kro-0	1	-	-	n.p.	-	-	n.p.
La-1	0	+	+	+	+	+	+
Ma-2	1-2	-	-	n.p.	-	-	n.p.
Mc-0	n.d.	-	-	n.p.	-	-	n.p.
Mh-0	0-1	+	+	+	+	+	+
Nd-0	2	+	+	+	+	+	n.p.
Nie-0	0-1	-	-	n.p.	-	-	n.p.
Nok-3	0	+	+	+	+	+	+
Nw-0	0	+	+	+	+	+	+
Nw-1	0	+	+	n.p.	+	+	+
Ob-0	0-1	+	+	+	+	+	+
Ob-3	0	+	+	+	+	+	+
Ove-0	0-1	+	+	+	+	+	+
Petergof	0	+	+	+	+	+	+
Pla-0	0	-	-	n.p.	+	-	n.p.
Pla-2	0	+	+	+	+	+	+
Pla-3	0	+	+	+	+	+	+
Pla-4	0	+	+	+	+	+	+
Po-0	0	+	+	+	+	+	+
Rak-2	0	+	+	+	+	+	+
Rsch-4	0	+	+	+	+	+	+
Sei-0	0	+	+	+	+	+	n.p.
Sha	0	+	+	+	+	+	+
Sorbo	0	+	+	+	+	+	+
Ste-0	0	-	-	n.p.	-	-	n.p.
Ty-0	0	-	-	n.p.	+	+	+
Uk-1	0	+	+	+	+	+	+
Uk-3	0	+	+	+	+	+	+
Uk-4	3	+	+	+	+	+	+
Wa-1	0	+	+	+	+	+	+
Wc-2	1	+	+	+	+	+	+
Wt-2	0	+	+	+	+	+	+

3.8.3.2 Sequence analysis

To obtain insights in the structure/function relationship of *RPW8* alleles, sequence analysis was performed. Nucleotide sequences of *RPW8.1* and *RPW8.2* cDNAs were determined in susceptible and resistant accessions in which *RPW8.1* or *RPW8.2* were found to be transcribed. *RPW8.1* was sequenced successfully in 57 accessions, and *RPW8.2* sequences could be obtained from 54 different accessions.

In general, at the nucleotide level, *RPW8* sequences were found to be surprisingly variable between the accessions ranging from 96,4 % to 100 % identity for *RPW8.1* and from 92 to 99,4 % identity on nucleotide level for *RPW8.2*. These values are similar to observations reported by Xiao et al. (2004).

a) *RPW8.1*

Based on the predicted amino acid sequence, the 57 recovered *RPW8.1* sequences could be assigned to 13 different groups or isoforms based on amino acid level (Table 11, Table 12 and Figure 18). Members of a given group have identical sequences at the amino acid level. A majority of 20 accessions belong to the group of Ms-0-like *RPW8.1* sequences.

Table 11: RPW8.1 sequences: groups of different isoforms

Group	name	accessions included	infection phenotype*	origin: country
1	Ms-0 like	C24	*Go, Gcr, Gci*	Portugal
		Co-1	*Go, Gcr, Gci*	Portugal
		Ei-2	*Go, Gcr, Gci*	Germany
		Ei-4	*Go, Gcr, Gci*	Germany
		Ei-5	*Go, Gcr, Gci*	Germany
		Nw-0	*Go, Gcr, Gci*	Germany
		Pla-3	*Go, Gcr, Gci*	Spain
		Sha	*Go, Gcr, Gci*	Tajikistan
		Sorbo	*Go, Gcr, Gci*	Tajikistan
		Wa-1	*Go, Gcr, Gci*	Poland
		Wt-2	*Go, Gcr, Gci*	Germany
		Cnt-1	susceptible	United Kingdom
		Kin-0	susceptible	USA
		Ove-0	*Go, Gcr*	Germany
		Pla-4	*Go, Gcr*	Spain
		Pla-2	*Gcr, Gci*	Spain
		Is-0	*Go*	Germany
		Ita-0	*Go*	Marocco
		Po-0	*Go*	Germany
		Uk-3	*Go*	Germany
2	Bla-3 like	Bla-3	susceptible	Spain
		Di-0	susceptible	France
		Dijon G	susceptible	France
		In-0	susceptible	Austria
		Uk-4	susceptible	Germany
		Co-3	*Go*	Portugal
3	Bu-6 like	Bu-6	susceptible	Germany
		Chi-0	susceptible	Russia
4	Ob-0	Ob-0	*Go, Gci*	Germany
5	Bu-0 like	Petergof	*Go, Gcr, Gci*	Russia
		Bu-13	susceptible	Germany
		Bu-0	*Go, Gcr*	Germany
6	Rsch-4	Rsch-4	*Go*	Russia
7	Bur-0 like	Nok-3	*Go, Gcr, Gci*	Netherlands
		Bur-0	susceptible	Irland
		Ei-6	susceptible	Germany
		H55	susceptible	Czech Republic
8	Do-0	Do-0	*Go, Gcr*	Germany
9	An-2 like	An-2	susceptible	Belgium
		Bs-2	susceptible	Switzerland
		Bu-25	susceptible	Germany
		Da(1)-12	susceptible	Czech Republic
		Enkheim D	susceptible	Germany
		Ko-2	susceptible	Denmark
		Kl-2	*Go*	Germany
		Sei-0	*Go*	Italy
		Wc-2	*Go*	Germany
10	Ob-3	Ob-3	susceptible	Germany
11	Fr-6	Fr-6	susceptible	Germany
12	Ang-0 like	Bla-2	susceptible	Spain
		Bla-11	susceptible	Spain
		Blh-2	susceptible	Czech Republic
		Hl-2	susceptible	Germany
		Jl-4	susceptible	Czech Republic
		Nd-0	susceptible	Germany
		Ang-0	*Go, Gci*	Belgium
		Hh-0	*Go*	Germany
13	Kb-0	Kb-0	susceptible	Germany

* Infection phenotypes with the tested three powdery mildew species are indicated as: ***Go, Gcr, Gci*** **:** Resistant to *G. orontii, G. cruciferarum* and *G. cichoracearum* ; **susceptible**: Susceptible to all three *Golovinomyces* sp.; ***Go, Gci*** **:** Resistant to *G. orontii and G. cichoracearum* ; ***Go, Gcr*** : Resistant to *G. orontii* and *G. cruciferarum* ; ***Gcr, Gci*** **:** Resistant to *G. cruciferarum* and *G. cichoracearum* ; ***Go*** **:** Resistant to *G. orontii* only.

Table 12: Sequences per RPW8.1 isoform group

group name	# sequences in group
Ms-0 like	20
An-2 like	9
Ang-0 like	8
Co-3 like	6
Bur-0 like	4
Bu-0 like	3
Bu-6 like	2
Do-0	1
Ob-0	1
Rsch-4	1
Ob-3	1
Fr-6	1
Kb-0	1

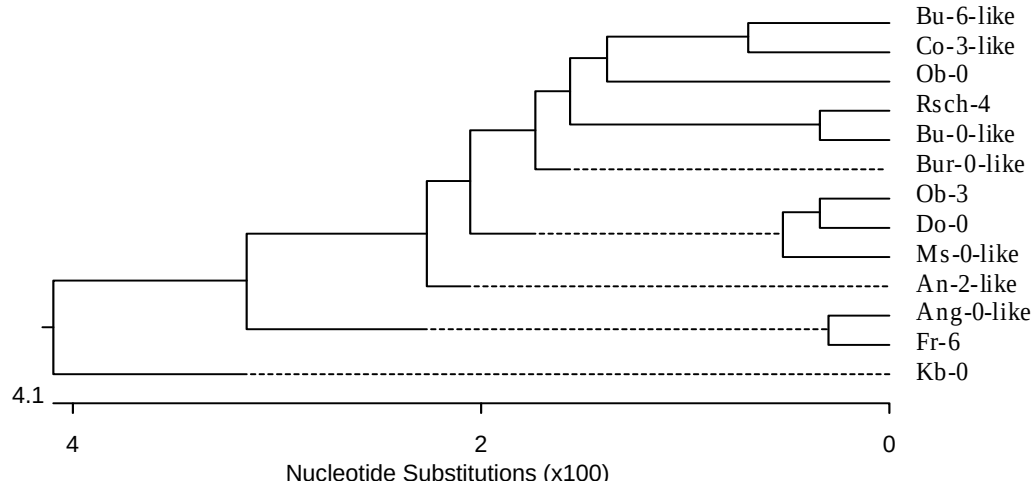

Figure 18: Phylogenetic relationship of RPW8.1 isoforms based on amino acid sequence. Nucleotide sequences were translated and aligned with the ClustalV function in the MegAlign program of the Lasergene software package. Isoforms recovered from more than one accessions are named according to the alphabetically first accession in that group.

A further dominant group harbouring nine members is the An-2-like class. Six groups contain only a single accession. The Ang-0-like group consisted of eight different accessions in which the RPW8.1 sequence is characterized by a C-terminal insertion event (see Figure 19). Starting from amino acid 132, there is an insertion of 21 amino acids, duplicating the previous 15 and 5 amino acids further downstream. This insertion does not lead to a frameshift.

An alignment of 169 amino acids of the different isoforms observed for RPW8.1 reveals that, at least on amino acid level, the region with the highest variability is located in the N-terminal part of the protein between amino acid position 31 and 45. This region is located between the predicted transmembrane (TM) and coiled-coil (CC) domains.

In addition, sequences were sorted with respect to the accession's phenotype. For example, all sequences from accessions resistant to only *G. orontii* or accessions susceptible to all three powdery mildew species were pooled together. Sequences from accessions resistant to all three powdery mildew species tend to cluster together; eleven sequences could be found in the Ms-0-like group and only two in other groups (Table 11). Sequences from mapping candidates were dispersed over three different groups (groups 1, 7 and 8), but most of them showed Ms-0-like RPW8.1 sequences (C24, Co-1, Sha and Sorbo). Accessions which were susceptible to all three powdery mildew species were distributed throughout ten groups. Nevertheless, groups 2, 3, 7 and 12 consisted mainly of sequences from these accessions. All other combinations of infection phenotypes considered showed no clustering.

In addition, no obvious correlation of infection phenotype combination with geographical origin could be noticed based on these data. Only sequences of accessions from Russia seemed to cluster in related groups 3, 5 and 6, but for no other country of origin a similar observation could be made.

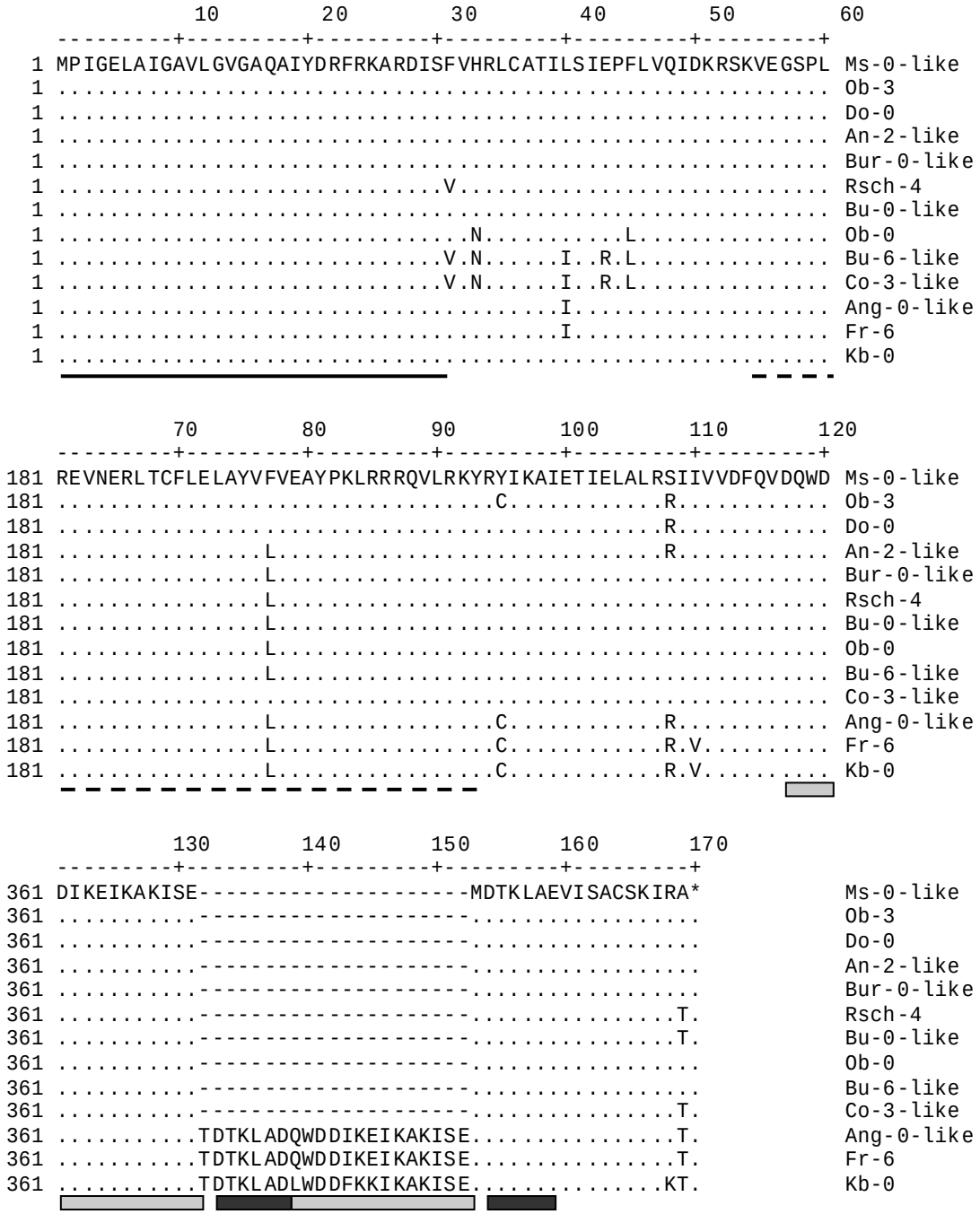

Figure 19: Alignment of 13 different RPW8.1 isoforms. Nucleotide sequences were translated and predicted amino acid sequences were aligned with the ClustalV function in the MegAlign program of the Lasergene software package. Isoforms recovered from more than one accessions were named according to the alphabetically first accession in that group. Amino acids identical to respective amino acid in Ms-0 are indicated with a "." , stop codons with a „*“.The gray and black bars mark the part of the sequence which is repeated in the insertion.

This is also true for the Ang-0-like group with the previously described insertion at the C-terminus. Members of this group have diverse origins: some originate from Spain and others from the Czech Republic, indicating a wide distribution of this allele throughout the European part of the native habitat of *A. thaliana*.

b) *RPW8.2*

RPW8.2 sequences were obtained for 54 different accessions and they show an even higher level of variation as compared to RPW8.1. They can be clustered into 26 different groups based on their amino acid sequence (see Table 13 and Figures 20 and 21) and represent again the biggest group with 16 accessions. Seven groups contain between two and five sequences, while the majority of 18 sequences were identified only once in this analysis (see Table 14).

Like in RPW8.1, the alignment of the of the predicted RPW8.2 protein sequences reveals a region of high variation at the amino acid level in the N-terminal part of the protein, between amino acid position 45 and 77. This region is located downstream of the TM domain and extends into the beginning of the CC domain. Another region of considerable variation is the C-terminus. In this region five different isoforms (corresponding to five accessions) had two identical insertions of two nucleotides each, leading to a frameshift and a premature STOP codon (Ei-5, Pla-4, Uk-4, Nw-1 and Rsch-4). Another isoform isolated from two accessions had a deletion of one nucleotide, which again resulted in a frame shift and a premature STOP codon (Bu-6-like). The same is true for three other isoforms representing five different accessions, but a different nucleotide was affected.

No correlation of phenotype distribution with the grouping could be discovered. It is notable that the Ms-0-like group consists of accessions with very diverse infection phenotypes: five sequences derived from accessions resistant

to all three powdery mildews (La-1, Sha, Sorbo, Uk-1 and Wt-2), while five stem from accessions susceptible to all of them (Bla-2, Bla-11, Ei-6, Ko-2 and Ob-3).

Three accessions are only resistant to *G. orontii* (Is-0, Ita-0 and Uk-3) and three accessions showed resistance to two different powdery mildew species (Bu-3, Ob-0 and Pla-2). In other groups with more than one accession, a similar picture could be observed. Likewise no strong correlation of sequence variation with geographical origin could be made, with the exception of the Russian accessions, which again cluster in related groups. However, groups of other isoforms, e.g. the Ms-0 like RPW8.2 sequence, are found in accessions from Spain (Bla-2, Bla-11 and Pla-2), Germany (Bu-3, Is-0, La-1, Ob-0, Ob-3, Uk-1, Uk-3 and Wt-2), Denmark (Ko-2), Morocco (Ita-0) and Tajikistan (Sha and Sorbo). Other groups show a similar wide distribution of geographical origins (e.g. Bs-2-like, Cnt-like, Bu-6-like and C24-like).

Intriguingly, in the phylogenetic analysis of RPW8.1 and RPW8.2 sequences the majority of accessions did not cluster in the same manner when the two genes were compared. Additionally, the sequence analysis of RPW8.1 and RPW8.2 regarded together with respect to sequence similarity to Ms-0 and phenotype constellation reveals several unexpected observations. The RPW8.1 sequence of Ms-0 controlled by a *35S* promotor was reported to be sufficient to confer broad-spectrum disease resistance to four different powdery mildew species. The same was true for 35S::*RPW8.2* alone and also for a vector containing both genes under control of their native promoters (Xiao et al. 2001). Consistent with this observation, the Ms-0-like RPW8.1 group contains sequences of many accessions which are resistant to three powdery mildews. A closer look, however, reveals that accessions reside in Ms-0-like groups of RPW8.1 or RPW8.2 which are susceptible to three powdery mildew species: Cnt-1 and Kin-0 for RPW8.1 and Ei-6, and Ko-2 for RPW8.2. No accession susceptible to three powdery mildews was found having Ms-0-like sequences for both RPW8.1 and RPW8.2.

Table 13: RPW8.2 sequences: groups of different isoforms

Group	name	accessions included	infection phenotype*	origin: country
1	Ms-0 like	La-1	*Go, Gcr, Gci*	Germany
		Sha	*Go, Gcr, Gci*	Tajikistan
		Sorbo	*Go, Gcr, Gci*	Tajikistan
		Uk-1	*Go, Gcr, Gci*	Germany
		Wt-2	*Go, Gcr, Gci*	Germany
		Bla-2	susceptible	Spain
		Bla-11	susceptible	Spain
		Ei-6	susceptible	Germany
		Ko-2	susceptible	Denmark
		Ob-3	susceptible	Germany
		Bu-3	*Go, Gci*	Germany
		Ob-0	*Go, Gci*	Germany
		Pla-2	*Gcr, Gci*	Spain
		Is-0	*Go*	Germany
		Ita-0	*Go*	Marocco
		Uk-3	*Go*	Germany
2	Co-1	Co-1	*Go, Gcr, Gci*	Portugal
3	Bu-0	Bu-0	*Go, Gcr*	Germany
4	Ei-2	Ei-2	*Go, Gcr, Gci*	Germany
5	Bur-0	Bur-0	susceptible	Irland
6	Kb-0	Kb-0	susceptible	Germany
7	Bu-13	Bu-13	susceptible	Germany
8	Bs-2 like	Bs-2	susceptible	Switzerland
		Bu-25	susceptible	Germany
		Do-0	*Go, Gcr*	Germany
		Rak-2	*Go, Gcr*	Czech Republic
		Wc-2	*Go*	Germany
9	Cnt-1 like	Nok-3	*Go, Gcr, Gci*	Netherlands
		Nw-0	*Go, Gcr, Gci*	Germany
		Cnt-1	susceptible	United Kingdom
		Ove-0	*Go, Gcr*	Germany
10	Ang-0 like	Ei-4	*Go, Gcr, Gci*	Germany
		Ang-0	*Go, Gci*	Belgium
11	Bla-3 like	Bla-3	susceptible	Spain
		Di-0	susceptible	France
12	Enkheim D	Enkheim D	susceptible	Germany
13	An-2	An-2	susceptible	Belgium
14	Wa-1	Wa-1	*Go, Gcr, Gci*	Poland
15	Blh-2	Blh-2	susceptible	Czech Republic
16	Po-0	Po-0	*Go*	Germany
17	Mh-0	Mh-0	*Go*	Poland
18	Nw-1	Nw-1	*Go*	Germany
19	Ei-5 like	Ei-5	*Go, Gcr, Gci*	Germany
	H55	H55	susceptible	Czech Republic
20	Uk-4	Uk-4	susceptible	Germany
21	Pla-4	Pla-4	*Go, Gcr*	Spain
22	Rsch-4	Rsch-4	*Go*	Russia
23	Bu-6 like	Petergof	*Go, Gcr, Gci*	Russia
		Bu-6	susceptible	Germany
24	C24 like	C24	*Go, Gcr, Gci*	Portugal
		Pla-3	*Go, Gcr, Gci*	Spain
		Gü-0	susceptible	Germany
25	Chi-0	Chi-0	susceptible	Russia
26	Ty-0	Ty-0	*Go*	United Kingdom

* Infection phenotypes with the tested three powdery mildew species are indicated as ***Go, Gcr, Gci*** **:** Resistant to *G. orontii, G. cruciferarum* and *G. cichoracearum* ; **susceptible**: Susceptible to all three *Golovinomyces* sp.; ***Go, Gci*** **:** Resistant to *G. orontii and G. cichoracearum* ; ***Go, Gcr*** : Resistant to *G. orontii* and *G. cruciferarum* ; ***Gcr, Gci*** **:** Resistant to *G. cruciferarum* and *G. cichoracearum* ; ***Go*** **:** Resistant to *G. orontii* only.

Table 14: Sequences per RPW8.2 isoform group

group name	# sequences in group
Ms-0 like	15
Bs-2 like	5
Cnt-1 like	4
C24 like	3
Ang-0 like	2
Bla-3 like	2
Bu-6 like	2
Ei-5 like	2
An-2	1
Blh-1	1
Bu-0	1
Bu-13	1
Bur-0	1
Chi-0	1
Co-1	1
Ei-2	1
Enkheim D	1
Kb-0	1
Mh-0	1
Nw-1	1
Pla-2	1
Pla-4	1
Po-0	1
Rsch-4	1
Ty-0	1
Uk-4	1
Wa-1	1

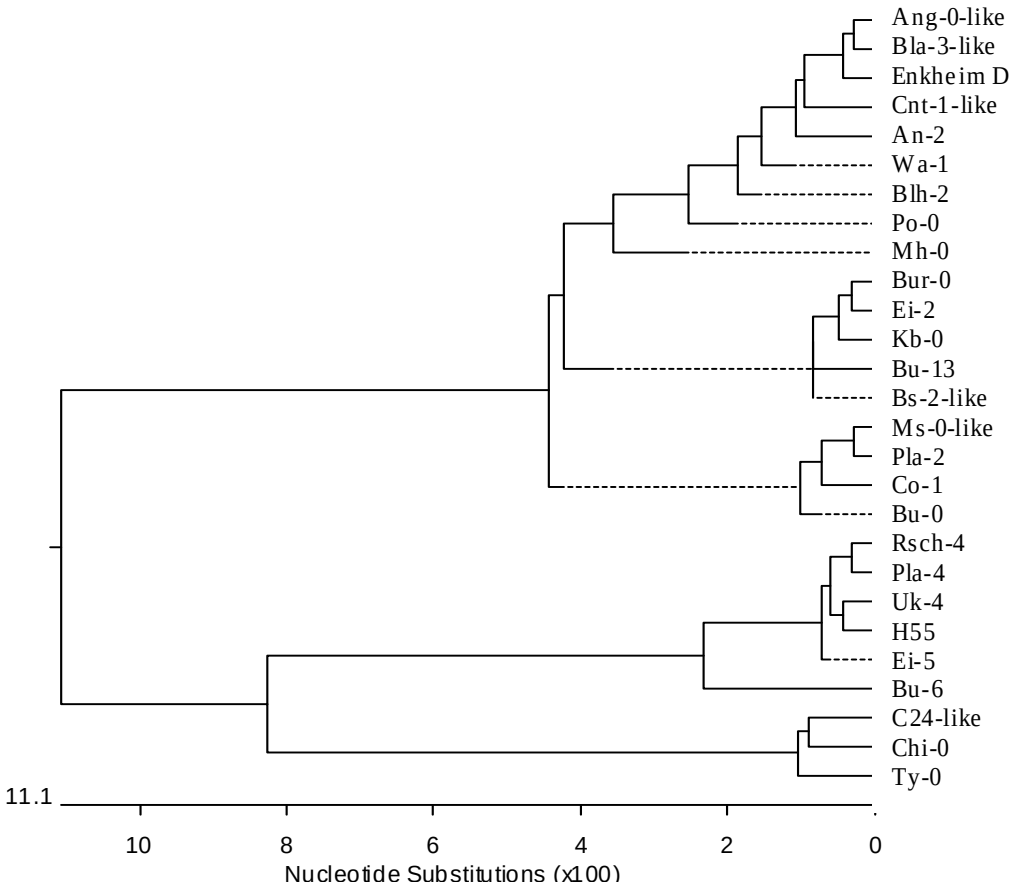

Figure 20: Phylogenetic relationship of RPW8.2 isoforms based on amino acid sequence. Nucleotide sequences were translated and aligned with the ClustalV function in the MegAlign program of the Lasergene software package. Isoforms recovered from more than one accessions are named according to the alphabetically first accession in that group.

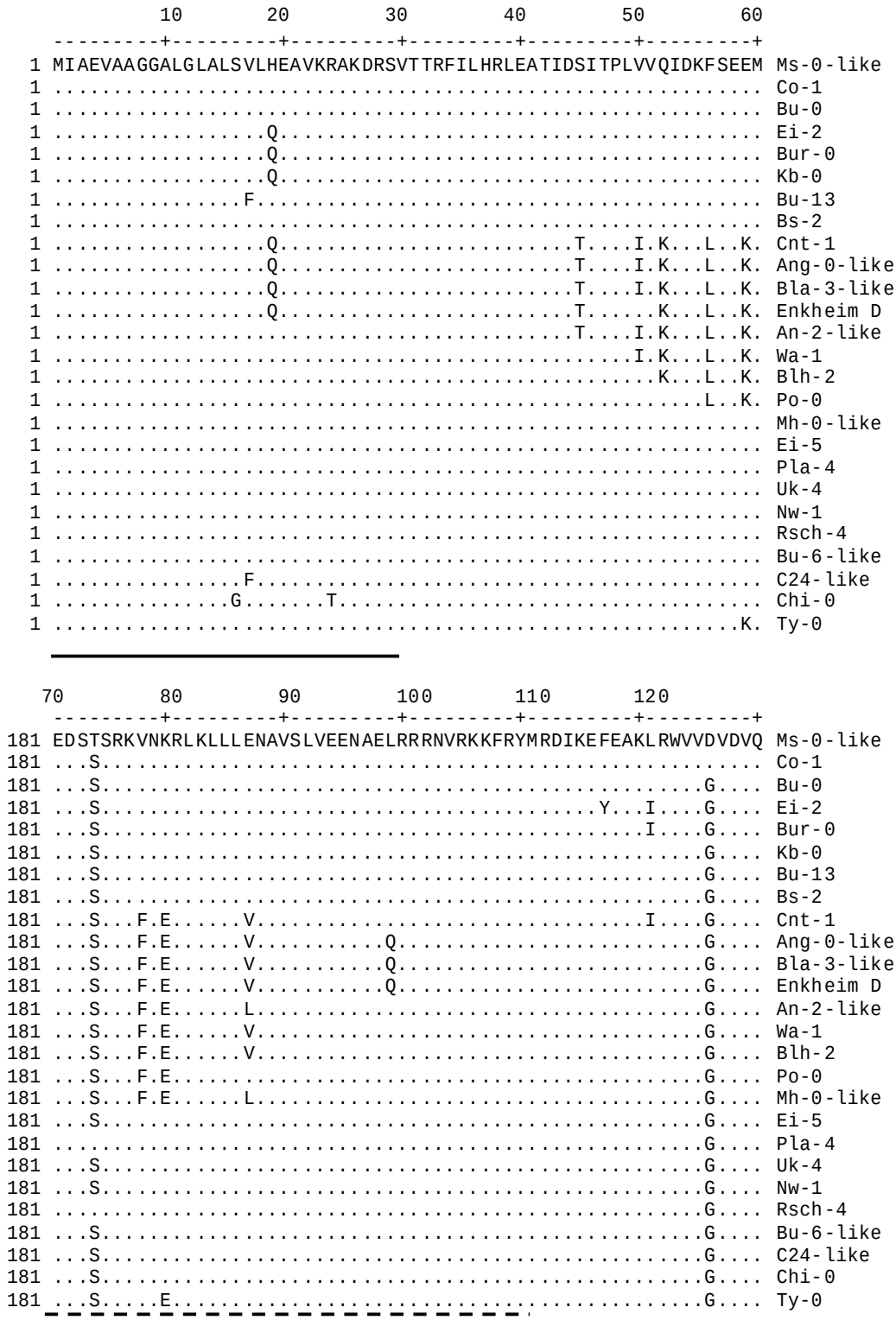

Figure 21: Alignment of 26 different RPW8.2 isoforms. Nucleotide sequence were translated and predicted amino acid sequences were aligned with the ClustalW function of the Lasergene software package. Isoforms recovered from more than one accessions were named according to the alphabetically first accession in that group. Amino acids identical to the respective amino acid in Ms-0 are indicated with a „.“, stop codons with a „*“. The black line indicates the transmembrane domain, the dashed line the coiled-coil domain.

```
            130       140       150       160       170
    ---------+---------+---------+---------+---------+-----
361 VNQLADIKELKAKMSEISTKLDKIMPQPKFEIHIGWCSGKTNRAIRFTFCSDDS*   Ms-0-like
361 ........................................Q.............   Co-1
361 ..................................................... .   Bu-0
361 ........................................K.............   Ei-2
361 ........................................K.............   Bur-0
361 ........................................K.............   Kb-0
361 ........................................K.............   Bu-13
361 ........................................K.............   Bs-2
361 ........................................K.............   Cnt-1
361 ........................................K.............   Ang-0-like
361 ..................................................... .   Bla-3-like
361 ..................................................... .   Enkheim D
361 ........................................K.............   An-2-like
361 ..................................................... .   Wa-1
361 ........................................K.............   Blh-2
361 ........................................K.............   Po-0
361 ............................A...........K.............   Mh-0-like
361 .................R..F.STN*                               Ei-5
361 .................R..F.STN*                               Pla-4
361 .................R..F.STK*                               Uk-4
361 .................R..F.STN*                               Nw-1
361 .................R..F.STK*                               Rsch-4
361 .................RH*                                    Bu-6-like
361 .........................CLNRSLKSTSAGVQE*                  C24-like
361 .........................CLNRSLKSTSAGVQE*                  Chi-0
361 .........................CLNRSLKSTSAGVQE*                  Ty-0
```

Figure 21: Continued.

Other accessions have Ms-0-like RPW8.1 or RPW8.2 or both and are susceptible to at least one powdery mildew species. Ove-0 and Pla-4 are susceptible to *G. cichoracearum*, Ob-0 to *G. cruciferarum*, Pla-2 to *G. orontii* and Po-0 is resistant to only *G. orontii*. All four accessions have Ms-0-like RPW8.1 but RPW8.2 is different. Ms-0-like RPW8.2 but a different version of RPW8.1 is found in accessions Ob-0 and Bu-3, both susceptible to *G. cruciferarum*. For three accessions, Is-0, Ita-0 and Uk-3, both RPW8.1 and RPW8.2 are Ms-0 like and all of them are resistant to only *G. orontii*.

In a comparative analysis of amino acid variation of sequences of the *RPW8* locus between Brassicacean species, Xiao et al. (2004) observed an elevated level of variation at the C-terminus of the proteins. As the alignments of RPW8.1 and

RPW8.2 sequences showed, this pattern of variability cannot be observed among *Arabidopsis* ecotypes.

Taken together, the sequences of RPW8.1 and RPW8.2 show variability on both nucleotide and amino acid level with slightly more amino acid exchanges in RPW8.2. The sequences can be grouped according to identical amino acid sequences, resulting in 13 groups for RPW8.1 and 26 groups for RPW8.2. A correlation of amino acid sequence with infection phenotype is possible for RPW8.1, but less obvious for RPW8.2. Thus, no strong correlation of *RPW8* sequence with infection phenotypes could be observed.

3.8.3.3 Correlation of presence or absence of *RPW8.1* and *RPW8.2* with infection phenotypes of RI lines

Another way of testing *RPW8* as a candidate gene is to analyze, whether presence of the *RPW8* locus strictly correlates with resistance in a mapping population. For mapping of resistance in the Sha accession, a population of RI lines derived from a cross between Bay-0 and Sha was employed. While Sha was tested positive for the presence of *RPW8.1* and *RPW8.2* by PCR, in Bay-0 none of them could be detected.

This observation allows testing for a correlation of the presence of *RPW8.1* and *RPW8.2* with infection phenotypes obtained with *G. orontii*. For these aims, a subset of 124 RI lines with known infection phenotypes (DR 0, 2 or 3) were analyzed by PCR with markers specific for *RPW8.1* or *RPW8.2*. The PCR was conducted twice to exclude false positive and false negative PCR results. Only samples for which no product was obtained in both PCR reactions were regarded as not having *RPW8*.

Consequently, the majority of RI lines (85 %) support the hypothesis that *RPW8* is the gene responsible for resistance (see Figure 22). Most of the conflicts were observed for plants with DR 0 (73 % association). The majority of these plants were genotyped Bay-0 like with markers close to *RPW8*. For plants with DR 2 and 3 the association between susceptibility and *RPW8* absence was high (92 %). In summary, the presence of *RPW8.1* and *RPW8.2* in the RIL population was correlated to the respective infection phenotypes obtained with *G. orontii*, emphasizing the hypothesis, that *RPW8* is responsible for resistance in Sha.

3.8.3.4 Depletion of *RPW8* transcript accumulation via dsRNAi

Sequence analysis of the *RPW8* candidate gene at the genome or transcript level provides correlative but no functional data about a potential role of this locus in disease resistance. Depletion of *RPW8* transcript accumulation by dsRNAi should result in susceptibility in those accessions, in which *RPW8* was responsible for resistance. In total, 45 ecotypes were transformed with the 35S::*RPW8.1*-dsRNAi and 35S::*RPW8.2*-dsRNAi vectors (see Supplementary Data, Table SD 9), including susceptible controls Col-0, Bay-0 and Ler (assumed to express no *RPW8*-mediated resistance; negative control) as well as accessions Ms, Ms-0, Kas-1 and Kas-1 Sh (accessions known or suspected to express *RPW8*-mediated powdery mildew resistance; positive control; Xiao et al. 2000; Wilson et al. 2001).

Line	DR*	Sorb50	Presence of *RPW8.1*	Presence of *RPW8.2*	Sorb52
Bay	2	B	-	-	B
Sha	0	S	+	+	S
13	0	S	+	+	S
15	0	S	+	+	S
29	0	S	+	+	S
30	0	S	+	+	S
33	0	S	+	+	S
34	0	S	+	+	S
48	0	S	+	+	S
49	0	S	+	+	S
55	0	S	+	+	S
59	0	S	+	+	S
65	0	S	+	+	S
67	0	S	+	+	S
70	0	S	+	+	S
77	0	S	+	+	S
83	0	S	+	+	S
102	0	S	-	+	S
107	0	S	+	+	S
110	0	S	+	+	S
112	0	S	+	+	S
116	0	S	+	+	S
122	0	S	+	+	S
123	0	S	+	+	S
129	0	S	+	+	S
137	0	S	+	+	S
143	0	S	+	+	S
150	0	S	+	+	S
157	0	S	+	+	S
170	0	S	+	+	S
171	0	S	+	+	S
173	0	S	+	+	S
179	0	S	+	+	S
180	0	S	-	-	S
183	0	S	+	+	S
186	0	S	+	+	S
191	0	S	+	+	S
194	0	S	+	-	S
195	0	S	+	+	S
197	0	S	+	+	S
200	0	S	+	+	S
204	0	S	+	+	S
220	0	S	+	+	S
220	0	S	+	+	S
232	0	S	+	+	S
236	0	S	+	+	S
271	0	S	+	+	S

Figure 22: Presence of *RPW8.1* and *RPW8.2* in RIL population Bay-0 x Sha (subset). Plants are sorted according to their infection phenotype. *Disease resistance score: 3 is fully, 2 intermediate susceptible, 1 is intermediate and 0 fully resistant (macroscopically).

Line	DR*	Sorb50	Presence of *RPW8.1*	Presence of *RPW8.2*	Sorb52
18	0	B	-	-	B
37	0	B	-	-	B
62	0	B	-	-	B
75	0	B	+	+	S
80	0	B	+	+	S
106	0	B	-	-	B
118	0	B	-	-	B
125	0	B	-	-	B
130	0	B	+	+	B
133	0	B	-	-	B
134	0	B	-	-	B
140	0	B	-	-	B
175	0	B	-	-	B
176	0	B	-	+	B
182	0	B	-	-	B
214	0	B	-	-	B
226	0	B	-	-	B
252	0	B	+	-	B
320	0	B	-	-	B
2	2	B	-	-	B
3	2	B	-	-	B
7	2	B	-	-	B
16	2	B	-	-	B
25	2	B	-	-	B
42	2	B	-	-	B
58	2	B	-	-	B
61	2	B	-	-	B
81	2	B	-	-	B
90	2	B	-	-	B
92	2	B	-	-	B
93	2	B	-	-	B
94	2	B	-	-	B
100	2	B	-	-	B
114	2	B	-	-	B
147	2	B	-	-	B
152	2	B	-	-	B
253	2	B	-	-	B
262	3	B	-	-	B
17	3	B	-	-	B
19	3	B	+	+	B
22	3	B	-	-	B
24	3	B	-	-	B
32	3	B	-	-	B
35	3	B	-	-	B
44	3	B	-	-	B

Figure 22: Continued.

Line	DR*	Sorb50	Presence of *RPW8.1*	Presence of *RPW8.2*	Sorb52
45	3	B	-	-	B
47	3	B	-	-	B
53	3	B	-	-	B
71	3	B	-	-	B
73	3	B	-	-	B
78	3	B	-	-	B
84	3	B	-	-	B
97	3	B	-	-	B
98	3	B	-	-	B
98	3	B	-	-	B
99	3	B	-	+	B
104	3	B	-	-	B
111	3	B	-	-	B
120	3	B	-	-	B
121	3	B	-	-	B
126	3	B	-	-	B
127	3	B	-	-	B
131	3	B	-	-	B
136	3	B	-	-	B
162	3	B	-	-	B
190	3	B	-	-	B
198	3	B	-	-	B
210	3	B	-	-	B
234	3	B	-	-	B
240	3	B	-	-	B
248	3	B	-	-	B
273	3	B	-	-	B
279	3	B	-	-	B
289	3	B	-	-	B
342	3	B	-	-	B
202	3	BS	-	-	BS
187	2	S	+	+	S
135	3	S	+	+	S
165	3	S	+	+	S

Figure 22: Continued.

3.8.3.4.1 Infection phenotypes of selected T_1 plants

Between 2 and 49 Basta-resistant T_1 plants were recovered per accession for 35S::*RPW8.1*-dsRNAi and only between 1 and 13 plants for 35S::*RPW8.2* dsRNAi (see Supplementary Data, Table SD 9). Transformation with 35S::*RPW8.2* dsRNAi was therefore drastically less effective. Due to time constrains, it was not possible to test whether this low transformation rate was due to differences in the genes or rather due to the selected *Agrobacterium* clone.

In total, 780 T_1 plants were recovered after Basta-selection, 733 from 45 different accessions including controls transformed with 35S::*RPW8.1*-dsRNAi and 47 from 14 different accessions including controls with 35S::*RPW8.2*-dsRNAi T_1 plants were inoculated with *G. orontii* and macroscopically screened for enhanced susceptibility. The infection phenotypes of the susceptible controls Bay-0 and Col-0 were unaltered in all Basta-selected T_1 plants. The majority of T_1 plants of the resistant accessions remained resistant: For 34 out of 40 resistant accessions (including resistant controls), no susceptible T_1 plants were identified with 35S::*RPW8.1*-dsRNAi. Likewise, 8 out of 13 different resistant accessions (including resistant controls) had no susceptible T_1 plant for 35S::*RPW8.2*-dsRNAi. Notably, T_1 plants transformed with 35S::*RPW8.1*-dsRNAi originating from eight accessions (Bu-0, Bu-17, Do-0, Fr-5, Nok-3, Ob-0, Ove-0 and Sha), were scored susceptible in the T_1 generation (for examples in T_2 generation see Figure 24). Additionally, T_1 plants derived from six different accessions (C24, Co-3, Ei-4, Ei-5, Kas-1 Sh and Sha) transformed with 35S::*RPW8.2*-dsRNAi showed enhanced susceptibility compared to the respective non-transformed wildtype.

3.8.3.4.2 Analysis of *RPW8* transcript levels by semi-quantitative RT-PCR

A subset of T_1 plants was selected for analysis by semi-quantitative RT-PCR to determine whether the dsRNAi construct was successful in reducing *RPW8* transcript levels. For Col-0, Ms, Ms-0, Ang-0, C24, Co-1, Do-0, La-1, Nok-3, Sha and Sorbo, all recovered T_1 plants were analyzed, while for other accessions only susceptible plants were selected for analysis. Transcript levels of *RPW8.1* were determined for total 210 T_1 plants originating from 17 different accessions (see Supplementary Data, Supplementary Data, Table SD 10). For 15 accessions (including resistant controls), T_1 plants were identified, in which *RPW8.1* mRNA levels were depleted, four of which were originally transformed with 35S::*RPW8.2*-dsRNAi. 27 (33 %) T_1 plants with reduced *RPW8.1* transcript levels were scored susceptible (DR 2 or DR 3). A selected subset of susceptible transformed T_1 plants is shown in Figure 22. Except for Co-3 T_1.1A transformed with the *RPW8.2*-dsRNAi construct, all other plants shown have reduced levels of either *RPW8.1* or *RPW8.2* or both transcript levels. Co-3 T_1.1A was not analyzed with Real-Time PCR (see below, 3.8.3.4.3), therefore very marginal reductions in transcript levels could be enough to induce susceptibility in this accession. Only in two susceptible Sha T_1 plants transformed with the R*PW8.1*-dsRNAi construct, transcript levels of *RPW8.1* and not *RPW8.2* are reduced, all other plants show reduction of transcript levels for both genes.

In plants which were recovered after Basta-selection, but did not show lower transcript levels, the insert could have been integrated in a truncated version or into regions of low transcription activity. Interestingly, for plants of which samples of two independent RNA-preparations were analyzed, some plants gave different results when comparing the two RNA-preparations. This could reflect the fact that sample collection and preparation may have occurred at different time points.

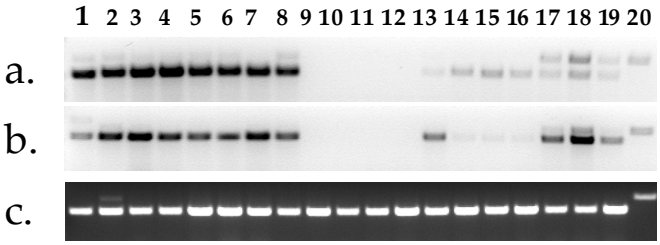

Figure 23: Transcript levels of *RPW8*-dsRNAi T_1 plants. 1-7 controls; 1: Co-3, 2: Do-0, 3: Ei-4, 4: Ei-5, 5: Kas-1 Sh, 6: Nok-3, 7: Sha. 8-19: dsRNAi-T_1 plants; 8: Co-3 T_1.1 A^2, 9: Do-0 T_1.2D^1, 10: Ei-4 T_1.1J^2, 11: Ei-5 T_1.1i^2, 12: Kas-1 Sh T_1.1M^2, 13: Nok-3 T_1.1D^1, 14: Sha T_1.1A^2, 15: Sha T_1.2A^2, 16: Sha T_1.3A^2, 17: Sha T_1.1C^1, 18: Sha T_1.3C^1, 19: Sha T_1.16C^1; 20: genomic control (Do-0). Plants were transformed with dsRNAi constructs for *RPW8.1* (^{1}) or *RPW8.2* (^{2}).

3.8.3.4.3 Analysis of transcript levels of by Real Time PCR

The high percentage of plants in which *RPW8.1* transcript accumulation was depleted but which were still resistant could be either due to an only minor decrease of transcript amount or to a compensating *RPW8.2* gene, whose expression might not be impaired by the vector specific for *RPW8.1*. Therefore, Real-Time PCR was employed to detect also minor differences in transcript levels. As a control some plants were included in the Real-Time analysis, which did not show a transcript reduction in the semi-quantitative RT-PCR. 65 plants of 10 different accessions were analyzed with Real-Time PCR, 51 of them as well in a second run (see Supplementary Data, Supplementary Data, Table SD 11). For the majority of plants showing reduced *RPW8.1* mRNA levels in semi-quantitative RT-PCR, the results could be confirmed in the Real Time PCR. Only some plants did not exhibit a similar reduction of *RPW8.1* transcript levels, probably due to the higher sensitivity of the Real Time method compared to the

RT-PCR. Some plants had different results in two Real-Time PCR runs, although the same cDNA preparation was used.

In addition, four susceptible T_1 plants were analyzed for reduced *RPW8.1* transcript, which had been transformed with the *RPW8.2* dsRNAi vector. In Ei-4 T_1.1 J and Ei-5 T_1.1. i *RPW8.1* transcript accumulation was reduced, in Sha T_1.2 A and Sha T_1.3 A not, although semiquantitative RT-PCR indicated that also *RPW8.1* transcript levels were reduced in these plants (see Figure 23). Real-Time-PCR analysis with primers specific for *RPW8.2* was not yet performed for these plants, but semiquantitative RT-PCR indicates a reduction of *RPW8.2* transcript levels (see Figure 23). Nevertheless, depletion of mRNA accumulation of *RPW8.1* or *RPW8.2* in Sha seems to be sufficient to induce susceptibility. Interestingly, the accessions identified in this experimental approach did not show similar segregation patterns in the F_2 generation: while resistance in Sha was segregating 1 : 2 : 1, the accepted ratio in Do-0 and Nok-3 was 3 : 1. In Bu-0 and Ob-0, none of these segregation patterns were significant and the F_2 progeny of Ei-4 and Ei-5 as well as Kas-1 Sh were not analyzed.

3.8.3.4.4 Confirmation of infection phenotypes in the T_2 progeny

In the previous analysis, infection phenotypes were determined for T_1 plants after Basta selection. To avoid false positive or negative results due to the stressful Basta treatment and uncontrolled growth conditions, progeny of T_1 plants, which showed a reduced accumulation of *RPW8.1* transcript in the RT-PCR and/or which were scored susceptible to *G. orontii*, were analyzed in the T_2 generation. In total 38 T_2 progeny were inoculated with *G. orontii* (see Supplementary Data, Table 12). Of 23 T_1 plants with reduced *RPW8.1* transcript levels, which were scored resistant in the T_1 generation, the majority of

corresponding T_2 families was resistant. However, for four T_2 families, some plants with at least intermediate susceptible plants were observed.

In five of eight accessions with susceptible T_1 plants susceptibility was confirmed in the T_2 progeny: In six T_2 families susceptibility segregated (Co-3 T_1.1 A, Nok-3 T_1.1 D, Sha T_1.16 C, Sha T_1.1 A, Sha T_1.2 A and Sha T_1.3 A) and in three T_2 families all plants were susceptible (Ei-4 T_1.1 J, and Kas-1 Sh T_1.1 M), suggesting the existence of more than one transgene copy in these plants (see Figure 24 and Supplementary Data, Table SD 12). The susceptible transgenic Kas-1 Sh line indicates that depletion of *RPW8* transcript accumulation by the *RPW8.2* dsRNAi construct is successful in an accession, in which *RPW8* is mainly responsible for resistance. For some other accessions like C24, Bu-17, Fr-5 and Ove-0, at least one susceptible T_1 plant was analyzed in the T_2 progeny, but did not confirm the previously observed susceptible infection phenotype. Progeny of other susceptible T_1 plants (Bu-0, Do-0 and Ei-5) were not yet analyzed in the T_2 progeny.

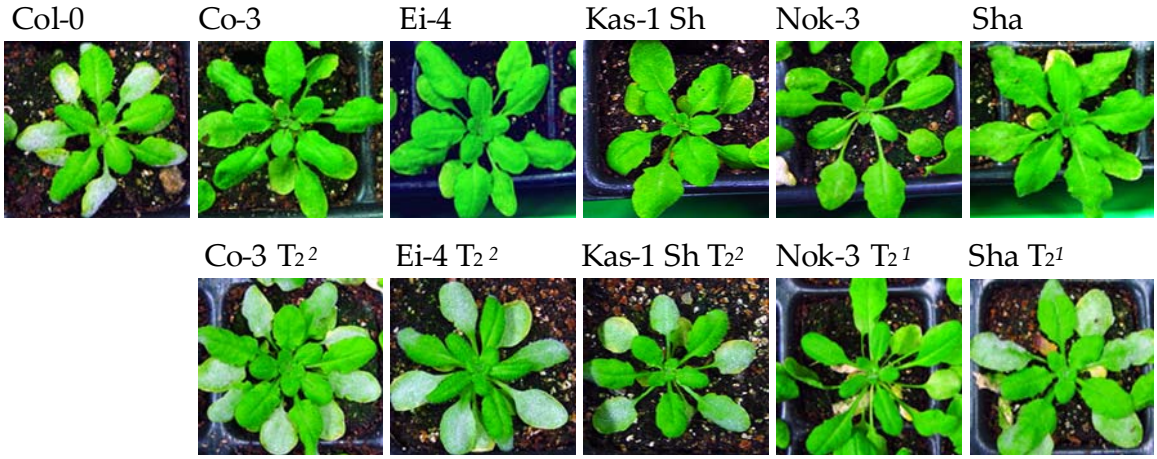

Figure 24: A subset of T_2 plants of selected resistant accessions transformed with *RPW8*-dsRNAi constructs. Plants are shown at eight days post inoculation with *G. orontii*. T_2 plant originates from Co-3: Co-3 T_1.1A^2 , Ei-4: Ei-4 T_1.1J^2 , Kas-1 Sh: Kas-1 Sh T_1.1M^2 ; Nok-3: Nok-3 T_1.1C^1 and Sha: Sha T_1.2A^2 . ^{1} and ^{2} indicate plants transformed with *RPW8.1*– and *RPW8.2*-dsRNAi construct, respectively.

To exclude possible contaminations and therefore false positive T_1 and T_2 plants, the T_2 progeny of susceptible T_1 plants should by analyzed by PCR and genomic markers to confirm that they derive from the respective resistant accession and are not a contamination with a susceptible ecotype. Nevertheless, since ecotypes Sha and Col-0 differ not only in infection phenotypes but also in overall morphology from each other, the susceptible Sha T_1 and T_2 plants are likely to be derived from Sha, since the plants look very similar to Sha wildtype plants.

These results suggest that determination of infection phenotypes in the T_1 generation provides a first impression of the resistance status. However, this early determination of the infection phenotype could lead to false positive results, probably due to uncontrolled growth conditions in combination with Basta-selection. Therefore, a confirmation of the infection phenotypes in the T_2 progeny of the respective plants was necessary. The analysis of T_2 plants indicated that the gene silencing effect is stable in the following generation. Analysis of T_3 progeny of susceptible T_2 plants will clarify whether stability is enduring several generations. In summary, dsRNAi-mediated depletion of *RPW8* transcript accumulation induces susceptibility to *G. orontii* in the previously resistant accessions Bu-0, Co-3, Do-0, Ei-4, Ei-5 Kas-1 Sh, Nok-3, Ob-0 and Sha (see Table 15), supporting the hypothesis that *RPW8* is responsible for resistance in these accessions.

Table 15: Summary of dsRNAi-mediated depletion of *RPW8* transcript accumulation

Accession with susceptible T_1 plant	construct # for	susceptible T_1 plants	T_2 susceptible	lowered transcript levels		
				RPW8.1		*RPW8.2*
				RT-PCR	Real-Time-PCR	RT-PCR
Bu-0	*RPW8.1*	*3*	n.d.	n.d.	n.d.	n.d.
Co-3	*RPW8.2*	*1*	+	-	n.d.	-
Do-0	*RPW8.1*	*1*	n.d.	+	+	+
Ei-4	*RPW8.2*	*1*	+	+	+	+
Ei-5	*RPW8.2*	*1*	n.d.	+	+	+
Kas-1 Sh	*RPW8.2*	*1*	+	+	+	+
Nok-3	*RPW8.1*	*2*	1 of 2	+	+	-
Ob-0	*RPW8.1*	*2*	n.d.	n.d.	n.d.	n.d.
Sha	*RPW8.1*	*7*	4 of 6	7 of 9	3 of 5	1 of 3
	RPW8.2	*3*	2 of 2	1 of 2	-	+

n.d. not determined

4. DISCUSSION

Using an approach based on natural genetic variation, resistance to powdery mildew was analyzed in *A. thaliana* with an emphasis on the identification of new compatibility factors required for this interaction. In six accessions a target gene locus on the lower arm of chromosome III was identified. These plants differed in the strength of resistance and defense responses. Several candidate genes were analyzed. The previously identified unusual *R*-gene *RPW8* was shown by dsRNAi-mediated gene silencing to be responsible for resistance in several accessions. In F_2 mapping populations and in the progeny of crossings between accessions and between accessions and a mutant of the candidate *PMR6*, several contradictory plants were identified.

4.1 SEGREGATION OF RESISTANCE

The majority of F_1 populations of crosses between resistant accessions and the susceptible Col-0 ecotype that were analyzed were found to show intermediate resistance. Only the F_1 progeny of two accessions were scored as fully susceptible (Nok-3 and Wt-2; Table 2). Since inoculation density with the pathogen was not controlled, it cannot be excluded that a high amount of inoculum could be responsible for the observed infection phenotypes of F_1 plants in these two accessions. These results indicate that likely no truly recessively inherited compatibility factor has been identified in this study. There are several potential scenarios for the inability to identify true compatibility factors in this study. Firstly, a loss of function in one of these factors may be disadvantagous for the plant under non-challenged conditions, and so the possibility that this genotype would be fixed is very low. Secondly, the pathogen does not exclusively rely on single plant factors for successful growth

and reproduction, but contributes to a compatible interaction using its own components, resulting in only weak resistance phenotypes when one of these plant-factors is non-functional. Furthermore, an absence of selection pressure on natural variation of the powdery mildew resistance mediating loci, which has been suggested for this interaction based also results of this study, could contribute to the situation. Additionally, the absence of a compatibility factor or of mutations in essential and invariant amino acids could result in lethality of the plant.

Segregation patterns for the resistance phenotpyes were determined for 16 accessions that had been crossed to the Col-0 ecotype. For 12 of them, a segregation pattern of either 3 : 1 (susceptible : resistant) or 1 : 2 : 1 (susceptible : intermediate : resistant) was observed (Table 3). In four accessions these segregation patterns deviated from a 3 : 1 or 1 : 2 : 2 ratio, indicating that resistance was most likely not caused by a single gene, but is rather a consequence of several loci acting together (polygenic resistance). In four accessions, it was not possible to determine the chromosomal location of the resistance-mediating genes, despite their segregation ratios following a 3 : 1 or 1 : 2 : 1 distribution. In these accessions, resistance may also be inherited in a more complex manner and be therefore polygenic as the contribution of many different genes to the trait could potentially result in a false positive 3 : 1 or 1 : 2 : 1 segregation, especially when the scoring of infection phenotypes is performed in a qualitative rather than quantitative manner. As summarized in Table 4, inheritance of resistance in most accessions could also be explained by different scenarios that involved two or three different loci.

Recombinant inbred lines represent a useful tool to determine the potential contribution of multiple genes. In the RIL population Bay-0 x Sha used in this study, the QTL analysis suggested a monogenic mode of inheritance of resistance in the resistant accession (Sha). Since this was the only RIL population

available, mapping of loci contributing to suspected polygenic resistance was not possible in any of the other selected accessions.

In summary, for eight accessions resistance is proposed to be monogenic and for the remaining eight, polygenic. In an earlier study it was suggested that polygenic resistance to powdery mildews appears to be over-represented in *Arabidopsis* compared to other described plant-pathogen interactions (Schulze-Lefert and Vogel, 2000). Results from the analysis presented here underline that polygenic resistance may contribute significantly to powdery mildew resistance in *Arabidopsis*. Nevertheless, *RPW8* and other so far unidentified loci are also thought to provide resistance to many accessions (see below).

4.2 THE ROLE OF PROTOTYPICAL *R*-GENES IN POWDERY MILDEW RESISTANCE OF *ARABIDOPSIS*

During the analysis of accessions with specific resistance to *G. orontii* but susceptibility to two other powdery mildew species, only two candidates for prototypical dominantly inherited *R*-gene mediated resistance were identified. The majority of the pre-selected accessions analyzed showed intermediate susceptible F_1 progeny after crossing with the susceptible Col-0 ecotype, suggesting that a semi-dominantly inherited locus is likely mediating resistance in these accessions. In earlier studies of interactions of *Arabidopsis* accessions with powdery mildews, no dominantly inherited powdery mildew resistance gene except *RPW8* was identified either (Adam and Somerville 1996; Xiao et al. 2001).

This situation clearly differs from the frequency of *R*-gene mediated resistance to other pathogens in *Arabidopsis*, where several genes each with multiple alleles mediate resistance to *Pseudomonas syringae* or *Peronospora parasitica*. For *Rps2*-mediated resistance to *P. syringae* strain DC3000 carrying the avirulence gene

avrRpt2, 17 out of 21 *A. thaliana* accessions representing the major geographic regions in the species distribution had a functional allele of the *Rps2* gene (Mauricio et al. 2003). Twenty-six *A. thaliana* accessions originating from the whole range of its distribution were analyzed for *Rps1*, an *R*-gene conferring resistance to *P. syringae* carrying *avrRpm1* or *avrB* (Grant et al. 1995). This *R*-gene was found with a frequency of 0,52 (Stahl et al. 1999). Consequently, *R*-genes providing resistance to bacterial pathogen *P. syringae* or the oomycete *P. parasitica* occur with much higher frequency than putative *R*-genes in the *Arabidopsis* – *G. orontii* interaction.

Resistance to powdery mildew in *Arabidopsis* seems to be either polygenic and/or mediated by *RPW8*, but not by typical *R*-genes with a NBS-LRR structure. This observation could reflect that *Arabidopsis* is not the primary host for powdery mildews (Schulze-Lefert and Vogel 2000), implying that there might have been not enough time or opportunity, possibly also due to other predominant pathogens, for establishing resistance which is based either on lack or non-function of compatibility factors or even relies on co-evolution and *R*-gene-based gene-for-gene-interactions. However, *RPW8* has possibly adopted the role of *R*-genes in this interaction. Thus, the situation is not indicative of an arms race or co-evolution between host and pathogen. Instead, the *Arabidopsis*-powdery mildew-pathosystem might represent a "naïve" plant-microbe interaction particularly suitable to study basal defense. Since only a subset of resistant accessions has been analyzed so far, the determination of loci responsible for resistance in the remaining accessions might still reveal additional resistance mechanisms.

4.3 THE CANDIDATE GENE *PMR6*

The first candidate gene, *PMR6*, conferring recessively inherited resistance to several powdery mildew species when non-functional, was located in the target

gene region on the lower arm of chromosome III. However, sequence and transcription analysis revealed no obvious defect in *PMR6* in accessions Sha and Sorbo. Additionally, a marker designed in the second intron of *PMR6* genomic region (Sorb40, see Figure 13) did not show convincing co-segregation with resistance in Sorbo x Col-0 F_2 progeny. Complementation analysis was successful for the *pmr6* mutant in a Col-0 genetic background, but failed to restore susceptibility in Do-0, Sha and Sorbo. These results provide further evidence that resistance in these accessions is not due to a defective *PMR6*.

Nevertheless, for the two other accessions in which no susceptible T_1 plant was recovered in the *RPW8* dsRNAi analysis and in which resistance mapped to the lower arm of chromosome III (e.g. C24/Co-1 and La-1), it cannot be principally excluded that resistance is due to loss of PMR6 function. This scenario, however, seems quite unlikely based on the comparative microscopic analysis of resistance, in which cell death, callose deposition phenotypes, hydrogen peroxide accumulation and host cell entry rates in Co-1 and La-1 differed significantly from *pmr6* (Table 5).

4.4 WHY WERE NONE OF THE ALREADY KNOWN INDUCED SUSCEPTIBILITY MUTANTS LIKE THE *PMR* MUTANTS OR *ATMLO2* RECOVERED IN THIS APPROACH?

During this survey of natural powdery mildew resistance in *A. thaliana* no evidence for the contribution of one of the compatibility factors identified so far in mutagenesis screens has been found. Most of the *pmr* mutants have pleiotropic effects expressed as altered phenotypes even in non-infected states, except *pmr1* (Vogel 2000, 2002, 2004). This could imply that a naturally occurring mutation in one of these genes would result in fitness costs, which may be too deleterious to lead to fixation of this allele in a population, which therefore will

prevent the spreading of this mutation. In addition, natural alleles of the *Atmlo2* mutant were not recovered, although this mutant shows similar pleiotropic effects to the *mlo* mutant in barley (Consonni et al. 2006), for which the *mlo-11* allele was isolated from natural barley accessions (Jørgensen et al. 1992; Piffanelli et al. 2004). However, since the *mlo-11* polymorphism arose quite recently (Piffanelli et al. 2004), it is not clear, if this allele is advantageous under natural conditions.

At this point it is of note to add that during microscopic analysis of accessions resistant to *G. orontii* several accessions were identified in which resistance is occurring at the penetration stage (Do-0, La-1 and Ms-0), as it has been shown for *Atmlo2* (Consonni et al 2006). Nevertheless, the determined target gene region in these accessions is not close to *AtMLO2*, which is located on chromosome I. Since the penetration phenotype of the *Atmlo2* single mutant allows a small level of colony formation and conidiophore production of *G. orontii* (Consonni et al 2006). Given this, natural mutant alleles of *AtMLO2* could be recovered from further accessions resistant to three powdery mildew species, which have already been microscopically analyzed but in which the resistance loci have not been mapped yet.

4.5 COMPARATIVE ANALYSIS OF RESISTANCE BETWEEN ACCESSIONS

For a comparative analysis of resistance between the accessions tested, the responses of both the pathogen and the plant were analyzed at different time points during fungal development. Host cell entry rates were quantified and the course of hyphal expansion was analyzed up to 63 hpi. Later stages of fungal development were assessed by determining the amount of conidiophores per colony. On the plant side, accumulation of hydrogen peroxide at early time points after inoculation, as well as cell death and callose deposition late in fungal development were compared between different accessions.

4.5.1 Microscopic analysis of fungal development

Microscopic analysis suggested that in all accessions resistance resulted in a reduced number of conidiophores per colony, although to different degrees. In all accessions, resistance was also associated with the accumulation of hydrogen peroxide in infected epidermal cells at 48 hpi, however in only few accessions this resulted in resistance at early time points of fungal development, such as via the reduction of successful host entry. In Co-1, Do-0, La-1 and Ms-0 fungal development was affected already at 48 hpi, but not in Ang-0, Nok-3, Sha and Sorbo. Hydrogen peroxide accumulation as a reaction of the plant might not be sufficient for inhibiting fungal growth. The effect on fungal host cell entry was reflected by a reduced level of hyphal growth and number of fungal structures between 48 and 63 hpi in Do-0 and Ms-0 compared to the susceptible Col-0 ecotype. In contrast, the amount of hyphal growth on Sha and Sorbo showed very little observable difference to Col-0. Therefore it seems that hydrogen peroxide accumulation as a response by the plant might not be sufficient for inhibiting fungal growth.

4.5.2 Cell death in response to powdery mildew attack

The analysis cell death and callose deposition at later time points of fungal development, when the asexual reproduction of the fungus takes place, indicated that the level of cell death is in general high in the tested resistant accessions compared to the susceptible Col-0 ecotype. In Nok-3 and Sha, however, the intensity of cell death was not as intense as in the other resistant accessions Ang-0, Co-1, Do-0, La-1, Ms-0 and Sorbo (Figure 8, Table SD8). Interestingly, cell death occurred mainly in the mesophyll cells below fungal colonies, but not in attacked epidermal cells, which are the only cells that are in direct contact with the powdery mildew pathogen.

This suggests that a signal from the attacked epidermal cell is transmitted to underlying mesophyll cells with which it is in physical contact. Fast cell death is in general associated with *R*-gene mediated resistance (reviewed in Shirasu and Schulze-Lefert 2000). With regard to the pathogen lifestyle, the fast cell death of the hypersensitive reaction (HR) might deplete biotrophic pathogens from nutrient supply and may stop growth of necrotrophic pathogens by releasing toxic host molecules. Nevertheless, HR is generally associated with epidermal cell death only, but not with mesophyll cell death as observed in this study. An exception seems to be the resistance conferred by the *Mla12 R*-gene of barley. Here, host cell death begins in the attached epidermal cell and then spreads into the adjacent mesophyll layer (Hückelhoven et al. 2000).

A similar mesophyll reaction to powdery mildew attack has been observed for *mlo* mutants in barley and the *edr1* mutant in *Arabidopsis*, in which after pathogen attack not the attacked epidermal cells but mainly clusters of mesophyll cells below the site of attack undergo a cell death reaction at later time points (Piffanelli et al. 2002; Frye and Innes 1998). It was suggested that absence of *Mlo* could lower the threshold for cell death in mesophyll cells, leading to an enhanced sensitivity to triggers of cell death or senescence (reviewed in Shirasu and Schulze-Lefert 2000). However, the *mlo* mutant also shows developmentally controlled spontaneous cell death in non-inoculated plants, which was not observed in the selected accessions.

In the *edr1* mutant of *Arabidopsis*, mesophyll cells were also suggested to be hair-triggered or sensitized towards cell death, which would indicate a function of *EDR1* in controlling homeostasis in mesophyll cells (reviewed in Shirasu and Schulze-Lefert 2000). This late cell death in *edr1* could induce a reduced nutrient supply of the adjacent epidermal cells and could thereby affect later stages of fungal development. This correlates well with the observation that resistance in *edr1* is associated with a reduced amount of conidiophores and conidia, similar

to the accessions analyzed in this study. The infection phenotype of *edr1* plants is similar to that conferred by the *Pm2* and *pm5* genes of wheat and the *Mla3* and *Mla7* genes of barley. Here, powdery mildew growth is affected after the development of secondary hyphae but before conidiophore production and the infection phenotypes are associated with the accumulation of large masses of dead mesophyll cells (Hyde and Colhoun 1975; Boyd et al. 1995). Interestingly, also *edr1* is resistant to at least two powdery mildew species, *G. cichoracearum* and *G. cruciferarum* (Frye and Innes 1998). Nevertheless, it can be ruled out as a candidate gene for resistance in the accessions selected here due to the location of *EDR1* on chromosome I.

Even though resistance in the selected accessions seems to correlate to mesophyll cell death, the intensity of cell death observed in this study was not exactly correlated to the amount of conidiophores produced per colony in the different accessions: Although Sorbo showed a higher percentage of strong cell death indicated by Trypan Blue staining compared to Sha, it produced on average more conidiophores per colony. Intriguingly, the intermediate susceptible accession Bay-0 also showed a level of cell death associated with fungal colonies comparable to the highly resistant accessions Sorbo and La-1. This could indicate that cell death might be involved in inhibiting fungal growth and reproduction at later stages of the infection cycle, but other defense responses of the plant might still be necessary for establishing full macroscopic resistance.

4.5.3 Callose deposition

The deposition of callose in infected cells was observed in all tested lines, susceptible or resistant, at penetration sites and around haustoria. Accumulation of this β-1,4-glucan possibly contributes to basal resistance (Gomez-Gomez and Boller 2000). The differences in callose deposition observed between the

accessions were not correlated with the production of conidiophores per colony, but did coincide roughly with the observed intensities of cell death visualized by Trypan Blue staining.

In summary, the comparative analysis of resistance in the selected accessions does not provide a strong indication whether resistance is due to the same genes and mechanisms in all tested accessions. The influence of the different genetic backgrounds of the analyzed accessions could lead to quantitative and/or qualitative differences in the reaction of the plant to the pathogen, even if resistance was mediated by the same locus, e.g. due to differences in transcript levels or timing of expression. For *RPW8* this was shown for accessions Ms-0 and Wa-1. In Ms-0, *RPW8*-mediated resistance is characterized by cell death while in Wa-1, no cell death was observed, although it was hypothesized that the same locus is responsible for resistance in these two accessions (Xiao et al. 1997; Xiao et al. 2001; Schiff et al. 2001).

Nevertheless, the introgression of *NahG* into Sorbo plants indicated that resistance might be SA-dependent in this accession, since in these plants resistance was impaired and susceptibility restored. However, it cannot be excluded that the latter effect is caused by the degradation product of salicylic acid, catechol, and not by the lack of SA itself (Van Wees 2003). Therefore, Sorbo was crossed to several mutants defective in SA and JA/ethylene signaling pathways. Forthcoming analysis will unravel whether resistance in Sorbo is truly SA-dependent. At least for the candidate gene *RPW8*, a dependency on SA-signaling has been described (Xiao et al. 2003), while resistance in pmr6 mutants was shown to be independent of SA signaling (Vogel et al. 2002). Since allelism analysis did not unequivocally support the hypothesis that resistance is mediated by the same locus in all accessions mapped to chromosome III, the results for Sorbo cannot be extrapolated to the other ecotypes.

4.6 CONTRADICTORY RESULTS IN ALLELISM TESTS AND MAPPING POPULATIONS

A surprising observation was made during the allelism tests as well as in the mapping populations: In crossings of Sorbo and Do-0 with *pmr6-3* as well as Sha with Sorbo, the observed resistance in the F_1 progeny and segregation of resistance in the F_2 generations did not match any expected simple Mendelian segregation pattern. A resistant F_1 generation between accessions would imply that the F_2 progeny should be also resistant, and suggests that resistance is due to the same locus in both lines. In contrast to the expected pattern for either two alleles of the same gene or two closely linked genes in the same region, 52 % of all F_2 plants were susceptible in the Sorbo x *pmr6-3* crossing and 46 % in the Do-0 x *pmr6-3* crossing as well as 19 % in the crossing between Sha and Sorbo and 14 % between Do-0 and Sha (see 3.7).

The F_2 generations derived from crossings of Sorbo x Col-0 and Sorbo x *pmr6-3* as well as Do-0 x Col-0 and Do-0 x *pmr6-3* differ only in the defective *pmr6* gene, since the *pmr6-3* allele was introduced in Col-0 genetic background. Consequently, the high amount of susceptible plants in the Sorbo x *pmr6* and Do-0 x *pmr6* F_2 progeny could be due to an effect of the defect *pmr6* allele on resistance in Sorbo mediated by *RPW8* or another gene from that genomic region. This effect would render the F_1 population resistant and result in more susceptible F_2 plants than expected. Effects of heterosis on resistance in F_1 plants can be excluded, since no resistance was observed in F_1 plants of crossings Sorbo and Col-0 or Do-0 and Col-0. The *pmr6-3* T-DNA insertion mutant used in this study was reported to possess only one single insertion (Vogel et al. 2000). Consequently, disruption of other genes by additional T-DNAs influencing the phenotype can be excluded as well. However, the segregation scenarios calculated for different crossings indicate that the segregation pattern in the Do-

0 x *pmr6* F_2 generation is significant for two recessive and two semi-dominant genes. If the resistance to *G. orontii* mediated by *pmr6* was not truly recessive but had a small dosage effect, then the F_1 could be resistant due to the additive effects of *RPW8* and *pmr6*, both present in one copy.

Based on preliminary mapping results, Do-0, Sha and Sorbo were expected to be resistant due to the same locus and therefore no susceptible F_2 plants should arise from crosses between these accessions. A possible explanation for susceptible plants identified in crosses between Do-0 and Sha and between Sha and Sorbo is that different loci are responsible for resistance in each of the accessions. If two different but truly recessive genes are responsible for resistance in Do-0, Sha and Sorbo the F_1 progeny should be susceptible, which was not the case. If both genes are semi-dominant but acting in the same pathway, then the F_1 progeny could be resistant as observed. Nevertheless, in both cases nearly all F_2 plants should be resistant, since in both accessions resistance mapped to the same chromosomal region with the consequence that the corresponding genes are expected to show linkage. Interestingly, the F_2 progeny of crossings between Do-0 and Sorbo did not show any susceptible plant.

The high number of susceptible plants observed in other F_2 populations (Do-0 x Sha, Sha x Sorbo) could be explained by an extraordinarily high recombination frequency in that region mimicking independent inheritance. This could be the case with regard to the *RPW8* locus, where the two parental accessions Col-0 and Sorbo differ dramatically. These differences could lead to imperfect pairing of this region during meiosis, which may result in an enhanced recombination rate. An analysis of 322 Sorbo F_2 plants genotyped with the markers Sorb34 (18,24 Mb) and Sorb39 (20,41 Mb) on chromosome III, revealed a recombination rate of 19 % (Figure 13). This value is twice as high compared to the expected 8,7 % based on the marker distance of 2170 kb and an expected average of 250 kb

per 1 % recombination (Lin et al. 1999). However, this scenario would still not explain why the F_1 progeny of the crossings with *pmr6* were resistant or why in the crossing of Do-0 x Sorbo, no susceptible F_2 plants were observed.

Interestingly, not only did crosses between resistant accessions or resistant accessions and induced mutants led to contradictory results, but a high number of individuals (19 % of all recombinants) were also identified in the mapping population of Sorbo x Col-0 where the observed phenotype did not match the expected, given the genotype in the region containing the predicted resistance locus (Figure 13). In the RIL population, the phenotype of 18 % of all plants also showed aberrant phenotypes given their genotype for the predicted target gene region (Figure 14). Although the analysis of Sorbo F_3 families could remove many of these contradictions, in two plants discrepancies between phenotype and genotype were confirmed (plants II.A9 and II.C12; Table 7) and in three more, new discrepancies were found (plants III.E3, IV.A5 and IV.C12; Table 7). In most cases segregation patterns in F_3 families did not fit the expected ratio, sometimes by lacking fully susceptible or fully resistant plants. However, this may indicate that the F_3 families were too small to identify all possible phenotypes for resistance loci following polygenic inheritance. No non-segregating F_3 progeny from heterozygous F_2 plants was observed, indicating that the issue is likely not due to scoring difficulties or unequal inoculation densities.

When considering the crosses between two resistant accessions, it might be that additional factor(s) are needed for the establishment of *RPW8*-mediated resistance, which differ between Sorbo and Sha and only the "own version" or constellation confers functionality of the respective *RPW8* copy. Assuming that these factors are not located in the *RPW8* region, but segregate independently, the expression of resistance could be affected by those means.

Such an epistatic interaction between different loci is observed in many species, including *A. thaliana* (Juenger et al 2005; Syed and Chen 2004; Hausmann et al. 2005; Kearsey et al. 2003; Kroymann and Mitchell-Olds 2005; Malmberg et al. 2005). The importance of the genetic background for *R*-gene function was previously shown for *RPP13*, where two different accessions carrying the same *RPP13* gene conferred different infection specificities (Rose et al. 2004). This hypothesis is more probable when accessions are of geographically distinct origin; however, Sha and Sorbo both derive from Tajikistan.

To identify putative secondary regulators that may have been masked in the first QTL analysis by epistasis, the RIL plants that showed aberrant phenotypes were analyzed with WinQTLCartographer, but no significant peaks were found other than the original locus. With respect to *RPW8,* some interactors have already been identified: Xiao et al. (2005) has suggested that *EDR1,* located on chromosome I, acts as a negative regulator of *RPW8* transcription. It was proposed that when *RPW8* is not negatively regulated by EDR1, it may engage basal defense responses by employing a feedback amplification circuit consisting of EDS1, PAD4, EDS5 and SA. It is not known how this putative negative regulation of *RPW8* by EDR1 is accomplished.

Another possibility is that the low but still significant peak found on chromosome II in the first RIL analysis is important for resistance. However, neither phenotypes of Sorbo F_2 plants showing contradictory results nor 122 RILs analyzed with a marker located on chromosome II (PLS5) revealed any association with this marker (not shown). Besides the possibility of additional genes modulating resistance in Sha and Sorbo, a simple effect of timing could be important in the expression of the resistance response. If the defense reaction mediated by *RPW8* is activated too late, the pathogen might be able to colonize the plant. These response times might differ between the accessions. Timing of defense gene expression seems to be important also in interactions of tomato

with powdery mildew species, where expression analyses showed that incompatible interactions differ from compatible ones in an earlier activation of gene transcription (Li et al. 2006).

An additional possibility for the observed contradictions in the mapping populations might be the effect of epigenetic mechanisms. Some of these mechanisms, which include dsRNA (double strand RNA)-mediated reduction of transcript levels, DNA and histone methylation, paramutation and heterochromatin formation (reviewed in Grant-Downtown and Dickinson 2005 and 2006) have been recently shown to be involved in defense responses in plants (i.e. Stokes and Richards 2002; Matzke et al. 2002; Waterhouse 2006), especially against viral pathogens, and in animals (reviewed in Fritz et al. 2006). SiRNAs (small interfering RNAs) and miRNAs (micro-RNAs) have been reported to function as sequence-specific guides for the post-transcriptional regulation of genes, transposons and viruses and to modify chromatin and genome structure (Carrington and Ambros 2003; Finnegan and Matzke 2003; Lai 2003).

With regard to pathogen resistance in general or to *RPW8* specifically, a posttranscriptional depletion of transcripts by either miRNAs or siRNAs as well as transcriptional silencing of the gene by chromatin methylation or histone deacetylation could interfere with the expected phenotype of a mapping plant in certain genotypic combinations without altering the sequence of the resistance gene itself. This possibility is especially intriguing for the *RPW8* locus, which is known to contain several related genes in tandem. It is not known for a given F_2 plant, whether additional local tandem structures inside or in between the genes are present or if even a rearrangement or additional gene duplication has occurred at this locus, possibly leading to structures triggering methylation or RNA silencing.

With regard to other loci associated with pathogen resistance, an *R*-gene cluster on the lower arm of chromosome 4 was shown to be subject to epigenetic regulation (Stokes and Richards 2002): The mutants *cpr1-1* (*constitutive expressor of pathogenesis-related protein 1*) and *bal* both map to this region and show similar phenotypes such as constitutive pathogen response, dwarfism, twisted leaves and reduced fertility. The *bal* mutant but not *cpr1-1* has strongly elevated transcript levels one *R*-gene of this cluster.

When *bal* and *cpr1* are crossed, they fail to complement each other in the F_1 progeny, suggesting that both mutants are affected at the same locus. Interestingly, in the F_2 progeny derived from selfed F_1 plants of this crossing, not only plants with mutant phenotypes were observed as expected, but approximately 20 % of phenotypically normal plants were recovered. Due to this observed paramutation, *bal* and *cpr1-1* were defined as two different epigenetic alleles (Stokes and Richards 2002). The appearance of F_2 plants similar to wildtype was proposed to be derived from epigenetic destabilization and reversion mediated by pairing interactions in *bal*/*cpr1-1* hybrids (Stokes and Richards 2002). The *bal*/*cpr1-1* situation resembles the observations made in the crossing between Sha and Sorbo, where a resistant F_1 progeny and a F_2 generation with approximately 19 % of unexpectedly susceptible plants were identified.

During the analysis of putative mechanisms of epigenetic regulation of the *bal*/*cpr1-1* locus, the authors did not find changes in the retrotransposons inside the *R*-gene cluster with respect to movement, structural changes or new insertions (Stokes and Richards 2002). Furthermore, they could not determine polymorphisms in the methylation pattern between *cpr1-1* and *bal* or between *cpr1-1* and Col-0 wildtype by using different cytosine–methylation sensitive restriction enzymes, or structural changes due to genomic rearrangements in the *R*-gene cluster (Stokes and Richards 2002).

Also in recent studies, the pairing of certain alleles has been suggested to epigenetically control gene expression (reviewed in Grant-Downtown and Dickinson 2004). Although most cases were correlated with introduction of transgenes (i.e. Sidorenko and Peterson 2001; Quin et al. 2003; Scheid et al. 2003) the effect could not be explained by mechanisms of RNA-based gene silencing due to overexpression of the transgene, since the paramutagenic activity was retained even when the transgene was segregated away.

Evidence for endogenous genes regulated by pairing was observed in the male determinant of compatibility, the S locus cysteine-rich (SRC) protein from *A. lyrat*a (Kusaba et al. 2002). Here, the combination of two different *SRC* alleles in the heterozygous plant, *SRCa* and *SRCb,* led to dramatic differences in transcript levels due to dominance of *SRCa* over *SRCb*. This observation was not correlated to methylation differences or small RNA species, indicating that homologous pairing could regulate the expression of the alleles.

Could this paramutation phenomenon of allelic pairing be responsible for the susceptible plants in the Sha x Sorbo F_2 progeny or for the contradictory plants in the mapping populations or even in the *pmr6* allelism tests? Col-0 lacks *RPW8.1* and *RPW8.2* but possesses homologuous genes in that locus which might allow homologous pairing also in these constellations. Thus, homologous pairing could be responsible for these phenomena.

Although not significant plant pathogenesis, the *PAI1-PAI4* locus in the *Arabidopsis* accession Ws is correlated to epigenetic regulation by paramutation in a gene family (Bender and Fink 1995; Bradley et al. 1999). This accession has four methylated *PAI* genes at three sites (an inverted repeat and two singlet genes at unlinked loci) encoding the tryptophan biosynthesis enzyme phosphoribosylanthranilate isomerase, while Col-0 possesses only three singlet and unmethylated *PAI* genes. When the copy number in the *PAI* locus is

reduced by deletion of the two tandemly arranged genes (*MePAI1-PAI4*), a mutant with fluorescent, tryptophan-deficient phenotypes results because the two remaining methylated *PAI* genes (*MePAI2* and *MePAI3*) supply insufficient *PAI* activity (Bender and Fink 1995).

Additionally, the Ws inverted repeat locus *PAI1-PAI4* triggers methylation of unlinked identical sequences when inserted in the Col-0 background, suggesting that the inverted locus in Ws probably provides the primary signal for methylation of *PAI*-related sequences elsewhere in the genome (Luff et al. 1999). Interestingly, here two accessions differ in the methylation status of genes in addition to the number of copies and sequence differences, stressing the importance of epigenetic mechanisms during plant evolution (Jablonka and Lamb 1989; Yi et al. 2004).

Although metastability is a common feature of epigenetic variation, many epigenetic alterations can be inherited with a high degree of fidelity, close to that of traditional genetic Mendelian segregation (Holliday and Ho 1990). With regard to this observation, the F_2 progeny segregating 3 : 1 (susceptible : resistant) or 1 : 2 : 1 (susceptible : intermediate : resistant) in the mapping populations might not be that drastically altered by pairing, resulting in only some plants which contradict the expected results. This might possibly be due to lower homology between the alleles of the *RPW8* locus in the resistant and the *HR* genes in the susceptible Col-0 accession. If the different alleles have a higher homology, like between Sha and Sorbo, the pairing effect is more drastic and leads to a high number of plants with wildtype or intermediate infection phenotypes. Nevertheless the effect of *pmr6* on resistance in Do-0 and Sorbo could not be explained by this hypothesis.

The presence of transposons was shown to be often correlated to epigenetic gene silencing (Martienssen 1996). Additionally, it was reported that transposons

may especially contribute substantially to genomic variation (Martienssen 1996; Kidwell and Lisch 1997, 2001), e.g. by disruption of coding genes via insertion of a mobile element, the formation of 'footprints' in insertion and excision cycles and, more drastically, by ectopic recombination between homologous elements during meiosis. The latter may lead to chromosomal rearrangements, allowing the formation of 'macrotransposons', capable of shifting even relatively large sequences of trapped host DNA to new sites in the genome (Gray 2000). Interestingly, the *Arabidopsis* genome database TAIR, which presents the genome of the ecotype Col-0, suggests the presence of a copia-like retrotransposon (At3g50490) directly adjacent to the *RPW8* locus.

Furthermore, it is notable that the crossing of Sorbo to the resistant accession Do-0, in which resistance was also mapped to chromosome III, did not produce susceptible F_2 plants like in the Sha x Sorbo F_2 generation or in Do-0 x Sha F_2 progeny, although to a much lesser extent in the latter crossing. Could the alleles of these accessions differ in their paramutagenic capabilities? This would be an interesting observation of an epigenetic phenomenon that is subject to natural variation.The occurrence of variation of epigenetic regulation in a wild plant population was also shown in the genus *Linaria*, where naturally occurring variants with radially rather than bilaterally symmetric flowers were analyzed, a phenomenon was already described by Linné. The genetic basis of this phenotype was identified as epigenetic silencing associated with DNA hypermethylation of the *Linaria CYCLOIDEA* homolog *Lcyc* (Cubas et al. 1999).

Additionally, methylation polymorphisms were observed between different accessions of *A. thaliana* (Cervera et al. 2002; Riddle and Richards 2002). Besides general differences, especially variation in DNA methylation patterns of the nucleolus organizer regions (NOR), large regions of highly repeated genes for ribosomal RNA, were detected. However, they could not be correlated with phenotypic differences between these accessions.

To clarify the situation with regard to potential epigenetic regulation of *RPW8*, it would be important to determine whether *RPW8* transcript levels are in individuals from the mapping population that possess the locus but are susceptible to *G. orontii*, as well as in susceptible F_2 plants of the Sha x Sorbo or Do-0 x Sha crosses, in comparison to wildtype or unaffected plants. In addition, it would be important to determine the mechanisms of how this regulation is achieved. Therefore, a cross between the resistant accessions with *Arabidopsis* mutants impaired in epigenetic mechanisms would allow determination of the contribution of some epigenetic effects on the expression of resistance in the populations.

For this purpose, Sorbo and Sha were selected to be crossed with the *rdr6* mutant (RNA-dependent RNA polymerase 6) in *Arabidopsis*, which is impaired in transgene-mediated RNAi silencing. It was reported that the functional RDR6 protein is necessary for the formation of siRNA precursors in sense transgene-mediated RNAi, but not for silencing of constructs that encode transcripts with hairpins containing extensive dsRNA structure, such as in miRNA-mediated silencing (Dalmay et al. 2000; Mourrain et al. 2000; Beclin et al. 2002).

SiRNAs are double-stranded and usually 21 to 24 nucleotides long. They are processed from precursors containing extensive or exclusive double-stranded RNA (dsRNA) structure, such as transcripts containing inverted repeats or intermediates formed during RNA virus replication (Hannon 2002). By crossing the resistant accessions to this mutant and establishing a new mapping population, the percentage of plants with abberant phenotypes should be drastically reduced if siRNA-mediated silencing of *RPW8* causes these phenomena.

Another RNA species capable of interfering with gene expression are miRNAs. Their sequences are encoded in the genome and, when transcribed, they

naturally form hairpin double-stranded RNA. Subsequently, they are enzymatically processed by DCL1 in the nucleus to smaller RNAs of around 21 nucleotides. These processed small miRNA species are then incorporated into RISC complexes to catalyse degradation of homologous mRNAs, thereby inhibiting protein translation or facilitating cleavage of the mRNA (reviewed in Grant and Dickinson 2005).

Interestingly, several mutants several mutants have now been shown to contain nucleotide changes in regions of complementarity in their miRNA (e.g. dominant gain-of-function alleles of *PHABULOSA* and *PHAVOLUTA*; Rhoades et al. 2002), which results in reduced binding and abnormally increased levels of the transcript and protein. If *RPW8* was regulated by a miRNA, it would be interesting to analyze, whether nucleotide changes in the region encoding the miRNA would impair its function.

The *dcl1 (dicer like 1*) mutant is impaired in the accumulation of several miRNAs (Park et al. 2002; Reinhardt et al. 2002; Xie et al. 2004). However, analysis of F_2 progeny of a crossing to the *dcl1* mutant would likely not be informative, since *dcl1* has pleiotropic effects during many aspects of plant development (Schauer *et al*. 2002). Another approach is a bioinformatic screen for miRNAs with a homology to the *RPW8* locus of Ms-0. By searching databases of *Arabidopsis* miRNAs (Adai et al. 2005; Gustafson et al 2005) for homology to the *RPW8* locus of Ms-0, several miRNA candidates were identified that were potential candidates. To analyze whether one of these miRNAs is involved in the regulation of *RPW8*, it would be necessary to specifically block these miRNAs, e.g. by using anti-miRNA single strand antisense oligonucleotides (ASOs; Meister et al. 2004; Davis et al. 2006).

To further unravel potential epigenetic mechanisms, the *RPW8* locus should be analyzed with regard to polymorphisms in the methylation pattern between the

contradictory F_2 plants and F_2 plants with expected phenotypes in comparison to the resistant parental accession. Also accessions with Ms-0-like RPW8, which are only resistant to *G. orontii*, should be analyzed. Both qualitative and quantitative changes in methylation patterns might be important, since it was reported that hypermethylation at a locus was often found to correlate with a reduction in gene expression or even its complete silencing, like in the SUPERMAN locus (Jacobsen and Meyerowitz 1997) or in the *Lcyc* locus in *Linaria vulgaris* already mentioned above (Cubas et al. 1999). The analysis of methylation could be achieved with a Southern Blot of genomic DNA, using cytosine–methylation sensitive restriction enzymes (Cubas et al. 1999). It is also not clear whether chromosomal rearrangements could be responsible for the silencing effects. Again, Southern Blots could help to elucidate the situation.

Previously, the importance of transposons for epigenetic regulation has been emphasized (see above). The *Arabidopsis* genome database TAIR suggests the presence of a copia-like retrotransposon (At3g50490) directly adjacent to the *RPW8* locus. Although in the previous analysis of candidate genes this locus was excluded, it would be interesting, with respect to epigenetic regulation, to explore if any changes in transposon copy number, structure or movement occurred, which could be responsible for changes in the expression levels of adjacent genes. Nevertheless, it cannot be excluded that transposons may affect the expression of neighboring genes in the absence of structural changes (Argeson et al. 1996; Morgan et al. 1999; Barkan and Martienssen 1991; Wals et al. 1998).

In summary, although resistance in several analyzed accessions maps to the same region on chromosome III, more factors seem to be necessary for full establishment of this trait. These factors could not be identified by QTL mapping. It is hypothesized that epistatic or epigenetic effects influencing transcript levels or the timing of expression could affect the expression of the

RPW8 resistance genes, thus leading to contradictory plants in the mapping populations.

One possibility to likely overcome the effects of the genetic background and to effectively remove all contradictory plants from the mapping population could be the production of a NIL, a nearly isogenic line. If after several backcrosses with Col-0 a new mapping population is established, effects of genetic backgrounds should be largely removed. Since during the selection process only F_2 plants are selected for the next crossing round, for which both phenotype and genotype indicate resistance, the effect of pairing should also be overcome.

A similar though shorter way is the selection of a resistant Sorbo F_2 plant as a new parent for a mapping population, which is clearly not segregating for resistance in the F_3 progeny. Here, potentially segregating secondary regulators could have been crossed out and effects of epigenetic pairing did probably not affect this plant. Nevertheless, these approaches would not help to clarify the questions why these contradictory plants exist and what factors might be the basis of these phenomena.

4.7 THE CANDIDATE GENE *RPW8*

Based on sequence analysis, it has been previously suggested that *RPW8* represents the main source of natural broad-spectrum resistance to powdery mildews in *Arabidopsis* (Xiao et al. 2004) based on sequence analysis. In six out of ten accessions with suggested monogenic resistance the resistance-mediating loci were identified to be located in the same region on chromosome III, where also *RPW8* is located (Table 6). For only three of them, Do-0, Nok-3 and Sha, dsRNAi-mediated transcript reduction of the candidate gene *RPW8* led to susceptibility, indicating that *RPW8* is the responsible locus mediating resistance in these three accessions (Table 15). Likewise, gene silencing suggests a role for

RPW8 in accessions Bu-0, Co-3, Ei-4, Ei-5 and Ob-0, in which the resistance locus has not been mapped, but for which susceptible T_1 and/or T_2 plants were identified (Tables 15, SD10 and SD12). The susceptibility phenotype was confirmed in the T_2 progeny for Co-3, Ei-4, Nok-3 and Sha. For the other three accessions mapped to chromosome III (C24/Co-1, La-1 and Sorbo), no susceptible plants transformed with a *RPW8* dsRNAi-construct were isolated. Either resistance in these accessions is not mediated by *RPW8,* or the reduction of transcript levels was not strong enough in the tested transgenic lines of these accessions. At this point it cannot be excluded that also in accessions suggested to be polygenic for resistance, *RPW8* plays a major role. In Kas-1, for example, in which resistance was first suggested to be mediated by a single and semi-dominantly inherited locus (Adam and Somerville 1996), analysis of a population of RILs subsequently revealed one major (corresponding to *RPW8*) and two minor QTLs on other chromosomes (Wilson et al. 2001). The differences were explained with different inoculation densities used in the two studies. Moreover, also in Ms-0, a minor QTL besides *RPW8* was identified, which was located on another chromosome (Xiao et al. 1997).

Likewise, in this study, resistance in the accessions Bu-0 and Ob-0 was suggested to be of polygenic origin, as indicated by segregation patterns of the F_2 progeny. Although resistance in Ob-0 was not mapped, because neither 3 : 1 nor 1 : 2 : 1 segregation of resistance was significant, subsequent PCR analysis for presence of *RPW8* was positive for all 12 tested resistant F_2 plants (data not shown), which is in accordance to the dsRNAi results obtained for this accession. Allelism tests between different accessions did not reveal conclusive data in many cases (see below). Therefore, it is not possible to determine whether also in the other accessions, in which resistance does map to the lower arm of chromosome III, it is mediated by *RPW8*.

4.8 *RPW8* – DOMINANT OR SEMI-DOMINANT?

In this study, resistance segregated in either semi-dominant or recessive manner for all accessions tested including those ecotypes, in which dsRNAi analysis showed that *RPW8* might be responsible for resistance. *RPW8* was first identified in the Ms-0 ecotype and quantitative analysis of resistance to *G. cichoracearum* indicated a dominant inheritance (Xiao et al. 1997).

In later studies, it was observed that in Col-0 plants carrying a transgene comprising *RPW8.1* and *RPW8.2*, resistance was inherited in a semi-dominant manner (Xiao et al. 2005). The authors suggested that *RPW8* acts in a gene dosage-dependent manner to stimulate defense mechanisms, and that those different genetic backgrounds may result in different levels of *RPW8* transcript and, thus, *RPW8*-mediated resistance. Consequently, the observed semi-dominant inheritance, in part observed in this study, could also be due to a gene-dosage affect in the respective accessions.

4.9 WHY WAS A CORRELATION OF SEQUENCE WITH PHENOTYPE OR GEOGRAPHIC ORIGIN NOT POSSIBLE?

In earlier studies in *Arabidopsis,* no correlation between genetic and geographical distance was found and this lack of phylogeographic pattern has been ascribed to recent human-induced migrations (King et al. 1993, Tokodoro et al. 1995; Bergelson et al. 1998; Miyashita et al. 1999). Nevertheless, more recent studies with larger sample sizes suggest the existence of a large-scale population structure (Sharbel et al. 2000; Nordborg et al. 2005; Schmid et al. 2006).

During the selection of accessions resistant to *G. orontii* and the subsequent sequence analysis of the *RPW8* locus, it was neither possible to correlate *RPW8* alleles to the infection phenotype to three different powdery mildew species nor

the *RPW8* sequences to the geographical origin of the respective accession. With respect to the first point, a similar observation was made in earlier studies with other *R*-genes: Although *RPP13* alleles of 24 ecotypes conferred different resistance specificities to three different isolates of *P. syringae*, they differed in many amino acids. For this reason, the identification of residues responsible for pathogen recognition could not be determined. In many *R*-gene studies, however, protein sequence variation in the LRR (leucine-rich repeat) domain has been found to be correlated with different pathogen recognition specificities (Wang et al. 1998; Bryan et al. 2000; Hwang et al. 2000; Banerjee et al. 2001; Dodds et al. 2001; Van der Hoorn et al. 2001; Wulf et al. 2001).

Nevertheless, at least the *RPW8* sequence itself was expected to show a correlation to geographical distance, which was not the case. These observations could be due to the small sample size used, which in addition has a strong emphasis on Central Europe, but which does not densely cover all regions of the natural species range (Hoffmann 2002). However, it was shown in a recent study that this sampling bias does not affect estimates of genetic variation (Schmid et al. 2006).

A. thaliana is commonly found in agricultural fields and other disturbed sites associated with human activity. Therefore, human-induced migrations and disturbances could be responsible for masking effects of phylogeographic association, especially in Central Europe, where variability is higher than in any of the glacial refugia (e.g. Bergelson et al. 1998; Mauricio 1998; Le Corré 2005). Accessions of this region were shown to contain a general proportion of ~ 45 % of all molecular polymorphisms found in the *Arabidopsis* genome so far, while this proportion is much lower (~ 12 %) in less disturbed parts of Norway (Stenoien et al. 2005). This low variation is probably not due to post-Pleistocene founder events, since no changes in genetic variation were observed in a latitudinal transect of Norwegian accessions.

It has also been suggested that the variability between accessions of the glacial refugia should account for most of the variability contained in larger samples (Hanfstingl et al. 1994), which was confirmed in this analysis of some polymorphisms in *RPW8.1* and *RPW8.2* (Tables 11 and 13). Schmid et al. (2006) even suggested that individuals from the same local population can be genetically different, indicating that they originated from multiple source populations, an observation also confirmed in this study as well as in an analysis of genetic variation within *A. thaliana* populations (Bakker et al. 2006).

Moreover, the selected accessions are not a representative subset throughout the geographic range of the species but reflect a biased selection due to specific pre-selection criteria. Nevertheless, the accessions selected with these criteria had origins located in the entire distribution range. It is also discussed that *Arabidopsis* may not be the primary host for powdery mildews (Schulze-Lefert 2000), implicating that an association of resistance to powdery mildew together with geographical distribution may principally not be possible.

Nonetheless, based on *RPW8* sequence analysis, some observations with regard to biogeographical aspects of *A. thaliana* can be made. This is especially true for plants carrying a *RPW8.1* copy with a unique C-terminal insertion (Ang-0, Bla-2, Bla-11, Blh-2, Hh-0, Hl-2, Jl-4 and Nd-0). It is expected that accessions with such an insertion event have a common ancestor. Nevertheless, these plants do not originate from one region, but they were collected in Spain (Bla-2, Bla-11), Belgium (Ang-0), Germany (Hh-0 and Hl-2) and the Czech Republic (Blh-2, Jl-4), consequently from Western to Eastern Europe. Postglacial colonization of *A. thaliana* of Central and Northern Europe, which started ~18 000 years ago, originated from Asia, with some indications of an additional Mediterranean Pleistocene refugium. The latter likely comprised the Iberian Peninsula, as well as Southern Italy and the Balkans (Sharbel et al. 2000; Schmid et al. 2006).

Similar post-Pleistocene migration patterns were determined for a number of species (Hewitt 1996; Taberlet et al. 1998).

It was suggested that the northern part of Central Europe and Eastern Europe may be a suture zone (Taberlet et al. 1998) of *Arabidopsis* for the admixture between the Iberian and Asian refugia (Sharbel et al. 2000). Consequently, Central European accessions may show an east-to-west clinal distribution in genetic variation. Therefore, accessions from Western Europe should generally be more closely related to the Iberian ecotypes and Eastern European accessions being more closely related to those from Asia (Sharbel et al. 2000). Based on this hypothesis, it can be speculated that the unique C-terminal insertion in *RPW8.1* observed in accessions originating from Western to Eastern Europe, but not found in accessions from Asia could have originated in the Iberian refugium and successively spread throughout Europe. Alignment of all nucleotide sequences obtained in this study shows no sequence variation inside the C-terminal insertion observed in *RPW8.1* and a higher degree of variation in a region of ~ 70 bp upstream of the insertion (not shown). Although this sequence could be compared between only ten accessions, this observation underlines the idea that this insertion is of recent origin and probably arose before or during the postglacial recolonization. Interestingly, no accession with this C-terminal insertion was identified from locations between these collection sites, e.g. from France. Possibly, collections of *Arabidopsis* accessions in France were not dense enough to provide an overview of all existing ecotypes in this region.

In a group of six accessions (Ei-5, H55, Nw-1, Pla-4, Rsch-4 and Uk-4) have a common insertion of nucleotides in *RPW8.2* leading to a premature STOP codon at amino acid position 146. It was suggested that these accessions collected from Spain (Pla-4), Germany (Ei-5, Nw-2, Uk-4), the Czech Republic (H55) and Russia (Rsch-4) likewise have a common ancestor. Since the sequence downstream of the premature STOP codon shows variation between the accessions (Figure 21),

it can be assumed that this allele is of ancient origin and arose probably before the postglacial recolonization. However, the small sample size impedes a strong hypothesis at this point.

In another five accessions (C24, Chi-0, Gü-0, Pla-3 and Ty-0) a nucleotide deletion led to a completely altered polypeptide sequence starting from amino acid 145 and to a premature STOP codon at position 160. These accessions had also diverse origins, comprising Spain (Pla-3), Portugal (C24), United Kingdom (Ty-0), Germany (Gü-0) and Russia (Chi-0).

Either the suture zone of postglacial colonization of Europe is positioned more far east as suggested by Sharbel et al. (2000), or these isoforms arose already before *Arabidopsis* was forced to withdraw into glacial refugia and spread during the following recolonization throughout Europe in both colonization waves. In this group no sequence variation was observed downstream of the deletion. In a region of ~ 80 bp ustream of the nucleotide deletion, two nucleotide exchanges unique for this group of accessions indicated a likely recent origin of this allele (not shown). In addition, two other accessions with yet another C-terminal alteration were collected in Germany (Bu-6) and Russia (Petergof), indicating an origin of this group in the Asian refugium.

In several further studies, the variability of *R*-genes of *A. thaliana* was determined, mainly to access whether they are subject to selection. *RPM1* has a long-lived presence/absence polymorphism, which arose 9,7 million years ago and is maintained by balancing selection. A fitness cost is associated with maintaining this locus in the absence of the pathogen but a fitness advantage in its presence (Stahl et al. 1999, Tian et al. 2003). The correlation of alleles to geographic origin of the accessions was not determined, since the distribution of the *RPM1* allele in the selected accessions did not show an obvious pattern.

Similar to *RPM1, RPS5* is also characterized by an old absence/presence polymorphism subjected to balancing selection. It was attempted to correlate the distribution of the *RPS5* allele to the infection phenotype in the selected accessions but this did again not reveal an obvious pattern (Tian et al. 2002). Additionally, the level of nucleotide polymorphism has been determined at the *RPS*2 *R*-gene locus in *A. thaliana* (Caicedo et al. 1999; Mauricio et al. 2003), where similar to the *RPM1* and *RPS5* loci, two different allelic classes were found. Haplotypes of one class were found in plants susceptible to *Pseudomonas syringae* pv. *tomato* expressing avrRpt2 (Mauricio et al. 2003), while alleles of the other were present in resistant and partially resistant plants. This indicates a correlation of haplotype differentiation with the observed phenotypic variation in these plants. Nevertheless, it was observed that accessions from the same geographic region are scattered throughout the *Rps2* phylogenetic tree, indicating that for *Rps2* there was no correlation of sequence variation to geographical origin of the accession.

4.10 STUDIES OF VARIABILITY OF *RPW8*

In prototypical *R*-genes of the NBS-LRR-class, the LRR domain is thought to be responsible for specificity of recognition (Staskawicz et al. 1995). Therefore, usually the main part of variation can be found in this region, which was previously shown to be subject to selection, e.g. in *RPP13* (Rose et al. 2004), *RPS2* (Tian et al. 2002) and *Xa21* in rice (Song et al. 1995). For *Xa21* it was suggested that especially the solvent-exposed residues of the LRR play a role in ligand binding (Parniske et al. 1997). In contrast, the broad-spectrum powdery mildew resistance genes *RPW8.1* and *RPW8.2* from *A. thaliana* accession Ms-0 encode novel proteins with a putative N-terminal transmembrane (TM) domain and a central coiled-coil (CC) domain (Xiao et al. 2001) that lack the LRR domain described for other *R*-genes.

Analysis of the distribution of non-synonymous nucleotide substitutions among *Arabidopsis RPW8.1*, *RPW8.2* and two additional homologous genes of that locus (*HR3* and *HR4)* revealed three regions, in which non-synonymous substitutions were more frequent (Xiao et al. 2004). Additionally, alignment of 17 protein sequences from *RPW8* homologues of different plant species suggested a region of higher sequence variation at the C-termini of the encoded proteins, close to the predicted coiled-coil domain. Interestingly, the CC domain of *AtRPW8.1* was abolished by extensive sequence polymorphisms. However, *AtRPW8.1* had a different putative CC domain at the C-terminus of the gene. The average ratio of nonsynonymous substitutions (*Ka*) to synonymous substitutions (*Ks*), an informative parameter to assess whether the evolution of genes is under strong selection, was > 1, indicative of diversifying selection.

Nevertheless, the authors did not perform this analysis on *RPW8* sequences from different *A. thaliana* accessions. These were analyzed during this study with respect to variability in amino acid sequence. In both, RPW8.1 and RPW8.2, the stretch of highest sequence variability resides between the predicted N-terminal TM domain and the CC region. In RPW8.2 the region of variability extends to the first part of the CC domain. This domain is supposed to be involved in signaling (Martin et al. 2003). The high amino acid variability in the region mostly between the two domains could indicate a function in pathogen recognition or signal transduction. This hypothesis would also explain, why variability in this area was not observed in the previous interspecies analysis of *RPW8*-homologous genes, since for most of them no function in the context of plant-microbe interactions has been described. In terms of evolution, this would be in accordance with the model of gene duplication and diversification for the emergence of *RPW8.1* and *RPW8.2* in *A. thaliana* and *A. lyrata*, which derived from an *HR3*-like progenitor gene. It was suggested that *HR3* retained the

original function of the progenitor gene, while the other copies developed new functions, probably in mediating disease resistance (Xiao et al. 2004).

4.11 SEQUENCE ANALYSIS IN *RPW8* WITH REGARD TO THE INFECTION PHENOTYPE CONSTELLATION

According to Xiao et al. (2001), *RPW8* can confer resistance under its native promoter only when both gene copies are present; (i.e., in homozygous plants). Probably a certain threshold transcript level is needed for establishment of resistance.

In this study, some resistant accessions carry a non Ms-0-like *RPW8.1* and/or *RPW8.2*. Either these different alleles of *RPW8* are functional or resistance is not mediated by *RPW8.* The Nok-3 ecotype, for example, has *RPW8* copies that both differ from Ms-0, but resistance was mapped to the *RPW8* region. This could mean that the sequence changes observed in this ecotype do not interfere with functionality.

Another group of accessions has Ms-0-like sequences for *RPW8.1* or *RPW8.2* or both, although they are susceptible to three powdery mildew species. Among these, accessions with only one Ms-0-like sequence could have a non-functional copy of the other gene, which may result in susceptibility. However, a different explanation is necessary for accessions with Ms-0-like sequences for both genes but without broad-spectrum disease resistance, like Is-0, Ita-0 and Uk-3, which are resistant to only *G. orontii*. This could be explained by reduced transcript levels, maybe due to epigenetic mechanisms, in these accessions. However, none of them had an obviously lower transcript accumulation for one or both genes in the semi-quantitative RT-PCR analysis (data not shown). Moreover, posttranslational mechanisms or degradation at the protein level is possible.

In the same manner, these accessions could have differences or a defect in downstream signaling. In all these cases, the specific resistance to only *G. orontii* could be mediated by another gene, for example a dominant *R*-gene. With regard to this aspect, F_1 and F_2 progeny of Is-0 crossed to Col-0 were analyzed with *G. orontii*, showing intermediate susceptible F_1 progeny and an F_2 generation segregating 1 : 2 : 1 (susceptible : intermediate : resistant). This pattern of inheritance is not expected for a dominant *R*-gene. Ita-0 and Uk-3 have not been crossed so far.

These observations may again stress the importance of the genetic background for expression of a trait; epistatic effects could be responsible to impair *RPW8*-mediated resistance in these accessions. Nevertheless, it has been not shown for these accessions that their resistance is truly mediated by *RPW8*. A similar observation was made with regard to the *R*-gene *RPP13* (Rose al. 2003), for which the *RPP13* allele recovered from the Col-0 ecotype did not show obvious defects like truncations but did also not provide resistance to the tested *P. parasitica* isolates. Downstream signaling was not compromised, since it became resistant when transformed with *RPP13* alleles of other resistant accessions. In addition, some accessions had the same *RPP13* allele but showed a different reaction to the tested *P. parasitica* isolates. The authors stress the fact that only for two accessions tested resistance was shown to be due to *RPP13*, leaving the possibility that resistance in the other accessions is not caused by *RPP13*. Therefore, they suggest that identifying residues involved in pathogen recognition can only be made by comparing alleles of susceptible accessions with those from resistant accessions, for which their resistance-mediating function has been shown by transformation. Likewise, it is necessary for the susceptible accessions carrying an Ms-0-like *RPW8* sequence to confirm that resistance is really mediated by *RPW8*.

Another possibility to explain the lacking correlation between *RPW8* genotypes and infection phenotypes could be the comparison of results for infection phenotypes obtained in different labs. Different growth conditions of plants and pathogens could interfere with aggressiveness of the pathogen and susceptibility of the plant, respectively. It has already been reported that certain QTLs are expressed in one environment but not in the other, or they differ in the magnitude of their allelic effects (Ungerer 2003; Juenger 2006). This could also explain the observation that Is-0 has been scored resistant to *G. orontii* in Germany but not in California, as reported by Xiao et al. 2004. Nevertheless, it is not clear whether the employed *G. orontii* is the same isolate in both labs or whether the accessions identical despite having the same designation.

4.12 SUMMARY AND OUTLOOK

In summary, natural resistance of *Arabidopsis* to the powdery mildew *G. orontii* is probably either of polygenic origin or mediated by the *RPW8* locus. Evidence for resistance conferred by prototypical NBS-LRR proteins with dominant inheritance was not observed. Some candidate accessions remain to be analyzed for dominantly inherited resistance.

Sequence analysis of *RPW8* in accessions with different infection phenotypes showed variability at the protein level in a central region of the protein between the TM and the CC domain. The sequence variability did not strongly correspond to geographic origin of the accessions or to the constellation of infection phenotypes to different powdery mildew species. Studies of variability at the nucleotide level could indicate whether genes or gene regions of the *RPW8* locus are subject to selection pressure.

Unexpected results were obtained in allelism tests between accessions and mutants, which might be due to locally increased recombination frequencies, or

epistatic or epigenetic effects, which may influence timing and levels of transcription. Several approaches were suggested to identify the nature of these phenomena.

V. SUPPLEMENTARY DATA

Table SD 1: Accessions of *A. thaliana* used in this study

	origin		
accession	city	country	NASC stock
Ag-0	Argentat	France	N936
An-1	Antwerpen	Belgium	N945
Ang-0	Angleur	Belgium	N948
Bay-0	Bayreuth (FRG)	Germany	N954
Bla-4	Blanes/Gerona	Spain	N977
Bla-10	Blanes/Gerona	Spain	N983
Bu-0	Burghaun/Rhön	Germany	N1006
Bu-3	Burghaun/Rhön	Germany	N1010
Bu-9	Burghaun/Rhön	Germany	N1022
Bu-11	Burghaun/Rhön	Germany	N1024
Bu-15	Burghaun/Rhön	Germany	N1034
Bu-17	Burghaun/Rhön	Germany	N1036
Bu-18	Burghaun/Rhön	Germany	N1038
Bu-21	Burghaun/Rhön	Germany	N1045
Bu-23	Burghaun/Rhön	Germany	N1048
C24	Coimbra	Portugal	N906
Cit-0	Citou/Aude	France	N1080
Co-1	Coimbra	Portugal	N6669
Co-3	Coimbra	Portugal	N6671
Co-4	Coimbra	Portugal	N1090
Ct-1	Catania	Italy	N1094
Di-2	Dijon	France	N1110
Do-0	Donsbach/Westerwald	Germany	N1112
Dra-0	Drahonin	Czech Republic	N1116
Dra-1	Drahonin	Czech Republic	N1118
Dra-2	Drahonin	Czech Republic	N1120
Ei-4	Eifel	Germany	N1126
Ei-5	Eifel	Germany	N1128
Ep-0	Eppenheim/Taunus	Germany	N1140
Es-0	Espoo	Finland	N1144
Et-0	Etragyes	France	N6702
Fl-1	Finland	Finland	N1160
Fr-5	Frankfurt	Germany	N1174
Ga-0	Gabelstein	Germany	N1180
Gr-3	Graz	Austria	N1202
Gr-5	Graz	Austria	N1206
Gy-0	La Minière	France	N1216
Ha-0	Hannover	Germany	N1218
Hl-0	Holtensen	Germany	N1228
Is-0	Isenburg/Neuwied	Germany	N6741
Ita-0	Ibel Tazekka	Marocco	N1244
Je-0	Jena	Germany	N1246
Je54	Relichova	Czech Republic	N924
Jl-2	Vranov u Brno	Czech Republic	N1250
Jl-5	Vranov u Brno	Czech Republic	N1256
Jm-2	Jamolice	Czech Republic	N1262
Kä-0	Kärnten	Austria	N1266
Kas-1	Kashmir	India	N903
Kl-3	Koeln	Germany	N1280
Ko-2	Kopenhagen	Denmark	N1288

Table SD 1 Continued

	origin		
accession	city	country	NASC stock
Kro-0	Krotzenburg	Germany	N1300
Ksk-1	Keswick	United Kingdom	N1634
La-1	Landsberg-Warthe	Germany	N1302
Li-1	Limburg	Germany	N1310
Ll-1	Llagostera	Spain	N1340
Ll-2	Llagostera	Spain	N1342
Mh-0	Mühlen	Germany	N904
Mir-0	Miramare/Trieste	Italy	N1378
Nie-0	Niederlauken/Ts.	Germany	N1392
Nok-0	Noordwijk	Netherlands	N1398
Nok-1	Noordwijk	Netherlands	N1400
Nok-3	Noordwijk	Netherlands	N1404
Nw-0	Neuweilnau	Germany	N1408
Nw-1	Neuweilnau	Germany	N1410
Nw-3	Neuweilnau	Germany	N1414
Ob-0	Oberursel/Hasen	Germany	N1418
Ob-1	Oberursel/Friedhof	Germany	N1420
Ove-0	Ovelgoenne	Germany	N1434
Oy-1	Oystese (N)	Norway	N1643
Pa-1	Palermo	Italy	N1438
Pa-2	Palermo	Italy	N1440
Pa-3	Palermo	Italy	N1442
Per-1	Perm	Russia	N1444
Petergof	Petergof	Russia	N926
Pf-0	Pfrondorf	Germany	N1452
Pla-0	Playa de Aro	Spain	N1458
Pla-2	Playa de Aro	Spain	N1462
Pla-3	Playa de Aro	Spain	N1464
Pla-4	Playa de Aro	Spain	N1466
Po-0	Poppelsdorf	Germany	N1470
Rak-2	Raksice	Czech Republic	N1484
Rou-0	Rouen	France	N1488
Rsch-4	Rschew/Starize	Russia	N1494
Sav-0	Slavice	Czech Republic	N1514
Sei-0	Seis am Schlemm	Italy	N1504
Sg-1	St. Georgen	Germany	N1518
Sha	Palmiro-Alay	Tajikistan	N929
Si-0	Siegen	Germany	N1524
Sorbo	Tajikistan	Tajikistan	N931
Ste-0	Stendal	Germany	N1536
Sy-0	Isle of Skye	United Kingdom	N1546
Ta-0	Tabor	Czech Republic	N1548
Ts-1	Tossa del Mar	Spain	N1552
Ts-7	Tossa de Mar	Spain	N1562
Tu-1	Turin	Italy	N1568
Ty-0	Taynuilt	United Kingdom	N1572

Table SD 1 Continued

	origin		
accession	**city**	**country**	**NASC stock**
Uk-1	Umkirch	Germany	N1574
Uk-3	Umkirch	Germany	N1576
Van-0	Vancouver	Canada	N1584
Wa-1	Warsaw	Poland	N1586
Wt-2	Wietze	Germany	N1606
Wt-3	Wietze	Germany	N1608
Wt-5	Wietze	Germany	N1612
XX-0	unknown	unknown	N1618
Zü-1	unknown	unknown	N1628

Table SD 2: Mutant lines used in this study

Mutant allele	ecotype	reference
Atmlo2/6/12	Col-0	Consonni et al. 2006
eds1- 2	La-er	Aarts et al. 1998; Falk et al. 1999
eds5- 1 (=*sid1* -1)	Col-0	Rogers and Ausubel 1997; Nawrath et al. 2002
ein2- 1	Col-0	Guzmán and Ecker 1990; Roman et al. 1995
etr1- 1	Col-0	Bleecker et al. 1988; Chang et al. 1993
NahG	Col-0	You et al ., 1991; Delaney et al ., 1994
ndr1- 1	Col-0	Century et al. 1995; Century et al. 1997
npr1-1	Col-0	Cao et al. 1994; Cao et al. 1997
pad2-1	Col-0	Glazebrook et al. 1994
pad3- 1	Col-0	Glazebrook et al. 1994; Zhou et al. 1998
pad4- 1	Col-0	Glazebrook et al. 1994; Jirage et al. 1999
pen1 -1	Col-0	Collins et al . 2003
pen2- 1	Col-3	Lipka et al. 2005
pmr4- 1	Col-0	Vogel and Somerville 2000; Nishimura et al. 2003
pmr6- 3	Col-0	Vogel et al. 2004
pmr6- 4	Col-0	Vogel et al. 2004
rar1- 10	La-er	Muskett et al. 2002
sid2 -1 (= *eds16-* 1)	Col-0	Nawrath and Métraux 1999; Wildermuth et al. 2001

Table SD 3: Oligonucleotides used in this study

Type	Primer name	Sequence 5' - 3'	Description
SSLP	Sorb02-1	TTGGCATCAGTGGAGGTGTA	Chromosome 3, 22,75 Mb
	Sorb02-2	AATCCAAACCGTAGTGCAAA	
	Sorb03-1	CAACAAATTTGACCTTTCAGTCA	Chromosome 3, 22,8 Mb
	Sorb03-2	TGGAAACAAGACTTACATAAGAAAAA	
	Sorb10-1	TCCATTAACAAGCTTTTCTTCGT	Chromosome 3, 22,91Mb
	Sorb10-2	TCCTTCTCAAGCTTTGCTAGAAC	
	Sorb13-1	CATTGTGTAAGTTTAGTTTTAATTCAT	Chromosome 3, 22,65
	Sorb13-2	CTGCACATTTCCTTGCAAAA	
	Sorb15-1	ATTCCCCGCATCTCCAAT	Chromosome 3, 20971200 bp
	Sorb15-2	TGTTTTAAACCACCGGAAAGA	
	Sorb18_1	GAG ACA CTC CGA GTA TGA GCA	Chromosome 3, 20037144 bp
	Sorb18_2	TGA CGA AGC AAG TGA CAA CC	
	Sorb27-1	TCAGGCTGCGATACTTTGTT	Chromosome 3, 21341213 bp
	Sorb27-2	AACCTTGAAGCGGTTGAGAA	
	Sorb32-1	GGAGATATCATCCGCCCTCT	Chromosome 3, 23320041 bp
	Sorb32-2	ATGAGCAAGTGCTTGTGTGC	
	Sorb34-1	GGAGAAGCGCTTTGTTCAGA	Chromosome 3, 18235770 bp
	Sorb34-2	TGCTTCTTCTTCTTCTTCTTCG	
	Sorb39-1	CGAGATTGTCGACCAAGGTT	Chromosome 3, 20405889 bp
	Sorb39-2	GGACTCAGTGTCAAGGAATCG	
	Sorb40-1	CAAATTTTACATTTTATTGGATTACCC	Chromosome 3, 20,35 Mb
	Sorb40-2	GCTACTAAAGAAAGTGTTCCACACC	
	Sorb42_2	CAT TGG CAC ACT CCT TCT GA	Chromosome 3, 19551364 bp
	Sorb42_1	TCA AAC AAA GAA TGT CTG GAA TTT	
	Sorb44_2	TTC TGT TGT GGA AGA CGA AAA A	Chromosome 3, 19778497 bp
	Sorb44_1	AGC ATC GGT TGC AGT CTC TT	
	Sorb47_2	TGA GCG TTG ATG GTA ACG AG	Chromosome 3, 20329442 bp
	Sorb47_1	AAA GTC AAA GGA AAA GGA CGA	
	Sorb50_1	CAGATTCCAAAACAAAACAAAAA	Chromosome 3, 18 493 161 bp
	Sorb50_2	TCACCAGAGTCTTTCTTCTTCG	
	Sorb51_1	GATCCTCCTTCCTTGGTATGG	Chromosome 3, 18664275 bp
	Sorb51_2	TTCGCATTTCTTGATTTACTATTTG	
	Sorb52_1	TTCTTGCAACAACAAAAAGGA	Chromosome 3, 18776433 bp
	Sorb52_2	TTACCAATATCTGGTCCCATGT	
	Sorb54_1	TTTGCAACTTTACATTCTCCATC	Chromosome 3, 18971800 bp
	Sorb54_2	TCCAAGGCAAAAGCCAATAC	
	Sorb55_1	AAAGTTTGCGTTGTATCATTAAAAA	Chromosome 3, 19142706 bp
	Sorb55_2	CTTTCGGTTCTTCCGAGTTG	
	Do2_1	GACCTTATCAATTGAGTTTTGAAGAA	Chromosome 3, 16369410 bp
	Do2_2	CTTCCCTCTGCTTCATCCAG	
	Do11_1	CCATCCTTTTCTTCCTGCAC	Chromosome 3, 16833507 bp
	Do11_2	CCAAAGACCAAAGAGACACG	
	NGA707_1	CTCTCTGCCTCTCGCTGG	Chromosome 3,21763494 bp
	NGA707_2	TGAATGCGTCCAGTGAGAAG	
	NGA112_1	CTCTCCACCTCCTCCAGTACC	Chromosome 3, 23179348 bp
	NGA112_2	TAATCACGTGTATGCAGCTGC	
	CIW4_F	GTTCATTAAACTTGCGTGTGT	Chromosome 3, 18901818 bp
	CIW4_R	TACGGTCAGATTGAGTGATTC	
	PLS5_F	GAT GCC TTT CTC CTG GTT G	Chromosome 2, 9178570 bp
	PLS5_R	AAT ATA GCC GTC GTC TTC ATC A	
PMR6	35Sreadout2_F	CGCACAATCCCACTATCCTT	35S-readout primer
analysis	pmr6_s	AAGAGGGCAGAGAAGCAGAGA	for sequencing, in 5'UTR.
	pmr6_as	CCAATCAATTGAACGGTCCA	for sequencing, in 3'UTR

Table SD 3 Continued

Type	Primer name	Sequence 5' - 3'	Description
PMR6	pmr6i_s	GAGAGAGTGTGAGAGAGAAAGCAG	for sequencing, in 5'UTR.
analysis	pmr6i_as	TTTGTTATCCATTCGTCACAATAA	for sequencing, in 3'UTR.
	pmr6i2_s	CACACTGCAACTTCCACAGC	for sequencing, in 5'UTR.
	p62_as	CGACGTAGATCTTGCCGTTT	for sequencing, in second exon .
	p62i_as	CGAAGCCGATTGAACAGTCT	for sequencing, in second exon .
	pmr6_ex3_s	CTCGAAGGACGATGGTGAAT	for sequencing, in third exon .
	pmr6_ex3_as	TCGAGTGAGCTGGTCTACGA	for sequencing, in third exon .
	pmr6_ex4_s	AGGTACGACGAGAGGAAGCA	for sequencing, in fourth exon .
	5UTR1_s	ACCCACTCATCAAAGCGTTC	for sequencing of promotorregion
	5UTR1_as	TTAGTGGCGGAGAGGAGTGT	for sequencing, in 5'UTR
	5UTR2_s	AACCCGAAACCCCAAGTTAC	for sequencing of promotorregion
	intr2.1_s	TGTTGTTGTTGTTATATGGTGTGG	for sequencing, in second intron.
	intr2.2_as	ATTCACCATCGTCCTTCGAG	for sequencing, in second exon.
	intr3.1_as	AAGAACCTCAAACAAGAAAGAACC	for sequencing, in third intron.
	intr3.2_s	GAAAAAGAAAACTCTAAACTGACGAA	for sequencing, in third intron.
	intr3.3_as	CCAATCGTCCTTAACCTCCA	for sequencing, in third intron.
	intr3.4_s	GGGTGGTGGAAATGTCTGTC	for sequencing, in third intron.
	intr3.5_as	TGCTTCCTCTCGTCGTACCT	for sequencing, in third intron.
	intr3.6_s	CTGATCCTAGCGCCAAAGAG	for sequencing, in second exon.
	intr3.7as	TGAGCTACTTTTTCAAATAAACCAA	for sequencing, in third intron.
	PMR6 _F-GW	GWF-GCCACC-ATGCTTCTTCAAAACTTCTCC	Gateway-Primer
	PMR6 _R-GW	GWR-TCACAATAATAGAGTTGATAACGAC	Gateway-Primer
other	Frbp-8-R	AAA CTT GGT TTC TTG GTC TAT AGT TTT	for sequencing
candidate	Frbp-7-R	TGA GTT ACC TCA GTC GGT TGG	for sequencing
genes	Frbp-6-R	CTT GCG TGA GGA CAA ACT CA	for sequencing
	Frbp-5-R	CCA ACA AAA GCA GAG CAT GT	for sequencing
	Frbp-4-F	TAT GCG AGT GGA GTG AGG TG	for sequencing
	Frbp-2-F	CCG CAA GGT ACA TTG GAA GT	for sequencing
	Frbp-1-F	AGT GGA ACC CAC ATG AAT CG	for sequencing
	Pal2-10-R	CAA CAA CAC TAA CAT TGT CC	for sequencing
	Pal2-9-R	AAG AGC CGG TGT GAA AGC TA	for sequencing
	Pal2-8-R	TTG AGA AAG CCG GAA TCA GT	for sequencing
	Pal2-7-R	GCG ATT CAC GGT GGT AAC TT	for sequencing
	Pal2-6-R	ACG GTG AGT TAC ATC CGT CA	for sequencing
	Pal2-5-F	TTG GTT CTC CGG TGA GAA GT	for sequencing
	Pal2-4-F	GTC CGG CGA TGT AAG AGA GA	for sequencing
	Pal2-3-F	GCC TTG TTC CTC GAA ACA TC	for sequencing
	Pal2-2-F	GCA AGT CCT TCT CGC AAA AC	for sequencing
	Pal2-1-F	TTG TCA ACG GTG TCA AAT CC	for sequencing
	PSII-6-F	GGA CTG TGA CTG CTG CGT AA	for sequencing
	PSII-5-F	CGT TAG CCT CTT TGG TGC TC	for sequencing
	PSII-4-R	TGG CTA GGG CTA ATA GCT TAG TG	for sequencing
	PSII-3-R	TTA CGC AGC AGT CAC AGT CC	for sequencing
	PSII-2-R	TCC GAC CTT ATC TTA GCG AAA	for sequencing
	PSII-1-F	AAA CTT GAT TTC TCA AAT TAT TCA CTG	for sequencing
RPW8	GW-R81_F	GWF-GCCACC-ATGCCGATTGGTGAGCTTG	Gateway-Primer
analysis	GW-R81_R	GWR-TCAAGCTCTTATTTTACTAC	Gateway-Primer
	GW-R82_F	GWF-GCCACC-ATGATTGCTGAGGTTGCCG	Gateway-Primer
	GW-R82_R	GWR-TCAAGAATCATCACTGCAGAAC	Gateway-Primer
	R81_5UTRa	TTCACCTCGAGAGCTAAACAAC	for sequencing
	R82_5UTRa	TCTTCACCTCGAGAGCTAACAA	for sequencing
reference	Actin1_F	TGCGACAATGGAACTGGAATG	for Real-Time and RT-PCR
gene	Actin1_R	CTGTCTCGAGTTCCTGCTCG	for Real-Time and RT-PCR
NahG	NahG_F	ATG AAA AAC AAT AAA CTT GGC TTG C	for crossing to NahG plants
	NahG_R	GCG TCG ATG AAA TCC GCC CG	

Table SD 4 : Quantitative assessment of percentage of secondary hyphal growth at 48 hpi.

	total host cell entry		branched hypae		one hypha	
	mean*	**s.d.***	**mean***	**s.d.***	**mean***	**s.d.***
Col-0	77,2	5,6	60,1	1,6	17,1	4,1
Bay-0	75,9	-	58,2	-	17,7	-
pmr6-3	63,7	-	45,7	-	18	-
Ms-0	31,6	12	7,1	12,9	24,4	1
Triple	0	0	0	0	0	0
Ang-0	68,9	-	26,3	-	42,6	-
Co-1	56,3	-	21,3	-	0	-
Do-0	44	15,8	11,2	22,6	32,9	6,9
La-1	49,8	-	18,3	-	31,4	-
Nok-3	65,9	-	32,9	-	32,9	-
Sha	63,9	1,9	41,2	0,6	22,7	2,5
Sorbo	66,6	1	44	10,3	22,6	11,3
Sha x Col-0 F_1	66,9	4,5	45,7	3	21,2	7,6
Sorbo x Col-0 F_1	71	6,7	44,8	11,3	26,3	4,6

* results are presented as mean valued of two independent experiments, except for lines where s.d. is represented with "-". For these accessions, only one experiment was performed.

Table SD 5: Analysis of hyphal growth of *G. orontii* at 24, 48 and 63 hpi

	Pixels per Image					
	24 hpi		**48 hpi**		**63 hpi**	
accession	**mean**	**s.d.**	**mean**	**s.d.**	**mean**	**s.d.**
Col-0	814,4	348,1	4642,0	1845,5	12364,7	3927,1
Ms-0	838,9	318,0	3375,5	1997,2	5832,8	2254,3
Do-0	909,2	305,7	2883,8	1600,5	7225,7	3708,0
Sha	803,4	259,5	4022,4	1808,4	11776,1	3411,6
Sha F_1	967,7	334,1	6279,1	1855,3	10330,8	3831,2
Sorbo	754,1	271,2	4872,4	1733,6	9289,6	2805,1
Sorbo F_1	1005,7	337,7	4000,5	1538,9	10267,6	3124,8

s.d. standard deviation.
Three plants per line and ~ 30 colonies per line and timepoint were analyzed.
The amount of pixels per fungal colony was determined with the HyphArea.

Table SD 6: Conidiophores per colony

	Conidophores per colony	
accession	**mean***	**s.d.***
Col-0	214,5	97,3
pmr6-3	4,2	5,8
Ms-0	0	0
Ang-0	3	6,3
Co-1	3	5,6
Do-0	4	8
La-1	0	0,1
Nok-3	1,6	3
Sha	5,6	10,8
Sorbo	17,3	26,6
Sorbo F_1	173,2	102,1
Sha F_1	120,1	78,1

* results are presented as mean values and standard deviation of one experiment.

Table SD 7: H_2O_2 accumulation in cells attacked by *G. orontii*

	positive DAB in %	
accession	**mean**	**s.d.**
Col-0	17,9	5,3
Bay-0	5,2	1,4
pmr6-3	13,9	0,9
Ms-0	81	3,8
Ang-0	70,1	6,9
Co-1	79,4	0,7
Do-0	79	2,7
La-1	96,7	0,6
Nok-3	81,9	1
Sha	80,7	13,5
Sorbo	88,8	0,5

Accessions were inoculated with *G. orontii* and analyzed for DAB production of infected tissue at 48 hpi. Three plants per line were analyzed microscopically, the data represents the results of one experiment.

Table SD 8: Cell death in resistant accessions visualized by Trypan Blue staining

	massive cell death		medium cell death	
accession	**mean***	**s.d.**	**mean***	**s.d.**
Col-0	2,2	3,1	4,1	3,4
pmr6-3	0,0	0,0	16,8	5,9
Bay-0	61,5	8,1	38,0	8,7
Ms-0	83,1	9,8	16,9	9,8
Ang-0	84,5	12,5	15,5	12,5
Co-1	78,5	12,0	21,5	12,0
Do-0	90,0	2,9	9,1	4,2
La-1	73,7	6,1	26,3	6,1
Nok-3	8,6	0,8	91,4	0,8
Sha	11,6	8,7	85,7	8,3
Sorbo	68,2	5,0	31,0	6,2

* results are mean values and standard deviation of two plants in one experiment.

Table SD 9: Infection phenotypes of *RPW8*- dsRNAi T_1 plants with *G. orontii*

Accession	gene in vector	tray[1]	total number of T_1 plants	susceptible[2] T_1 plants	
				number	name
An-1	*RPW8.1*	E	2	0	-
Ang-0	*RPW8.1*	C	16	0	-
Ang-0	*RPW8.1*	H	3	0	-
Bay-0	*RPW8.1*	C	21	21	T_1.1 - T_1.21
Bu-0	*RPW8.1*	C	20	20	-
Bu-0	*RPW8.1*	E	16	2	T_1.2, T_1.3 [3]
Bu-0	*RPW8.2*	i	13	0	-
Bu-15	*RPW8.1*	B	10	0	-
Bu-15	*RPW8.1*	i	4	0	-
Bu-17	*RPW8.1*	B	22	3	T_1.9, T_1.15, T_1.16
Bu-3	*RPW8.1*	i	16	0	-
C24	*RPW8.1*	C	8	0	-
C24	*RPW8.1*	M	14	0	-
C24	*RPW8.2*	M	6	4	T_1.1, T_1.2, T_1.5, T_1.6 [4]
Can-0	*RPW8.1*	J	24	0	-
Can-0	*RPW8.2*	M	5	0	-
Co-1	*RPW8.1*	M	8	0	-
Co-1	*RPW8.1*	A	10	0	-
Co-3	*RPW8.1*	A	5	0	-
Co-3	*RPW8.2*	A	1	1	T_1.1
Col-0	*RPW8.1*	E	7	7	T_1.1 - T_1.7
Col-0	*RPW8.2*	H	1	1	T_1.1
Col-0	*RPW8.2*	M	1	1	T_1.1
Do-0	*RPW8.1*	A	2	1	T_1.2
Do-0	*RPW8.2*	G	1	0	-
Do-0	*RPW8.1*	M	7	0	-
Ei-2	*RPW8.1*	i	16	0	-
Ei-4	*RPW8.1*	H	12	0	-
Ei-4	*RPW8.1*	i	11	0	-
Ei-4	*RPW8.2*	J	1	1	T_1.1
Ei-5	*RPW8.1*	i	1	0	-
Ei-5	*RPW8.2*	i	1	1	T_1.1
Fr-5	*RPW8.1*	A	7	1	T_1.3
Fr-5	*RPW8.1*	B	1	0	-
Fr-5	*RPW8.1*	i	1	0	-
Ha-0	*RPW8.1*	G	16	0	-
Hl-0	*RPW8.1*	A	22	0	-
Is-0	*RPW8.1*	D	18	18	all DR 1
Je-0	*RPW8.2*	i	6	0	-
Je-0	*RPW8.1*	K	16	0	-
Kas-1	*RPW8.1*	F	24	0	-
Kas-1 Sh[5]	*RPW8.1*	J	7	0	-
Kas-1 Sh[5]	*RPW8.2*	M	1	1	T_1.1
La-1	*RPW8.1*	D	27	0	-

T_1 plants recovered after Basta selection were inoculated with *G. orontii*

Trays contain controls Col-0 and Sorbo also growing in the Basta greenhouse.

[1] Tray L does not exist.

[2] susceptible indicates DR 3 or DR 2.

[3] have a different leaf shape compared to the other T_1 plants of Bu-0.

[4] susceptible T_1 from C24 are bigger and have more trichomes than untransformed C24.

[5] Kas-1 (Kashmir-1) obtained from Shauna Somerville's lab.

Table SD 9 Continued

Accession	gene in vector	tray[1]	total number of T_1 plants	susceptible[2] T_1 plants	
				number	name
La-1	*RPW8.1*	H	5	0	-
Ler	*RPW8.1*	A	1	1	T_1.1
Ler	*RPW8.1*	B	2	1	T_1.2
Ler	*RPW8.1*	K	12	9	T_1.1 - T_1.12 except T_1.4, T_1.9, T_1.11
Ms	*RPW8.1*	H	12	0	-
Ms-0	*RPW8.1*	H	12	0	-
Ms-0	*RPW8.1*	M	1	0	-
Ms-0	*RPW8.1*	M	18	0	-
Nok-3	*RPW8.1*	D	4	2	T_1.1, T_1.3
Nok-3	*RPW8.1*	H	5	0	-
Nok-3	*RPW8.1*	J	1	0	-
Nw-0	*RPW8.2*	B	2	0	-
Nw-0	*RPW8.1*	B	21	0	-
Ob-0	*RPW8.1*	E	12	1	-
Ob-0	*RPW8.1*	G	12	2	T_1.1, T_1.8 DR 1
Ob-0	*RPW8.1*	J	10	0	-
Ove-0	*RPW8.1*	A	24	5	-
Ove-0	*RPW8.1*	H	11	0	-
Petergof	*RPW8.1*	i	11	0	-
Pla-2	*RPW8.1*	K	16	0	-
Pla-3	*RPW8.1*	B	1	0	-
Pla-3	*RPW8.1*	J	8	0	-
Pla-4	*RPW8.1*	K	16	0	-
Rak-2	*RPW8.1*	H	4	0	-
Sendai-3	*RPW8.1*	H	6	0	-
Sha	*RPW8.2*	A	3	3	T_1.1, T_1.2, T_1.3
Sha	*RPW8.1*	C	24	7	T_1.12, T_1.15, T_1.16, T_1.18, T_1.21, T1.22, T1.24
Sha	*RPW8.2*	i	1	0	-
Sha	*RPW8.1*	M	16	3	T_1.7, T_1.11, T_1.13
Sorbo	*RPW8.1*	A	1	0	-
Sorbo	*RPW8.1*	H	7	0	-
Sorbo	*RPW8.2*	i	1	0	-
Sorbo	*RPW8.1*	K	18	0	-
Ts-7	*RPW8.1*	H	2	0	-
Ts-7	*RPW8.1*	H	1	0	-
Wa-1	*RPW8.1*	A	20	0	-
Wt-2	*RPW8.2*	A	3	0	-
Wt-2	*RPW8.1*	G	19	0	-
Wt-3	*RPW8.1*	H	6	0	-

Table SD 10: Results of T_1 plants in semi-quantitative RT-PCR of *RPW8* -dsRNAi

Accession	gene in construct	T_1 plant	Tray	DR* T_1 plant	tested with	low in RT-PCR
Ang-0	*RPW8.1*	T1.1	H	0	*RPW8.1*	+
Ang-0	*RPW8.1*	T1.1	C	0	*RPW8.1*	+
Ang-0	*RPW8.1*	T1.2	H	0	*RPW8.1*	-
Ang-0	*RPW8.1*	T1.2	C	0	*RPW8.1*	-
Ang-0	*RPW8.1*	T1.3	C	0	*RPW8.1*	-
Ang-0	*RPW8.1*	T1.5	C	0	*RPW8.1*	-
Ang-0	*RPW8.1*	T1.6	C	0	*RPW8.1*	-
Ang-0	*RPW8.1*	T1.7	C	0	*RPW8.1*	+
Ang-0**	*RPW8.1*	T1.8	C	0	*RPW8.1*	+
Ang-0**	*RPW8.1*	T1.8	C	0	*RPW8.1*	-
Ang-0	*RPW8.1*	T1.10	C	0	*RPW8.1*	-
Ang-0	*RPW8.1*	T1.12	C	0	*RPW8.1*	-
Ang-0	*RPW8.1*	T1.13	C	0	*RPW8.1*	-
Ang-0	*RPW8.1*	T1.14	C	0	*RPW8.1*	+
Ang-0	*RPW8.1*	T1.16	C	0	*RPW8.1*	-
Bu-0	*RPW8.1*	T1.1	C	2	*RPW8.1*	+
Bu-0	*RPW8.1*	T1.4	C	2	*RPW8.1*	+
Bu-0	*RPW8.1*	T1.5	C	2	*RPW8.1*	+
C24	*RPW8.1*	T1.1	M	0	*RPW8.1*	-
C24	*RPW8.1*	T1.2	C	0	*RPW8.1*	-
C24**	*RPW8.1*	T1.2	M	0	*RPW8.1*	-
C24**	*RPW8.1*	T1.2	M	0	*RPW8.1*	-
C24**	*RPW8.1*	T1.3	C	0	*RPW8.1*	-
C24**	*RPW8.1*	T1.3	C	0	*RPW8.1*	-
C24	*RPW8.1*	T1.4	C	0	*RPW8.1*	-
C24**	*RPW8.1*	T1.4	M	0	*RPW8.1*	-
C24**	*RPW8.1*	T1.4	M	0	*RPW8.1*	-
C24	*RPW8.1*	T1.5	C	0	*RPW8.1*	+
C24	*RPW8.1*	T1.5	M	0	*RPW8.1*	-
C24	*RPW8.1*	T1.6	C	0	*RPW8.1*	-
C24	*RPW8.1*	T1.6	M	0	*RPW8.1*	+
C24	*RPW8.1*	T1.7	C	0	*RPW8.1*	+
C24	*RPW8.1*	T1.7	M	2	*RPW8.1*	-
C24	*RPW8.1*	T1.8	C	0	*RPW8.1*	-
C24	*RPW8.1*	T1.8	M	0	*RPW8.1*	-
C24	*RPW8.1*	T1.9	M	0	*RPW8.1*	-
C24	*RPW8.1*	T1.11	M	0	*RPW8.1*	-
C24	*RPW8.1*	T1.12	M	0	*RPW8.1*	-
C24	*RPW8.1*	T1.13	M	0	*RPW8.1*	-
C24	*RPW8.1*	T1.14	M	0	*RPW8.1*	-
C24	*RPW8.2*	T1.1	M	0	*RPW8.1*	-
C24	*RPW8.2*	T1.3	M	0	*RPW8.1*	-
C24	*RPW8.2*	T1.5	M	0	*RPW8.1*	-
C24	*RPW8.2*	T1.6	M	0	*RPW8.1*	-
Co-1**	*RPW8.1*	T1.1	M	0	*RPW8.1*	-
Co-1**	*RPW8.1*	T1.1	M	0	*RPW8.1*	+
Co-1	*RPW8.1*	T1.1	A	0	*RPW8.1*	+

* DR is disease resistance score, DR 3 is fully susceptible and DR 0 fully resistant.
** plants with more than one RNA-preparation.
*** Tray unknown.

Table SD 10 Continued

Accession	gene in construct	T_1 plant	Tray	DR* T_1 plant	tested with	low in RT-PCR
Co-1	*RPW8.1*	T1.2	A	0	*RPW8.1*	+
Co-1	*RPW8.1*	T1.3	M	0	*RPW8.1*	+
Co-1	*RPW8.1*	T1.4	A	0	*RPW8.1*	-
Co-1	*RPW8.1*	T1.4	M	0	*RPW8.1*	-
Co-1	*RPW8.1*	T1.5	A	0	*RPW8.1*	+
Co-1	*RPW8.1*	T1.5	M	0	*RPW8.1*	+
Co-1	*RPW8.1*	T1.6	M	0	*RPW8.1*	+
Co-1	*RPW8.1*	T1.7	M	0	*RPW8.1*	-
Co-1	*RPW8.1*	T1.8	M	0	*RPW8.1*	+
Co-1	*RPW8.1*	T1.9	A	0	*RPW8.1*	-
Co-3	*RPW8.2*	T1.1	A	2	*RPW8.1*	-
Col-0**	*RPW8.1*	T1.1	E	3	*RPW8.1*	+
Col-0**	*RPW8.1*	T1.1	E	3	*RPW8.1*	+
Col-0	*RPW8.1*	T1.2	E	3	*RPW8.1*	+
Col-0	*RPW8.1*	T1.5	E	3	*RPW8.1*	+
Col-0	*RPW8.1*	T1.6	E	3	*RPW8.1*	+
Col-0	*RPW8.1*	T1.7	E	3	*RPW8.1*	+
Col-0	*RPW8.2*	T1.1	H	0	*RPW8.1*	+
Col-0	*RPW8.2*	T1.1	M	3	*RPW8.1*	+
Do-0	*RPW8.1*	T1.1	A	0	*RPW8.1*	+
Do-0	*RPW8.1*	T1.1	M	0	*RPW8.1*	+
Do-0**	*RPW8.1*	T1.2	A	2	*RPW8.1*	+
Do-0**	*RPW8.1*	T1.2	A	2	*RPW8.1*	-
Do-0	*RPW8.1*	T1.2	M	0	*RPW8.1*	+
Do.0	*RPW8.1*	T1.3	M	0	*RPW8.1*	+
Do-0	*RPW8.1*	T1.4	M	0	*RPW8.1*	+
Do-0	*RPW8.1*	T1.5	M	0	*RPW8.1*	+
Do-0	*RPW8.2*	T1.1	G	0	*RPW8.1*	-
Ei-4	*RPW8.1*	T1.9	H	0	*RPW8.1*	+
Ei-4	*RPW8.2*	T1.1	J	3	*RPW8.1*	+
Ei-5	*RPW8.2*	T1.1	i	3	*RPW8.1*	+
Kas-1 Sh	*RPW8.2*	T1.1	M	3	*RPW8.1*	+
La-1**	*RPW8.1*	T1.1	H	0	*RPW8.1*	-
La-1**	*RPW8.1*	T1.1	H	0	*RPW8.1*	-
La-1**	*RPW8.1*	T1.1	H	0	*RPW8.1*	-
La-1	*RPW8.1*	T1.2	***	0	*RPW8.1*	-
La-1	*RPW8.1*	T1.3	H	0	*RPW8.1*	-
La-1**	*RPW8.1*	T1.5	H	0	*RPW8.1*	+
La-1**	*RPW8.1*	T1.5	H	0	*RPW8.1*	+
La-1	*RPW8.1*	T1.7	D	0	*RPW8.1*	-
La-1	*RPW8.1*	T1.8	D	0	*RPW8.1*	-
La-1	*RPW8.1*	T1.10	D	0	*RPW8.1*	+
La-1	*RPW8.1*	T1.13	D	0	*RPW8.1*	-
La-1	*RPW8.1*	T1.14	D	0	*RPW8.1*	-
La-1	*RPW8.1*	T1.15	D	0	*RPW8.1*	-
La-1	*RPW8.1*	T1.16	D	0	*RPW8.1*	-
La-1	*RPW8.1*	T1.17	D	0	*RPW8.1*	-

Table SD 10 Continued

Accession	gene in construct	T_1 plant	Tray	DR* T_1 plant	tested with	low in RT-PCR
La-1	*RPW8.1*	T1.18	D	0	*RPW8.1*	-
La-1	*RPW8.1*	T1.19	D	0	*RPW8.1*	-
La-1	*RPW8.1*	T1.20	D	0	*RPW8.1*	-
La-1	*RPW8.1*	T1.21	D	0	*RPW8.1*	+
La-1	*RPW8.1*	T1.22	D	0	*RPW8.1*	-
La-1	*RPW8.1*	T1.23	D	0	*RPW8.1*	-
Ms	*RPW8.1*	T1.1	H	0	*RPW8.1*	-
Ms**	*RPW8.1*	T1.2	H	0	*RPW8.1*	+
Ms**	*RPW8.1*	T1.2	H	0	*RPW8.1*	+
Ms	*RPW8.1*	T1.3	H	0	*RPW8.1*	-
Ms	*RPW8.1*	T1.4	H	0	*RPW8.1*	-
Ms	*RPW8.1*	T1.5	H	0	*RPW8.1*	-
Ms**	*RPW8.1*	T1.6	H	0	*RPW8.1*	-
Ms**	*RPW8.1*	T1.6	H	0	*RPW8.1*	-
Ms	*RPW8.1*	T1.7	H	0	*RPW8.1*	-
Ms	*RPW8.1*	T1.8	H	0	*RPW8.1*	+
Ms	*RPW8.1*	T1.9	H	0	*RPW8.1*	-
Ms	*RPW8.1*	T1.9	H	0	*RPW8.1*	-
Ms**	*RPW8.1*	T1.10	H	0	*RPW8.1*	-
Ms**	*RPW8.1*	T1.10	H	0	*RPW8.1*	+
Ms**	*RPW8.1*	T1.11	H	0	*RPW8.1*	-
Ms**	*RPW8.1*	T1.11	H	0	*RPW8.1*	+
Ms**	*RPW8.1*	T1.11	H	0	*RPW8.1*	-
Ms	*RPW8.1*	T1.12	H	0	*RPW8.1*	+
Ms-0	*RPW8.1*	T1.1	H	0	*RPW8.1*	-
Ms-0	*RPW8.1*	T1.1	M	0	*RPW8.1*	-
Ms-0	*RPW8.1*	T1.2	M	0	*RPW8.1*	-
Ms-0	*RPW8.1*	T1.2	H	0	*RPW8.1*	+
Ms-0	*RPW8.1*	T1.3	M	0	*RPW8.1*	-
Ms-0	*RPW8.1*	T1.4	H	0	*RPW8.1*	-
Ms-0	*RPW8.1*	T1.4	M	0	*RPW8.1*	+
Ms-0	*RPW8.1*	T1.5	H	0	*RPW8.1*	-
Ms-0	*RPW8.1*	T1.5	M	0	*RPW8.1*	-
Ms-0**	*RPW8.1*	T1.6	M	0	*RPW8.1*	-
Ms-0**	*RPW8.1*	T1.6	M	0	*RPW8.1*	-
Ms-0	*RPW8.1*	T1.7	H	0	*RPW8.1*	-
Ms-0**	*RPW8.1*	T1.7	M	0	*RPW8.1*	+
Ms-0**	*RPW8.1*	T1.7	M	0	*RPW8.1*	+
Ms-0	*RPW8.1*	T1.8	H	0	*RPW8.1*	-
Ms-0**	*RPW8.1*	T1.8	M	0	*RPW8.1*	-
Ms-0**	*RPW8.1*	T1.8	M	0	*RPW8.1*	+
Ms-0	*RPW8.1*	T1.9	H	0	*RPW8.1*	-
Ms-0	*RPW8.1*	T1.9	M	0	*RPW8.1*	+
Ms-0	*RPW8.1*	T1.10	H	0	*RPW8.1*	-
Ms-0	*RPW8.1*	T1.10	M	0	*RPW8.1*	-
Ms-0	*RPW8.1*	T1.11	H	0	*RPW8.1*	+

Table SD 10 Continued

Accession	gene in construct	T_1 plant	Tray	DR* T_1 plant	tested with	low in RT-PCR
Ms-0	*RPW8.1*	T1.11	M	0	*RPW8.1*	-
Ms-0	*RPW8.1*	T1.12	H	0	*RPW8.1*	-
Ms-0**	*RPW8.1*	T1.12	M	0	*RPW8.1*	-
Ms-0**	*RPW8.1*	T1.12	M	0	*RPW8.1*	-
Ms-0	*RPW8.1*	T1.14	M	0	*RPW8.1*	-
Ms-0	*RPW8.1*	T1.16	M	0	*RPW8.1*	-
Ms-0	*RPW8.1*	T1.17	M	0	*RPW8.1*	-
Ms-0**	*RPW8.1*	T1.18	M	0	*RPW8.1*	-
Ms-0**	*RPW8.1*	T1.18	M	0	*RPW8.1*	-
Ms-0	*RPW8.1*	T1.16	M	0	*RPW8.1*	-
Ms-0	*RPW8.1*	T1.14	M	0	*RPW8.1*	-
Ms-0	*RPW8.2*	T1.1	M	0	*RPW8.1*	-
Nok-3**	*RPW8.1*	T1.1	H	0	*RPW8.1*	-
Nok-3**	*RPW8.1*	T1.1	H	0	*RPW8.1*	-
Nok-3	*RPW8.1*	T1.1	D	3	*RPW8.1*	+
Nok-3	*RPW8.1*	T1.2	H	0	*RPW8.1*	+
Nok-3	*RPW8.1*	T1.2	D	0	*RPW8.1*	-
Nok-3	*RPW8.1*	T1.3	H	0	*RPW8.1*	-
Nok-3	*RPW8.1*	T1.4	H	0	*RPW8.1*	-
Nok-3	*RPW8.1*	T1.4	D	1	*RPW8.1*	+
Nok-3	*RPW8.1*	T1.5	H	0	*RPW8.1*	-
Ove-0	*RPW8.1*	T1.5	A	2	*RPW8.1*	-
Ove-0	*RPW8.1*	T1.8	A	2	*RPW8.1*	+
Ove-0	*RPW8.1*	T1.10	A	2	*RPW8.1*	+
Ove-0	*RPW8.1*	T1.11	H	0	*RPW8.1*	-
Sha	*RPW8.1*	T1.1	C	0	*RPW8.1*	+
Sha	*RPW8.1*	T1.1	M	0	*RPW8.1*	+
Sha	*RPW8.1*	T1.2	C	0	*RPW8.1*	-
Sha	*RPW8.1*	T1.2	M	0	*RPW8.1*	+
Sha	*RPW8.1*	T1.3	C	0	*RPW8.1*	+
Sha	*RPW8.1*	T1.3	M	0	*RPW8.1*	+
Sha	*RPW8.1*	T1.4	M	0	*RPW8.1*	-
Sha	*RPW8.1*	T1.5	M	0	*RPW8.1*	+
Sha	*RPW8.1*	T1.6	C	0	*RPW8.1*	+
Sha	*RPW8.1*	T1.6	M	0	*RPW8.1*	-
Sha	*RPW8.1*	T1.7	C	0	*RPW8.1*	-
Sha	*RPW8.1*	T1.7	M	2	*RPW8.1*	-
Sha	*RPW8.1*	T1.8	C	0	*RPW8.1*	-
Sha	*RPW8.1*	T1.8	M	0	*RPW8.1*	+
Sha	*RPW8.1*	T1.9	C	0	*RPW8.1*	-
Sha	*RPW8.1*	T1.9	M	0	*RPW8.1*	+
Sha	*RPW8.1*	T1.10	C	0	*RPW8.1*	-
Sha	*RPW8.1*	T1.10	M	0	*RPW8.1*	+
Sha	*RPW8.1*	T1.11	C	0	*RPW8.1*	-
Sha	*RPW8.1*	T1.11	M	2	*RPW8.1*	+
Sha	*RPW8.1*	T1.12	C	2	*RPW8.1*	-

Table SD 10 Continued

Accession	gene in construct	T_1 plant	Tray	DR* T_1 plant	tested with	low in RT-PCR
Sha	*RPW8.1*	T1.12	M	0	*RPW8.1*	-
Sha	*RPW8.1*	T1.12	C	2	*RPW8.1*	+
Sha	*RPW8.1*	T1.13	C	0	*RPW8.1*	-
Sha	*RPW8.1*	T1.13	M	2	*RPW8.1*	+
Sha	*RPW8.1*	T1.14	C	0	*RPW8.1*	-
Sha	*RPW8.1*	T1.14	M	0	*RPW8.1*	+
Sha	*RPW8.1*	T1.15	C	2	*RPW8.1*	-
Sha	*RPW8.1*	T1.15	M	0	*RPW8.1*	+
Sha	*RPW8.1*	T1.16	C	2	*RPW8.1*	+
Sha	*RPW8.1*	T1.16	M	0	*RPW8.1*	-
Sha	*RPW8.1*	T1.18	C	2	*RPW8.1*	+
Sha	*RPW8.1*	T1.19	C	0	*RPW8.1*	+
Sha	*RPW8.1*	T1.20	C	0	*RPW8.1*	+
Sha	*RPW8.1*	T1.21	C	2	*RPW8.1*	-
Sha	*RPW8.1*	T1.22	C	2	*RPW8.1*	+
Sha	*RPW8.1*	T1.23	M	0	*RPW8.1*	-
Sha	*RPW8.1*	T1.24	C	2	*RPW8.1*	+
Sha	*RPW8.2*	T1.1	i	2	*RPW8.1*	-
Sha	*RPW8.2*	T1.1	i	2	*RPW8.1*	-
Sha	*RPW8.2*	T1.1.	A	2	*RPW8.1*	+
Sha	*RPW8.2*	T1.2	A	2	*RPW8.1*	-
Sha	*RPW8.2*	T1.3	A	2	*RPW8.1*	+
Sha	*RPW8.2*	T1.2	A	2	*RPW8.1*	-
Sha	*RPW8.2*	T1.3	A	2	*RPW8.1*	+
Sorbo	*RPW8.1*	T1.1	H	0	*RPW8.1*	-
Sorbo**	*RPW8.1*	T1.1	A	0	*RPW8.1*	+
Sorbo**	*RPW8.1*	T1.1	A	0	*RPW8.1*	+
Sorbo	*RPW8.1*	T1.2	***	0	*RPW8.1*	-
Sorbo	*RPW8.1*	T1.3	H	0	*RPW8.1*	-
Sorbo	*RPW8.1*	T1.4	H	0	*RPW8.1*	-
Sorbo	*RPW8.1*	T1.4	K	0	*RPW8.1*	-
Sorbo	*RPW8.1*	T1.5	H	0	*RPW8.1*	-
Sorbo	*RPW8.1*	T1.5	K	0	*RPW8.1*	-
Sorbo	*RPW8.1*	T1.6	H	0	*RPW8.1*	+
Sorbo	*RPW8.1*	T1.6	K	0	*RPW8.1*	-
Sorbo	*RPW8.1*	T1.7	H	0	*RPW8.1*	-
Sorbo	*RPW8.1*	T1.7	K	0	*RPW8.1*	-
Sorbo	*RPW8.1*	T1.8	K	0	*RPW8.1*	-
Sorbo	*RPW8.1*	T1.13	K	0	*RPW8.1*	-
Sorbo	*RPW8.1*	T1.14	K	0	*RPW8.1*	-
Sorbo	*RPW8.1*	T1.15	K	0	*RPW8.1*	-
Sorbo	*RPW8.1*	T1.16	K	0	*RPW8.1*	-
Sorbo	*RPW8.2*	T1.1	i	0	*RPW8.1*	-

Table SD 11: Results of T_1 plants in Real Time PCR of *RPW8* -dsRNAi

						lowered transcript in								
						RT	Real-Time							
	gene in						1st				2nd			
Accession	construct	T_1 plant	Tray	DR*	T_1			value	from	to	low	value	from	to
Ang-0	*RPW8.1*	T1.1	C	0	+	+		0,39	0,28	0,53	+	0,11	0,07	0,18
Ang-0	*RPW8.1*	T1.8	C	0	+	+		0,06	0,03	0,11	+	0,06	0,03	0,10
Ang-0***	*RPW8.1*	T1.14	C	0	+	+		0,06	0,04	0,08	+	0,11	0,00	0,00
Bu-0	*RPW8.1*	T1.1	C	2	+	+		0,44	0,31	0,63	n.p.	n.p.	n.p.	n.p.
Bu-0	*RPW8.1*	T1.4	C	2	+	+		0,80	0,47	1,37	n.p.	n.p.	n.p.	n.p.
Bu-0	*RPW8.1*	T1.5	C	2	+	+		0,67	0,26	1,68	n.p.	n.p.	n.p.	n.p.
C24	*RPW8.1*	T1.1	M	0	-	+		0,03	0,02	0,04	n.p.	n.p.	n.p.	n.p.
C24	*RPW8.1*	T1.5	C	0	+	+		0,00	0,00	0,00	n.p.	n.p.	n.p.	n.p.
C24	*RPW8.1*	T1.6	M	0	+	+		0,04	0,03	0,06	n.p.	n.p.	n.p.	n.p.
Co-1	*RPW8.1*	T1.1	M	0	+	+		0,32	0,24	0,42	+	0,30	0,22	0,43
Co-1	*RPW8.1*	T1.1	A	0	+	-		6,90	4,64	10,24	-	10,65	4,22	26,92
Co-1	*RPW8.1*	T1.3	M	0	+	+		0,06	0,04	0,09	+	0,19	0,09	0,38
Co-1	*RPW8.1*	T1.4	M	0	-	-		1,00	0,37	2,67	-	1,00	0,37	2,71
Co-1	*RPW8.1*	T1.5	A	0	+	+		0,01	0,01	0,01	+	0,01	0,01	0,02
Co-1	*RPW8.1*	T1.6	M	0	+	+		0,27	0,18	0,39	+	0,17	0,00	0,00
Co-1	*RPW8.1*	T1.8	M	0	+	+		0,09	0,06	0,13	+	0,09	0,08	0,10
Do-0	*RPW8.1*	T1.1	A	0	+	+		0,06	0,04	0,08	+	0,00	0,00	0,00
Do-0	*RPW8.1*	T1.1	M	0	+	+		0,75	0,51	1,09	n.a.d.	n.a.d.	n.a.d.	n.a.d.
Do-0	*RPW8.1*	T1.2	A	2	+	+		0,00	0,00	0,00	+	0,17	0,11	0,26
Do-0	*RPW8.1*	T1.2	M	0	+	+		0,28	0,20	0,41	+	0,18	0,09	0,36
Do.0	*RPW8.1*	T1.3	M	0	+	+		0,02	0,02	0,03	+	0,06	0,03	0,10
Do-0	*RPW8.1*	T1.4	M	0	+	-		1,00	0,66	1,51	-	1,00	0,74	1,35
Do-0	*RPW8.1*	T1.5	M	0	+	+		0,01	0,01	0,02	+	0,04	0,02	0,06
Ei-4	*RPW8.2*	T1.1	J	3	+	+		0,00	0,00	0,00	+	0,00	0,00	0,00
Ei-5	*RPW8.2*	T1.1	i	3	+	+		0,00	0,00	0,00	n.p.	n.p.	n.p.	n.p.
Ms-0	*RPW8.1*	T1.1	H	0	-	+		0,00	0,00	0,00	-	2,92	1,88	4,52
Ms-0	*RPW8.1*	T1.2	H	0	+	+		0,24	0,00	98,27	+	0,07	0,05	0,12
Ms-0**	*RPW8.1*	T1.7	M	0	+	+		0,09	0,05	0,15	-	1,83	1,09	3,08
Ms-0**	*RPW8.1*	T1.7	M	0	+	n.a.d.		n.a.d.	n.a.d.	n.a.d.	n.a.d.	0,01	0,00	0,01
Ms-0	*RPW8.1*	T1.8	H	0	-	-		1,00	0,00	>100	-	1,00	0,00	>100
Ms-0	*RPW8.1*	T1.9	M	0	+	+		0,49	0,00	>100	n.a.d.	n.a.d.	n.a.d.	n.a.d.
Ms-0	*RPW8.1*	T1.11	H	0	+	+		0,00	0,00	0,00	-	0,94	0,50	1,76
Nok-3***	*RPW8.1*	T1.1	D	3	+	+		0,07	0,05	0,10	+	0,45	0,00	0,00
Nok-3	*RPW8.1*	T1.2	H	0	+	-		1,77	1,25	2,49	+	0,21	0,14	0,31
Nok-3	*RPW8.1*	T1.4	D	1	+	+		0,00	0,00	0,00	+	0,00	0,00	0,00
Ove-0	*RPW8.1*	T1.5	A	2	-	-		28,71	14,08	58,53	-	1,18	0,84	1,68
Ove-0	*RPW8.1*	T1.8	A	2	+	+		0,11	0,06	0,19	n.a.d.	n.a.d.	n.a.d.	n.a.d.
Ove-0	*RPW8.1*	T1.10	A	2	+	-		1,00	0,86	1,17	-	1,00	0,93	1,08
Sha	*RPW8.1*	T1.1	C	0	+	+		0,03	0,00	0,21	+	0,07	0,01	0,41
Sha	*RPW8.1*	T1.1	M	0	+	+		0,11	0,09	0,14	+	0,15	0,11	0,22
Sha	*RPW8.1*	T1.2	M	0	+	-		1,00	0,90	1,11	n.p.	n.p.	n.p.	n.p.
Sha	*RPW8.1*	T1.3	C	0	+	-		0,89	0,22	3,60	-	1,61	0,66	3,92
Sha	*RPW8.1*	T1.3	M	0	+	-		1,00	0,48	2,07	n.p.	n.p.	n.p.	n.p.
Sha	*RPW8.1*	T1.5	M	0	+	+		0,45	0,26	0,79	+	0,90	0,54	1,49

* DR is disease resistance score, DR3 is fully susceptible and DR 0 fully resistant.

** plants with more than one RNA-preparation.

*** in second Real-Time run no s.d. because only one replicate gave analyzable data.

n.p.: not performed, n.a.d.: no analyzable data

Table SD 11 Continued

					lowered transcript in								
					RT	Real-Time							
	gene in					1st				2nd			
Accession	construct	T_1 plant	Tray	DR*	T_1		value	from	to	low	value	from	to
Sha	*RPW8.1*	T1.6	C	0	+	+	9,48	5,96	15,06	n.p.	n.p.	n.p.	n.p.
Sha	*RPW8.1*	T1.8	M	0	+	-	1,00	0,81	1,23	-	1,00	0,05	20,00
Sha	*RPW8.1*	T1.9	C	0	-	-	4,22	3,16	5,63	n.p.	n.p.	n.p.	n.p.
Sha	*RPW8.1*	T1.9	M	0	+	+	0,51	0,38	0,70	+	0,87	0,67	1,12
Sha	*RPW8.1*	T1.10	M	0	+	-	1,00	0,50	2,01	-	1,00	0,72	1,40
Sha	*RPW8.1*	T1.11	M	2	+	-	1,00	0,86	1,17	-	1,00	0,07	15,23
Sha	*RPW8.1*	T1.12	M	0	-	+	0,30	0,20	0,47	+	0,07	0,04	0,13
Sha	*RPW8.1*	T1.12	C	2	+	n.a.d.	n.a.d.	n.a.d.	n.a.d.	n.a.d.	n.a.d.	n.a.d.	n.a.d.
Sha	*RPW8.1*	T1.13	M	2	+	+	0,54	0,36	0,83	+	0,20	0,14	0,28
Sha	*RPW8.1*	T1.14	M	0	+	+	0,09	0,07	0,11	+	0,02	0,02	0,04
Sha	*RPW8.1*	T1.15	M	0	+	+	0,16	0,11	0,23	+	0,56	0,35	0,90
Sha	*RPW8.1*	T1.16	C	2	+	+	0,02	0,00	0,37	+	0,02	0,01	0,03
Sha	*RPW8.1*	T1.16	M	0	-	+	1,35	0,83	2,21	+	0,21	0,14	0,30
Sha	*RPW8.1*	T1.18	C	2	+	+	0,55	0,45	0,67	n.p.	n.p.	n.p.	n.p.
Sha	*RPW8.1*	T1.19	C	0	+	+	0,23	0,11	0,49	n.p.	n.p.	n.p.	n.p.
Sha	*RPW8.1*	T1.20	C	0	+	+	0,32	0,22	0,48	n.p.	n.p.	n.p.	n.p.
Sha	*RPW8.1*	T1.22	C	2	+	-	1,33	0,80	2,22	-	30,05	0,04	>100
Sha	*RPW8.2*	T1.2	A	2	-	-	0,92	0,23	3,64	n.p.	n.p.	n.p.	n.p.
Sha	*RPW8.2*	T1.3	A	2	+	-	1,00	0,19	5,33	n.p.	n.p.	n.p.	n.p.
Sorbo**	*RPW8.1*	T1.1	A	0	+	+	0,04	0,02	0,07	+	0,29	0,21	0,42
Sorbo**	*RPW8.1*	T1.1	A	0	+	-	1,00	0,59	1,71	-	1,00	0,06	17,95
Sorbo	*RPW8.1*	T1.14	K	0	-	+	0,01	0,00	0,04	+	0,16	0,09	0,27
Sorbo	*RPW8.1*	T1.7	H	0	-	-	2,12	0,42	10,79	-	4,29	2,52	7,30

Table SD 12: Infection phenotypes of T_2 plants of *RPW8* -dsRNAi with *G. orontii*

	gene in				low in	segregation in T_2**		
Accession	**construct**	**T_1 plant**	**Tray**	**DR* T_1 plant**	**RT-PCR**	**susceptible**	**intermediate**	**resistant**
C24	*RPW8.1*	T1.5	C	0	+	0	0	15
C24	*RPW8.1*	T1.6	C	0	-	0	0	15
C24	*RPW8.1*	T1.1	M	0	+	0	0	15
C24	*RPW8.1*	T1.6	M	0	+	0	0	15
Co-1	*RPW8.1*	T1.2	M	0	+	0	0	15
Co-1	*RPW8.1*	T1.3	M	0	+	0	0	16
Co-1	*RPW8.1*	T1.4	M	0	+	0	0	16
Co-1	*RPW8.1*	T1.8	M	0	+	0	0	16
Co-1	*RPW8.1*	T1.1	M	0	+	0	0	15
Co-3	*RPW8.2*	T1.1	A	2	-	15	0	0
Ei-4	*RPW8.1*	T1.9	H	0	+	10	4	0
Ei-4	*RPW8.2*	T1.1	J	3	+	16	0	0
Kas-1 Sh	*RPW8.2*	T1.1	M	3	+	15	0	0
La-1	*RPW8.1*	T1.5	H	0	+	0	0	15
Ms-0	*RPW8.1*	T1.2	H	0	+	0	0	15
Nok-3	*RPW8.1*	T1.1	D	3	+	0	2	13
Ove-0	*RPW8.1*	T1.5	A	2	-	0	0	15
Sha	*RPW8.1*	T1.1	C	0	+	0	2	14
Sha	*RPW8.1*	T1.2	C	0	-	0	0	15
Sha	*RPW8.1*	T1.2	M	0	+	0	0	15
Sha	*RPW8.1*	T1.3	C	0	+	0	2	14
Sha	*RPW8.1*	T1.3	M	0	+	0	0	15
Sha	*RPW8.1*	T1.6	C	0	+	0	2	13
Sha	*RPW8.1*	T1.12	M	0	-	0	0	15
Sha	*RPW8.1*	T1.14	M	0	+	0	0	15
Sha	*RPW8.1*	T1.15	M	0	+	0	0	15
Sha	*RPW8.1*	T1.16	C	2	+	0	1	14
Sha	*RPW8.1*	T1.16	M	0	-	0	0	15
Sha	*RPW8.1*	T1.1	M	0	+	0	0	15
Sha	*RPW8.1*	T1.7	M	2	-	0	0	15
Sha	*RPW8.1*	T1.8	M	0	+	0	0	15
Sha	*RPW8.1*	T1.9	M	0	+	0	0	15
Sha	*RPW8.1*	T1.10	M	0	+	0	0	15
Sha	*RPW8.1*	T1.12	M	2	-	0	0	16
Sha	*RPW8.2*	T1.1.	A	2	+	4	0	10
Sha	*RPW8.2*	T1.2	A	2	-	2	2	10
Sha	*RPW8.2*	T1.3	A	2	+	3	2	10
Sorbo	*RPW8.1*	T1.1	A	0	+	0	0	15

* DR is disease resistance score, DR3 is fully susceptible and DR 0 fully resistant.

** of susceptible or downregulated plants, ~15 T_2 plants were analyzed with *G. orontii.*

VI. LITERATURE CITED

Aarts, N., Metz, M., Holub, E., Staskawicz, B.J., Daniels, M.J., Parker, J.E. (1998). Different requirements for EDS1 and NDR1 by disease resistance genes define at least two *R* gene-mediated signaling pathways in *Arabidopsis*. Proc Natl Acad Sci U S A. **95**(17), 10306-11.

Abbott, R.J. and Gomes, M.F. (1989) Population genetic structure and outcrossing rate of *Arabidopsis thaliana* (L.) Heynh. Heredity. **62**, 411–418.

Abiola, O., Angel, J.M., Avner, P. et al. (2003). Complex Trait Consortium. The nature and identification of quantitative trait loci: a community's view. Nat Rev Genet. **4**(11),911-916.

Abramoff, M.D., Magelhaes, P.J., Ram, S.J. (2004). "Image Processing with ImageJ". Biophotonics International **11**(7), 36-42.

Adai, A., Johnson, C., Mlotshwa, S., Archer-Evans, S., Manocha, V., Vance, V. and Sundaresan, V. (2005). Computational Prediction of miRNAs in *Arabidopsis thaliana*. Genome Res. **15**(1), 78-91.

Adam, L. and Somerville, S.C. (1996). Genetic characterization of five powdery mildew disease resistance loci in *Arabidopsis thaliana* The Plant Journal **9**, 3 341

Adam, L., Ellwood, S., Wilson, I., Saenz, G., Xiao, S., Oliver, R.P., Turner, J.G. and 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. Mol Plant Microbe Interact. **12**(12), 1031-1043.

Aderem, A. and Ulevitch, R. (2000). Toll-like receptors in the induction of the innate immune response. Nature **406,** 782–787.

Allen, R.L., Bittner-Eddy, P.D., Grenville-Briggs, L.J. ,Meitz, J.C., Rehmany, A.P., Rose, L.E. and Beynon, J.L. (2004). Host–parasite co-evolutionary conflict between *Arabidopsis* and downy mildew. Science **306**, 1957–1960.

Alonso-Blanco, C. and Koornneef, M. (2000). Naturally occurring variation in *Arabidopsis*: an underexploited resource for plant genetics. Trends Plant Sci. **5**(1),22-29.

Amano (Hirata), K. (1986). Host range and geographic distribution of the powdery mildew fungi. Japan Scientific Societies Press, Tokyo, Japan. Annual Review of Plant Physiology and Plant Molecular Biology **48**, 251-275.

Apel, K. and Hirt, H. (2004). Reactive oxygen species: metabolism, oxidative stress, and signal transduction. Annu Rev Plant Biol. **55**, 373-99.

Argeson, A. C., Nelson, K. K., Siracusa, L. D. (1996).Molecular Basis of the Pleiotropic Phenotype of Mice Carrying the hypervariable yellow (A(hvy)) Mutation at the agouti Locus. Genetics **142**, 557-567.

Asai, T., Tena, G., Plotnikova, J., Willmann, M.R., Chou, W.-L., Gomez-Gomez, L., Boller, T., Ausubel, F. M., and Sheen, J. (2002). MAP kinase signalling cascade in *Arabidopsis* innate immunity. Nature **415**, 977-983.

Axtell, M.J. and Staskawicz, B.J. (2003). Initiation of RPS2-specified disease resistance in *Arabidopsis* is coupled to the AvrRpt2-directed elimination of RIN4. Cell **112**, 369–377.

Axtell, M.J., Chisholm, S.T., Dahlbeck, D. and Staskawicz, B.J. (2003). Genetic and molecular evidence that the *Pseudomonas syringae* type III effector protein

Bai, Y., van der Hulst, R., Bonnema, G., Marcel, T.C., Meijer-Dekens, F., Niks, R.E. and Lindhout, P. (2005) Tomato defense to *Oidium neolycopersici*: dominant Ol genes confer isolate-dependent resistance via a different mechanism than recessive ol-2. Mol Plant Microbe Interact. **18**(4), 354-62.

Baillieul, F., Genetet, I., Kopp, M., Saindrenan, P., Fritig, B. and Kauffmann, S. (1995). A new elicitor of the hypersensitive response in tobacco: a fungal glycoprotein elicits cell death, expression of defence genes, production of salicylic acid, and induction of systemic acquired resistance. Plant J **8,** 551–560.

Bakker, E.G., Stahl, E.A., Toomajian, C., Nordborg, M., Kreitman, M. and Bergelson, J. (2006). Distribution of genetic variation within and among local populations of *Arabidopsis thaliana* over its species range. Mol. Ecol. **15**, 1405–1418.

Bakker, E.G., Toomajian, C., Kreitman, M., Bergelson, J.A. (2006). Genome-Wide Survey of *R* Gene Polymorphisms in *Arabidopsis*. Plant Cell. (Epub ahead of print).

Banerjee, D., Zhang, X. and Bent, A.F. (2001). The leucine-rich repeat domain can determine effective interaction between RPS2 and other host factors in *Arabidopsis RPS2*-mediated disease resistance. Genetics **158**, 439-450.

Barkan, A. and Martienssen, R.A. (1991). Inactivation of Maize Transposon Mu Suppresses a Mutant Phenotype by Activating an Outward-Reading Promoter Near the End of Mu1. PNAS **88**, 3502-3506.

Barker, C.L., Donald, T., Pauquet, J., Ratnaparkhe, M.B., Bouquet, A., Adam-Blondon, A.F., Thomas, M.B. and Dry, I. (2005). Genetic and physical mapping of the grapevine powdery mildew resistance gene, *Run1*, using a bacterial artificial chromosome library. TAG Theoretical and Applied Genetics **111**(2), 370 – 377.

Barton, G.M. and Medzhitov, R. (2003). Toll-like receptor signalling pathways. Science **300,** 1524–1525.

Beclin, C., Boutet, S., Waterhouse, P., Vaucheret, H. (2002). A branched pathway for transgene-induced RNA silencing in plants. Curr Biol **12**, 684–688.

Bélanger, R. R., Bushnell, W. R., Dik, A J., and Carver, T. L. W. (2002). The Powdery Mildews: A Comprehensive Treatise. APS Press. St. Paul.

Bender, J. and Fink, G.R. (1995). Epigenetic control of an endogenous gene family is revealed by a novel blue fluorescent mutant of *Arabidopsis*. Cell, 83(5), 725-734.

Benson, G. (1999). Tandem repeats finder: a program to analyze DNA sequences. Nucleic Acids Research **27**(2), 573-580.

Bergelson, J., Kreitman, M., Stahl, E.A. and Tian, D.C. (2001). Evolutionary dynamics of plant *R*-genes. Science **292**, 2281-2285.

Bergelson, J., Stahl, E., Dudek, S. and Kreitman, M. (1998) Genetic variation within and among populations of *Arabidopsis thaliana*. Genetics **148**, 1311–1323.

Birnboim, H.C., Doly, J. (1979). A rapid alkaline extraction procedure for screening recombinant plasmid DNA. Nucleic Acids Res. **7**(6), 1513-23.

Bisgrove, S.R., Simonich, M.T., Smith, N.M., Sattler, A. and Innes, R.W. (1994). A disease resistance gene in *Arabidopsis* with specificity for two different pathogen avirulence genes. Plant Cell **6**, 927–933.

Bleecker, A.B., Estelle, M.A., Somerville, C., Kende, H. (1988). Insensitivity to ethylene conferred by a dominant mutation in *Arabidopsis thaliana*. Science **241,** 1086-1089.

Blume, B., Nürnberger, T., Nass, N., Scheel, D. (2000). Receptor-mediated increase in cytoplasmic free calcium required for activation of pathogen defense in parsley. Plant Cell **12,** 1425–1440.

Boedjin, K.B. (1956).Trypan blue as stain for fungi. Staining Technologies **31**(3), 115-116.

Boyd, L.A., Smith, P.H., Foster, E.M., and Brown, J.K.M. (1995). The effects of allelic variation at the *Mla* resistance locus in barley on the early development of *Erysiphe graminis* f. sp. *hordei* and host responses. Plant J. **7**, 959-968.

Braun, U. (1987). A monograph of the Erysiphales (powdery mildews). Beih. Nova Hedwigia. **89**, 1-700.

Braun, U. (1995). The Powdery Mildews (Erysiphales) of Europe. G. Fischer Verlag. Jena.

Brunner, F., Rosahl, S., Lee, J., Rudd, J., Geiler, C., Kauppinen, S., Rasmussen, G., Scheel, D., and Nürnberger, T. (2002). Pep-13, a plant defense-inducing pathogen-associated pattern from *Phytophthora* transglutaminases. EMBO J **21,** 6681-6688.

Bryan, G.T., Wu, K.-S., Farral, Y.J. and Hershey, H.P. (2000). A single amino acid difference distinguishes resistant and susceptible alleles of the rice blast resistance gene *Pi-ta*. Plant Cell **12**, 2033-2045.

Büschges, R., Hollricher, K., Panstruga, R., Simons, G., Wolter, M., Frijters, A., Caicedo, A.L and Schaal B.A. (2004). Heterogenous evolutionary processes affect *R* gene diversity in natural populations of *Solanum pimpinellifolium*. Proc Natl Acad Sci USA **101**, 17444–17449

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

Cao, H.., Bowling, S.A., Gordon, A.S., and Dong, X. (1994). Characterization of an A*rabidopsis* Mutant That Is Nonresponsive to Inducers of Systemic Acquired Resistance. Plant Cell **6,** 1583-1592.

Carrington, J.C. and Ambros, V. (2003). Role of microRNAs in plant and animal development. Science **301**, 336–338.

Century, K.S., Holub, E.B., and Staskawicz, B.J. (1995). *NDR1,* a locus of *Arabidopsis thaliana* that is required for disease resistance to both bacterial and fungal pathogens. Proc. Natl. Acad. Sci. USA **92**, 6597–6601.

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

Cervera, M.-T., Ruiz-García, L. and Martínez-Zapater, J. (2002). Analysis of DNA methylation in *Arabidopsis thaliana* based on methylation-sensitive AFLP markers, Molecular Genetics and Genomics **268** (4), 543 – 552.

Chang, C., Kwok, S.F., Bleecker, A.B., and Meyerowitz, E.M. (1993). *Arabidopsis* ethylene-response gene *ETR1*: similarity of product to two-component regulators. Science **262,** 539-544.

Chang, J.H., Urbach, J.M., Law, T.F., Arnold, L.W., Hu, A., Gombar, S., Grant, S.R., Ausubel, F.M. and Dangl, J.L. (2005). A high-throughput, near-saturating creen for type III effector genes from *Pseudomonas syringae*. Proc Natl Acad Sci U S A. **102**(7), 2549-2554.

Che, F.S., Nakajima, Y., Tanaka, N., Iwano, M., Yoshida, T., Takayama, S., Kadota, I. and Isogai, A. (2000). Flagellin from an incompatible strain of *Pseudomonas avenae* induces a resistance response in cultured rice cells. J Biol Chem **275,** 32347–32356.

Chu, Z., Fu, B., Yang, H., Xu, C., Li, Z., Sanchez, A., Park, Y.J., Bennetzen, J.L., Zhang, W. and Wang, S. (2006). Targeting *xa13,* a recessive gene for bacterial blight resistance in rice. TAG Theoretical and Applied Genetics **112**(3), 455 – 461.

Ciccarese, F., Amenduni, M., Ambrico, A. and Cirulli, M. (2000). The resistance to Oidium lycopersici conferred by ol-2 gene in tomato. Acta Physiol Plant 22, 266.

Ciccarese, F., Amenduni, M., Schiavone, Citrulli, M. (1998). Occurrence and inheritance of resistance to powdery mildew (*Oidium lycopersici*) in *Lycopersicon* species. Plant Pathology **47**(4), 417-419.

Clarke, J.D., Aarts, N., Feys, B.J., Dong, X.N. and Parker, J.E. (2001). Constitutive disease resistance requires *EDS1* in the *Arabidopsis* mutants *cpr1* and *cpr6* and is partially *EDS1*-dependent in *cpr5*. Plant J **26**, 409–420.

Clough, S.J. and Bent, A.F. (1998). Floral dip: a simplified method for *Agrobacterium*-mediated transformation of *Arabidopsis thaliana.* The Plant Journal 16:6 735.

Coaker, G., Falick, A. and Staskawicz, B. (2005). Activation of a phytopathogenic bacterial effector protein by a eukaryotic cyclophilin. Science. **308**(5721), 548-550.

Colum, P., Walsh, J., Chaillet, R. and Bestor, T.H. (1998). Transcription of IAP endogenous retroviruses is constrained by cytosine methylation. Nature Genetics **20**, 116 – 117.

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

Cubas P., Vincent, C. and Coen, E. (1999). An epigenetic mutation responsible for natural variation in floral symmetry. Nature **401**, 157.

Dalmay, T., Hamilton, A., Rudd, S., Angell, S. and Baulcombe, D.C. (2000). An RNA-dependent RNA polymerase gene in *Arabidopsis* is required for posttranscriptional gene silencing mediated by a transgene but not by a virus. Cell **101**, 543–553.

Dangl, J. L. and Jones, J. (2001) Plant pathogens and integrated defence responses to infection. Nature **411**, 826-833.

Davis, S., Lollo, B., Freier, S. and Esau, C. (2006). Improved targeting of miRNA with antisense oligonucleotides. Nucl. Acids Res. **34**, 2294-2304.

Day, B., Dahlbeck, D., Huang, J., Chisholm, S.T., Li, D. and Staskawicz, B.J. (2005). Molecular basis for the RIN4 negative regulation of RPS2 disease resistance. Plant Cell **17** , 1292–1305.

DebRoy, S., Thilmony, R., Kwack, Y.B., Nomura, K. and He S.Y. (2004). A family of conserved bacterial effectors inhibits salicylic acid-mediated basal immunity and promotes disease necrosis in plants, Proc. Natl. Acad. Sci. USA **101**, 9927–9932.

Deslandes, L., Olivier, J., Peeters, N., Feng, D.X., Khounlotham, M., Boucher, C., Somssich, I., Genin, S. and Marco, Y. (2003). Physical interaction between RRS1-R, a protein conferring resistance to bacterial wilt, and PopP2, a type III effector targeted to the plant nucleus. Proc. Natl. Acad. Sci. USA **100**, 8024–8029.

Deslandes, L., Olivier, J., Theulieres, F., Hirsch, J., Feng, D.X., Bittner-Eddy, P., Beynon J., Marco, Y. (2002). Resistance to *Ralstonia solanacearum* in *Arabidopsis thaliana* is conferred by the recessive *RRS1-R* gene, a member of a novel family of resistance genes. Proc Natl Acad Sci U S A **99**(4),2 404-2409.

Dietrich, R.A., Delaney, T.P., Uknes, S.J., Ward, E.R., Ryals, J.A. and Dangl, J.L. (1994). *Arabidopsis* mutants simulating disease resistance response. Cell **77**(4), 565-577.

Keogh, R.C., Deverall, B.J. and McLeod, S. (1980). Comparison of histological and physiological responses to *Phakospora pachyrhizi* in resistant and susceptible soybean. Trans. Br. Mycol. Soc. **74**, 329–333.

Dixon, R.A. (2001). Natural products and plant disease resistance. Nature **411,** 843-847.

Dodds, P.N., Lawrence, G.J. and Ellis, J.G. (2001). Six amino acid changes confined to the leucine-rich repeat beta-strand/beta-turn motif determine the difference between the P and P2 rust resistance specificities in flax. Plant Cell **13**, 163-178.

Dodds, P.N., Lawrence, G.J., Catanzariti, A.M., Ayliffe, M.A. and Ellis, J.G. (2004). The *Melampsora lini* AvrL567 avirulence genes are expressed in haustoria and their products are recognized inside plant cells. Plant Cell **16,** 755–768.

Doerge, R.W. (2002). Mapping and analysis of quantitative trait loci in experimental populations. Nat Rev Genet. **3**(1), 43-52.

Donald, T.M., Pellerone, F., Adam-Blondon, A.-F., Bouquet, A., Thomas, M. R. and Dry, I.B. (2002). Identification of resistance gene analogs linked to a powdery mildew resistance locus in grapevine. TAG Theoretical and Applied Genetics, 104(4), 610 – 618

Dong, X. (1998). Sa, Ja, ethylene, and disease resistance in plants. Curr Opin Plant Biol **1,** 316-323.

Donnelly, M.A. and Steiner, T.S. (2002). Two nonadjacent regions in enteroaggregative *Escherichia coli* flagellin are required for activation of toll-like receptor 5. J Biol Chem **277**, 40456–40461.

Dow, M., Newman, M.-A., von Roepenack, E. (2000). The induction and modulation of plant defense responses by bacterial lipopolysaccharides. Annu Rev Phytopathol **38,** 241–261.

Ebel, J. and Cosio, E.G. (1994). Elicitors of plant defense responses. Int. Rev. Cytol. **148**, 1-36.

Ebrahim-Nesbat, F., Heitefuss, R., and Rohringer, R. (1986). Ultrasctructural and histochemical studies on mildew of barley (*Erysiphe graminis* DC. f.sp. *hordei* Marchal) IV Characterization of papillae in fifth leaves exhibiting adult plant resistance. J. Phytopathol. **117**, 289-300.

Ehlers, J.D., Matthews, W.C., Jr., Hall, A.E., Roberts, P.A. (2000). Inheritance of a Broad-Based Form of Root-Knot Nematode Resistance in Cowpea. Crop Sci **40**, 611-618.

Espinosa, A. and Alfano, J.R. (2004). Disabling surveillance: bacterial type III secretion system effectors that suppress innate immunity, Cell Microbiol **6,** 1027–1040

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

Felix, G. and Boller, T. (2003). Molecular sensing of bacteria in plants. The highly conserved RNA-binding motif RNP-1 of bacterial cold shock proteins is recognized as an elicitor signal in tobacco. J Biol Chem **278,** 6201–6208.

Felix, G., Duran, J.D., Volko, S. and Boller, T. (1999). Plants have a sensitive perception system for the most conserved domain of bacterial flagellin. Plant J **18,** 265–276.

Fellbrich, G., Romanski, A., Varet, A., Blume, B., Brunner, F., Engelhardt, S., Felix, G., Kemmerling, B., Krzymowska, M., Nürnberger, T. (2002). NPP1, a *Phytophthora*-associated trigger of plant defense in parsley and *Arabidopsis*. Plant J **32,** 375–390.

Feys, B. and Parker, J. E. (2000). Interplay of signaling pathways in plant disease resistance . Trends Genet. **16**, 449-455

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

Finnegan, E.J. and Matzke, M.A. (2003). The small RNA world. J Cell Sci **116**, 4689–4693

Flor, H.H. (1971). Current status of the gene-for-gene concept. Annual Revies of Phytopathology **9**, 275-227.

Fritz, J.H., Girardin, S.E. and Philpott, D.J. (2006). Innate Immune Defense Through RNA Interference Sci. STKE **339**, pe27.

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

Frye, C.A., Tang, D. and Innes, R.W. (2001). Negative regulation of defense responses in plants by a conserved MAPKK kinase. PNAS **98**, 373-378.

Glazebrook J. (2001). Genes controlling expression of defense responses in *Arabidopsis*--2001 status. Curr Opin Plant Biol. **4**(4), 301-308.

Glazebrook, J. (2005). Contrasting mechanisms of defense against biotrophic and necrotrophic pathogens. Annual Review of Phytopathology **43**, 205-227.

Glazebrook, J., and Ausubel, F.M. (1994). Isolation of phytoalexin-deficient mutants of *Arabidopsis thaliana* and characterization of their interactions with bacterial pathogens. Proc Natl Acad Sci U S A **91,** 8955-8959.

Glazebrook, J., Rogers, E.E., and Ausubel, F.M. (1996). Isolation of *Arabidopsis* mutants with enhanced disease susceptibility by direct screening. Genetics **143,** 973-982.

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

Gomez-Gomez, L. and Boller, T. (2000). FLS2: an LRR receptor-like kinase involved in the perception of the bacterial elicitor flagellin in *Arabidopsis*. Mol Cell **5,** 1003–1011.

Grant-Downton, R.T. and Dickinson, H.G. (2005) Epigenetics and its implications for plant biology. 1. The epigenetic network in plants.Ann Bot (Lond). Dec; 96(7):1143-64.

Grant-Downton, R.T. and Dickinson, H.G. (2006). Epigenetics and its implications for plant biology 2. The 'epigenetic epiphany': epigenetics, evolution and beyond.Ann Bot (Lond) **97**(1):11-27.

Gray, Y.H.M. (2000). It takes two transposons to tango: transposable-element-mediated chromosomal rearrangements. *Trends in Genetics* **16**: 461–468.

Greenberg, J.T. and Yao, N. (2004). The role and regulation of programmed cell death in plant-pathogen interactions. Cellular Microbiology 6: 201-211

Gu, K., Yang, B., Tian, D., Wu, L., Wang, D., Sreekala, C., Yang, F., Chu, Z., Wang, G.L., White, F. and Yin, Z. (2005). *R* gene expression induced by a type-III effector triggers disease resistance in rice. Nature **435**. 1122–1125.

Gupta, V., Willits, M.J. and Glazebrook, J. (2000). *Arabidopsis thaliana EDS4* contributes to salicylic acid (SA)-dependent expression of defense responses: Evidence for inhibition of jasmonic acid signaling by SA. Mol Plant Microbe Interact **13**, 503–511.

Gustafson, A.M., Allen, E., Givan, S., Smith, D., Carrington, J.C. and Kasschau, K.D. (2005). ASRP: The *Arabidopsis* Small RNA Project Database. Nucleic Acids Res **33**, D637-40.

Guzman, P., and Ecker, J.R. (1990). Exploiting the triple response of *Arabidopsis* to identify ethylene-related mutants. Plant Cell **2,** 513-523.

Hannon, G.J. (2002). RNA interference. Nature **418**, 244–251.

Hauck, P., Thilmony, R. and He, S.Y. (2030). A *Pseudomonas syringae* type III effector suppresses cell wall-based extracellular defense in susceptible *Arabidopsis* plants. Proc. Natl. Acad. Sci. USA **100**, 8577–8582.

Hausmann, N.J., Juenger, T.E., Sen, S. et al. (2005) Quantitative trait loci affecting delta C-13 and response to differential water availability in *Arabidopsis thaliana*. Evolution **59**, 81–96.

Hayashi, F., Smith, K., Ozinsky, A., Hawn, T., Yi, E., Goodlett, D., Eng, J., Akira, S., Underhill, D., and Aderem, A. (2001). The innate immune response to bacterial flagellin is mediated by Toll-like receptor 5. Nature **410,** 1099-1103.

He, S.Y., Huang, H.-C,, Collmer, A. (1993). *Pseudomonas syringae* pv. *syringae* Harpin$_{Pss}$: a protein that is secreted via the Hrp pathway and elicits the hypersensitive response in plants. Cell **73,** 1255–1266.

Heringa, R. J., van Noveland, A. and Tazelaar, M. F. (1969). Resistance to powdery mildew (*E. polygoni*) in peas (*P. sativum* L.). Euphytica **18**, 163-169.

Hewitt, G.M. (1996). Some genetic consequences of ice ages, and their role in divergence and speciation. Biological Journal of the Linnaean Society **58**, 247–276.

Hippe-Sanwald, S., Hermanns, M., and Somerville, S.C. (1992). Ultrastructural comparison of incompatible and compatible interactions in the barley powdery mildew disease. Protoplasma **168**, 27-40.

Hoffmann, M. H. (2002). Biogeography of *Arabidopsis thaliana* (L.) Heynh. (*Brassicaceae*). J. Biogeogr. **29**, 125–134 (2002)

Holliday, R. and Ho, T. (1990) Evidence for allelic exclusion in Chinese hamster ovary cells. New Biol. **2**, 719.

Huang, C.C., Hoefs-Van De Putte, P.M., Haanstra-Van Der Meer, J.G., Meijer-Dekens, F. and 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**(6), 511-520.

Hückelhoven, R., Fodor, J., Preis, C. and Kogel, K.-H. (1999). Hypersensitive Cell Death and Papilla Formation in Barley Attacked by the Powdery Mildew Fungus Are Associated with Hydrogen Peroxide but Not with Salicylic Acid Accumulation. Plant Physiology **119**, 1251-1260.

Hückelhoven, R., Fodor, J., Trujillo, M. and Kogel, K.H. (2000). Barley *Mla* and *Rar* mutants compromised in the hypersensitive cell death response against *Blumeria graminis* f.sp. *hordei* are modified in their ability to accumulate reactive oxygen intermediates at sites of fungal invasion. Planta, **212** (1), 16 – 24.

Hughes, A. L., and Nei, M. (1988). Pattern of nucleotide substitution at major histocompatibility complex class I loci reveals overdominant selection. Nature **335**, 167-170.

Hulbert, S.H., Webb, C.A., Smith, S.M. and Sun, Q. (2001). Resistance gene complexes: evolution and utilization. Annu Rev Phytopathol **39**, 285–312.

Hwang, C.-F., Bhakta, A.V., Turesdell, G.M., Pudlo, W.M. and Williamwon, V.M. (2000). Evidence for a role of the N terminus and leucine-rich repeat region of the Mi gene product in regulation of localized cell death. Plant Cell **12**, 1319-1329.

Hyde, P.M., and Colhoun, J. (1975). Mechanisms of resistance of wheat to *Erysiphe graminis* f. sp. *tritici*. Phytopathol. Z. **82**, 185-206.

Jablonka, E. and Lamb, M. (1989). The inheritance of acquired epigenetic variations. Journal of Theoretical Biology **139**, 69–83.

Jacobs, A.K., Lipka, V., Burton, R.A., Panstruga, R., Strizhov, N., Schulze-Lefert, P. and Fincher, G.B. (2003). An *Arabidopsis* Callose Synthase, GSL5, Is Required for Wound and Papillary Callose Formation. Plant Cell **15,** 2503-2513.

Jacobsen, S.E. and Meyerowitz, E.M. (1997). Hypermethylated *SUPERMAN* epigenetic alleles in *Arabidopsis*. Science 277, 1100–1103.

Jander, G., Norris, S.R., Rounsley, S.D., Bush, D.F., Levin, I.M., Last, R.L. (2002). *Arabidopsis* map-based cloning in the post-genome era. Plant Physiol. **129**(2):440-450.

Jirage, D., Tootle, T.L., Reuber, T.L., Frost, L.N., Feys, B.J., Parker, J.E., Ausubel, F.M. and Glazebrook, J. (1999). *Arabidopsis thaliana* PAD4 encodes a lipase-like gene that is important for salicylic acid signaling. Proc Natl Acad Sci USA **96**, 13583–13588.

Jonak, C., Okresz, L., Bogre, L. and Hirt, H. (2002). Complexity, cross talk and integration of plant MAP kinase signalling. Curr Opin Plant Biol **5**, 415–424.

Jones, D.A. and Takemoto, D. (2004). Plant innate immunity – direct and indirect recognition of general and specific pathogen-associated molecules. Curr Opin Immunol **16**, 48–62.

Jørgensen, J.H. (1977). Spectrum of resistance conferred by Ml-o powdery mildew resistance genes in barley. Euphytica **26**, 55-62.

Jørgensen, J.H. (1992). Discovery, characterization and exploitation of mlo powdery mildew resistance in barley. Euphytica **63,** 141-152.

Jørgensen, J.H. (1994). Genetics of powdery mildew resistance in barley. Crit. Rev. Plant Sci. **13**, 97-119.

Jorgensen, S. and Mauricio, R.(2004). Neutral genetic variation among wild North American populations of the weedy plant *Arabidopsis thaliana* is not geographically structured. Mol. Ecol. **13**, 3403–3413.

Juenger, T. E. , Sen, S. , Stowe, K. A. & Simms, E. L.(2005). Epistasis and genotype–environment interaction for quantitative trait loci affecting flowering time in *Arabidopsis thaliana*. Genetica 123, 87–105.

Kamoun, S. (2001). Nonhost resistance to *Phytophthora*: novel prospects for a classical problem. Curr Opin Plant Biol **4,** 295–300.

Kearsey, M. , Pooni, H. and Syed, N. (2003). Genetics of quantitative traits in *Arabidopsis thaliana*. Heredity **91**, 456–464.

Kessler, A. and Baldwin, I.T. (2002). Plant responses to insect herbivory: the emerging molecular analysis. Annu Rev Plant Biol. **53**, 299-328.

Kidwell, M.G. amd Lisch, D.R. (1997). Transposable elements as sources of variation in plants and animals. Proceedings of the National Academy of Sciences of the USA **94**, 7704–7711.

Kidwell, M.G. and Lisch, D.R. (2001). Transposable elements, parasitic DNA and genome evolution. Evolution **55**, 1–24.

Kim, M.G., da Cunha, L., McFall, A.J., Belkhadir, Y., DebRoy, S., Dangl, J.L. and Mackey, D. (2005). Two *Pseudomonas syringae* type III effectors inhibit RIN4-regulated basal defense in *Arabidopsis*. Cell **121**, 749–759.

King, G., Niehnuis, J. and Hussey, C. (1993). Genetic similarity among ecotypes of *Arabidopsis thaliana* estimated by analysis of restriction fragment length polymorphisms. Theoretical and Applied Genetics, **86**, 1028–1032.

Koncz C., Schell J.(1986). The promoter of TL-DNA gene 5 controls the tissue-specific expression of chimaeric genes carried by a novel type of *Agrobacterium* binary vector. Mol. Gen. Genet. **204**, 383-396.

Koornneef, M., Alonso-Blanco, C. and Vreugdenhil, D. (2004). Naturally occurring genetic variation in *Arabidopsis thaliana*. Annu. Rev. Plant Biol. **55**, 141–172.

Korves, T. and Bergelson, J. (2004). A novel cost of *R*-gene resistance in the presence of disease. Am Nat. **163**(4), 489-504.

Kroymann, J. and Mitchell-Olds, T. (2005). Epistasis and balanced polymorphism influencing complex trait variation. Nature 435, 95–98.

Kumar, H. and Singh, R. B. (1981). Genetic analysis of adult plant resistance to powdery mildew in peas (*Pisum sativum* L.). Euphytica **30**, 147-151.

Kunkel, B.N., Bent, A.F., Dahlbeck, D., Innes, R.W., and Staskawicz, B.J. (1993). *RPS2*, an *Arabidopsis* disease resistance locus specifying recognition of *Pseudomonas syringae* strains expressing the avirulence gene *avrRpt2*. Plant Cell **5**(8), 865-875.

Kunoh, H., Matsuoka, K., and Kobayashi, I. (1996).. Ultrastructure of papillae induced in barley coleoptile cells by a pathogen, *Erysiphe graminis,* and a non-pathogen, *E. pisi*. Fitopatol. Brasil **21**, 418-425.

Kusaba, M., Tung, C.W., Nasrallah, M.E., Nasrallah, J.B. (2002). Monoallelic expression and dominance interactions in anthers of self-incompatible *Arabidopsis lyrata*. *Plant Physiol.* **128**, 17–20.

Kwok, P.Y. (2001). Methods for genotyping single nucleotide polymorphisms. Annu Rev Genomics Hum Genet. **2**, 235-258.

Lai, E.C. (2003). microRNAs: Runts of the genome assert themselves. Curr Biol. **13**, 925–936.

Lamb, C. and Dixon, R.A. (1997). The oxidative burst in plant disease resistance. Annu Rev Plant Physiol Plant Mol Biol. 48, 251-275.

Le Corré, V. (2005). Variation at two flowering time genes within and among populations of *Arabidopsis thaliana*: comparison with markers and traits. Mol. Ecol. **14**, 4181-40192.

Lecellier, C.H. and Voinnet, O. (2004). RNA silencing: no mercy for viruses? Immunol Rev **198**, 285-303.

Lee J., Klessig D.F., Nürnberger, T. (2001). A harpin binding site in tobacco plasma membranes mediates activation of the pathogenesis-related gene HIN1 independent of extracellular calcium but dependent on mitogen-activated protein kinase activity. Plant Cell **13,** 1079–1093.

Lellis, A.D., Kasschau, K.D., Whitham, S.A. and Carrington, J.C. (2002). Loss-of-Susceptibility Mutants of *Arabidopsis thaliana* Reveal an Essential Role for *eIF(iso)4E* during Potyvirus Infection. Current Biology **12**(12), 1046-1051.

Levine, A., Tenhaken, R., Dixon, R. and Lamb, C. (1994). H_2O_2 from the oxidative burst orchestrates the plant hypersensitive disease resistance response. Cell **79**, 583-593.

Li, C., Bai, Y., Jacobsen, E., Visser, R., Lindhout, P., and Bonnema, G. (2006) Tomato defense to the powdery mildew fungus: differences in expression of genes in susceptible, monogenic- and polygenic resistance responses are mainly in timing. Plant Mol Biol. (Epub ahead of print).

Lin, X., Kaul, S., Rounsley et al. (1999). Sequence and analysis of chromosome 2 of the plant *Arabidopsis thaliana*. Nature. **402**(6763), 761-768.

Linde, M. and 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*. TAG Theoretical and Applied Genetics **107**(2), 256–262.

Linde, M., Mattiesch, M. and Debener, T. (2004) *Rpp1,* a dominant gene providing race-specific resistance to rose powdery mildew (*Podosphaera pannosa*): molecular mapping, SCAR development and confirmation of disease resistance data. TAG Theoretical and Applied Genetics **109** (6), 1261 – 1266.

Lipka, V., Dittgen, J., Bednarek, P., Bhat, R., Wiermer, M., Stein, M., Landtag, J., Brandt, W., Rosahl, S., Scheel, D., Llorente, F., Molina, A., Parker, J., Somerville, S., Schulze-Lefert P. (2005). Pre- and Postinvasion Defenses Both Contribute to Nonhost Resistance in *Arabidopsis.* Science **310**, 1180-1183.

Loudet, O., Chaillou, S. Camilleri, C. Bouchez, D. and Daniel-Vedele, F. (2002). Bay-0 × Shahdara recombinant inbred line population: a powerful tool for the genetic dissection of complex traits in *Arabidopsis*. TAG Theoretical and Applied Genetics **104** (6–7),1173 – 1184.

Luff, B., Pawlowski, L. and Bender, J. (1999). An Inverted Repeat Triggers Cytosine Methylation of Identical Sequences in Arabidopsis. Molecular Cell, 3(4), 505-511.

Lukowitz, W., Gillmor, C.S., and Scheible, W.R. (2000). Positional cloning in *Arabidopsis*. Why it feels good to have a genome initiative working for you. Plant Physiol **123,** 795-805.

Mackey, D., Belkhadir, Y., Alonso, J.M., Ecker, J.R. and Dangl, J.L. (2003). *Arabidopsis* RIN4 is a target of the type III virulence effector AvrRpt2 and modulates RPS2-mediated resistance. Cell **112**, 379–389.

Maleck, K., Neuenschwander, U., Cade, R.M., Dietrich, R.A., Dangl, J.L. and Ryals, J.A. (2002). Isolation and Characterization of Broad-Spectrum Disease-Resistant *Arabidopsis* Mutants. Genetics **160,** 1661-1671

Malmberg, R. L. , Held, S. , Waits, A. and Mauricio, R. (2005). Epistasis for fitness-related quantitative traits in *Arabidopsis thaliana* grown in the field and in the greenhouse. Genetics 171, 2013–2027.

Maloof, J.N. (2003). Genomic approaches to analyzing natural variation in *Arabidopsis thaliana*. Curr Opin Genet Dev. **13**(6), 576-582.

Maor, R. and Shirasu, K. (2005). The arms race continues: battle strategies between plants and fungal pathogens. Current Opinion in Microbiology **8**(4), 399-40.

Martienssen, R. (1996) Epigenetic phenomena: paramutation and gene silencing in plants. Curr. Biol. **6**, 810-813.

Martin, G.B., Bogdanove, A.J. and Sessa, J. (2003). Understanding the functions of plant disease resistance proteins. Annual Review of Plant Biology **54**, 23-61.

Mateo, A., Muhlenbock, P., Rustérucci, C., Chang, C.C., Miszalski, Z., Karpinska, B., Parker, J.E., Mullineaux, P.M. and Karpinski, S. (2004). *LESION SIMULATING DISEASE 1* is required for acclimation to conditions that promote excess excitation energy. Plant Physiol **136**, 2818–2830.

Matzke, M.A., Aufsatz, W., Kanno, T., Mette, M.F. and Matzke, A.J. (2002). Homology-dependent gene silencing and host defense in plants. Adv Genet. **46**, 235-75.

Mauricio, R. (1998) Costs of resistance to natural enemies in field populations of the annual plant *Arabidopsis thaliana*. American Naturalist **151**, 20–28.

McDowell, J.M., Cuzick, A., Can, C., Beynon, J., Dangl, J.L .and Holub, E.B. (2000). Downy mildew (*Peronospora parasitica*) resistance genes in *Arabidopsis* vary in functional requirements for NDR1, EDS1, NPR1 and salicylic acid accumulation. Plant J **22**(6):523-529.

McGuinness, D.H., Dehal, P.K and Pleass, R.J. (2003). Pattern recognition molecules and innate immunity to parasites. Trends Parasitol **19,** 312–319.

Medzhitov, R. and Janeway, Jr. C.A. (2002). Decoding the patterns of self and nonself by the innate immune system. Science **296,** 298–300.

Meister, G., Landthaler, M., Dorsett, Y. and Tuschl, T. (2004). Sequence-specific inhibition of microRNA- and siRNA-induced RNA silencing. RNA**10**, 544-550.

Mendgen, K. and Hahn, M. (2002). Plant infection and the establishment of fungal biotrophy.Trends Plant Sci. **7**(8), 352-356.

Meyers, B.C., Kaushik, S., Nandety, R.S. (2005). Evolving disease resistance genes Curr Opin Plant Biol **8**(2), 129-134.

Meyers, B.C., Kozik, A., Griego, A., Kuang, H. and Michelmore, R.W. (2003). Genome-wide analysis of NBS-LRR-encoding genes in *Arabidopsis*. Plant Cell **15**, 809–834.

Michelmore, R.W. and Meyers, B.C. (1998). Clusters of resistance genes in plants evolve by divergent selection and a birth-and-death process. Genome Res **8**, 1113–1130.

Mitchell-Olds, T., Schmitt, J. (2006). Genetic mechanisms and evolutionary significance of natural variation in *Arabidopsis*. Nature **441**(7096), 947-952.

Mittelsten Scheid, O., Afsar, K. and Paszkowski, J. (2003). Formation of stable epialleles and their paramutation-like interaction in tetraploid *Arabidopsis thaliana*. *Nat. Genet.* **34** (2003), pp. 450–454.

Miyashita, N.T., Kawabe, A. and Innan, H. (1999) DNA variation in the wild plant *Arabidopsis thaliana* revealed by amplified fragment length polymorphism analysis. Genetics, 152, 1723–1731.

Morgan, H.D., Sutherland, H.G.E., Martin. D.I.K and Whitelaw, E. (1999). Epigenetic inheritance at the agouti locus in the mouse. Nature Genetics **23**, 314 – 318.

Mourrain, P., Beclin, C., Elmayan, T., Feuerbach, F., Godon, C., Morel, J.B., Jouette, D., Lacombe, A.M., Nikic, S., Picault, N., Remoue, K., Sanial, M., Vo, T.A., Vaucheret, H. (2000). *Arabidopsis SGS2* and *SGS3* genes are required for posttranscriptional gene silencing and natural virus resistance. Cell 101: 533–542.

Muskett, P.R., Kahn, K., Austin, M.J., Moisan, L.J., Sadanandom, A., Shirasu, K., Jones, J.D., and Parker, J.E. (2002). *Arabidopsis* RAR1 exerts rate-limiting control of *R* gene-mediated defenses against multiple pathogens. Plant Cell **14,** 979-992. Nature. **402**(6763), 761-168.

Nawrath, C., and Metraux, J.P. (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,** 1393-1404.

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

Neff, M.M., Turk, E. and Kalishman, M. (2002). Web-based Primer Design for Single Nucleotide Polymorphism Analysis. Trends in Genetics **18,** 613-615.

Nei, M., Gu, X. and Sitnikova, T. (1997). Evolution by the birth-and-death process in multigene families of the vertebrate immune system. Proc. Natl. Acad. Sci. USA **94,** 7799-7806.

Newman, M.A., von Roepenack-Lahaye, E., Parr, A., Daniels, M.J. and Dow, J.M. (2002). Prior exposure to lipopolysaccharide potentiates expression of plant defenses in response to bacteria. Plant J **29**, 487–495.

Nimchuk, Z., Eulgem, T., Holt III, B.F. and Dangl, J.L. (2003). Recognition and response in the plant immune system. Annu Rev Genet **37**, 579–609.

Nishimura, M.T., Stein, M., Hou, B.H., Vogel, J.P., Edwards, H. and Somerville, S.C. (2003). Loss of a callose synthase results in salicylic acid-dependent disease resistance. Science **301**(5635), 969-972.

Nomura, K., Debroy, S., Lee, Y.H., Pumplin, N., Jones, J. and He, S.Y. (2006). A bacterial virulence protein suppresses host innate immunity to cause plant disease. Science. **313**(5784), 220-223.

Nordborg, M., Hu, T.T., Ishino, Y., Jhaveri, J., Toomajian, C., Zheng, H., Bakker, E., Calabrese, P., Gladstone, J., Goyal, R., Jakobsson, M., Kim, S., Morozov, Y., Padhukasahasram, B., Plagnol, V., Rosenberg, N.A., Shah, C., Wall, J.D., Wang, J., Zhao, K., Kalbfleisch, T., Schulz, V., Kreitman, M., Bergelson, J. et al. (2005). The pattern of polymorphism in *Arabidopsis thaliana*. PLoS Biol. **3**, e196.

Nürnberger, T. and Scheel, D. (2001). Signal transmission in the plant immune response. Trends Plant Sci **6,** 372–379.

Nürnberger, T., Brunner, F., Kemmerling, B. and Piater, L. (2004). Innate immunity in plants and animals: striking similarities and obvious differences. Immunological Reviews **198** (1), 249-266.

Nürnberger, T., Colling, C., Hahlbrock, K., Jabs, T., Renelt, A., Sacks, W.R. and Scheel, D. (1994). Perception and transduction of an elicitor signal in cultured parsley cells. Biochem Soc Symp **60**, 173-182.

Ochsenbein, C., Przybyla, D., Danon, A., Landgraf, F., Gobel, C., Imboden, A., Feussner, I., Apel, K. (2006). The role of EDS1 (enhanced disease susceptibility) during singlet oxygen-mediated stress responses of *Arabidopsis*. Plant J. (Epub ahead of print).

O'Kane, S.L. and Al-Shehbaz, I.A. (1997). A Synopsis of *Arabidopsis* (*Brassicaceae*). Novon **7**, 323–327.

Osbourn, A.E. (1996). Preformed Antimicrobial Compounds and Plant Defense against Fungal Attack. Plant Cell **8**, 1821-1831.

Panstruga, R. (2005). Serpentine plant MLO proteins as entry portals for powdery mildew fungi. Biochem. Soc. Trans. **33**, 389–392.

Park, W., Li, J., Song, R., Messing, J. and Chen, X. (2002). CARPEL FACTORY, a Dicer homolog, and HEN1, a novel protein, act in microRNA metabolism in *Arabidopsis thaliana*. Curr Biol **12**, 1484–1495

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

Parniske, M., Hammond Kosack, K.E., Golstein, C., Thomas, C.M., Jones, D.A., Harrison, K., Wulff, B.B.H. and Jones, J.D.G. (1997). Novel disease resistance specificities result from sequence exchange between tandemly repeated genes at the Cf-4/9 locus of tomato. **Cell** 91, 821-832.

Peterhänsel, C., Freialdenhoven, A., Kurth, J., Kolsch, R., and 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.

Petersen, M., Brodersen, P., Naested, H., Andreasson, E., Lindhart, U., Johansen, B., Nielsen, H.B., Lacy, M., Austin, M.J. and Parker, J.E. *(2000). Arabidopsis* MAP kinase 4 negatively regulates systemic acquired resistance. Cell **103**, 1111–1120.

Pfaffl, M.W. (2001). A new mathematical model for relative quantification in real-time RT-PCR. Nucleic Acids Res. **29**(9), e45.

Pfund, C.,Tans-Kersten, J., Dunning, F.M., Alonso, J.M., Ecker, J.R., Allen, C. and Bent, A.F. (2004). Flagellin is not a major defense elicitor in *Ralstonia solanacearum* cells or extracts applied to *Arabidopsis thaliana*. Mol Plant Microbe Interact **17,** 696–706.

Pieterse, C.M. and van Loon, L.C. (1999). Salicylic acid-independent plant defence pathways.Trends Plant Sci. **4**(2), 52-58.

Piffanelli, P., Ramsay, L., Waugh, R., Benabdelmouna, A., D'hont, A., Hollricher, K., Jørgensen, J., Schulze-Lefert, P., and Panstruga, R. (2004). A barley cultivation-associated polymorphism conveys resistance to powdery mildew. Nature **430,** 887-891.

Price, R.A., Al-Shehbaz, I.A. and Palmer, J.D. (1994). Systematic relationships of *Arabidopsis*: a molecular and morphological approach. In: *Arabidopsis* (eds Meyerowitz E, Somerville C), pp. 7–19. Cold Spring Harbor Press, Cold Spring Harbor, NY.

Qin, H., Dong, Y. and von Arnim, A.G.H. (2003). Epigenetic interactions between *Arabidopsis* transgenes: characterization in light of transgene integration sites. Plant Mol. Biol. **52**, 217–231.

Qutob, D., Kamoun, S., Tyler, B.M. and Gijzen, M. (2002). Expression of a *Phytophthora sojae* necrosis inducing protein occurs during transition from biotrophy to necrotrophy. Plant J **32,** 361–373.

Ramos, H.C., Rumbo, M. and Sirard, J.C. (2004). Bacterial flagellins: mediators of pathogenicity and host immune responses in mucosa, Trends Microbiol **12**, 509–517.

Redei, G.P. (1975). *Arabidopsis* as a genetic tool. *Annual Review of Genetics* **9**, 111–127.

Reinhart, B.J., Weinstein, E.G., Rhoades, M.W., Bartel, B. and Bartel, D.P. (2002). MicroRNAs in plants. Genes Dev **16**, 1616–1626.

Reymond, P. and Farmer, E.E. (1998). Jasmonate and salicylate as global signals for defense gene expression. Curr Opin Plant Biol. **1**(5), 404-411.

Rhoades, M.W., Reinhart, B.J., Lim, L.P., Burge, C.B., Bartel, B., Bartel, D.P. (2002). Prediction of plant microRNA targets. Cell **110**, 513–520.

Richly, E., Kurth, J. and Leister, D. (2002). Mode of amplification and reorganization of resistance genes during recent *Arabidopsis thaliana* evolution. Mol Biol Evol **19**, 76–84.

Riddle, N.C. and Richards, E. J. (2002): The control of natural variation in cytosine methylation in *Arabidopsis*. Genetics **162**, 355.

Robaglia, C. and Caranta, C. (2006). Translation initiation factors: a weak link in plant RNA virus infection. Trends in Plant Science **11**(1), 40-45.

Rogers, E.E., and Ausubel, F.M. (1997). *Arabidopsis* enhanced disease susceptibility mutants exhibit enhanced susceptibility to several bacterial pathogens and alterations in *PR -1* gene expression. Plant Cell **9,** 305-316.

Roman, G., Lubarsky, B., Kieber, J.J., Rothenberg, M., and Ecker, J.R. (1995). Genetic analysis of ethylene signal transduction in *Arabidopsis:* Five Novel Mutant Loci Integrated into a Stress Response Pathway**.** Genetics **139**, 1393-1409.

Rose, L.E., Bittner-Eddy, P.D., Langley, C.H., Holub, E.B., Michelmore, R.W. and Beynon, J.L. (2004). The maintenance of extreme amino acid diversity at the disease resistance gene *RPP13* in *Arabidopsis thaliana*. Genetics **166**,1517–1527.

Rozen, S. and Skaletsky, H. (2000). Primer3 on the WWW for general users and for biologist programmers. In: Krawetz S, Misener S (eds) Bioinformatics Methods and Protocols: Methods in Molecular Biology. Humana Press, Totowa, NJ, 365-386.

Ruffel, S., Dussault, M.H., Palloix, A., Moury, B., Bendahmane, A., Robaglia, C. and Caranta, C. (2002). A natural recessive resistance gene against *Potato virus Y* in pepper corresponds to the *eukaryotic initiation factor 4E (eIF4E)*. Plant J **32**, 1067–1075.

Ruffel, S., Gallois, J.L., Lesage, M.L. and Caranta, C. (2005). The recessive potyvirus resistance gene *pot-1* is the tomato orthologue of the pepper *pvr2-eIF4E* gene. Mol Genet Genomics **274**(4), 346-353.

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

Saenz, G.S. and Taylor, J.W. (1999). Phylogeny of the Erysiphales (powdery mildews) inferred from internal transcribed spacer ribosomal DNA. Can J Bot **77**, 150-168.

Salamini, F., and Schulze-Lefert, P. (1997). The barley Mlo gene: a novel control element of plant pathogen resistance. Cell **88,** 695-705.

Sanger, F., Nicklen, F. and Coulson, A.R. (1977). DNA sequencing with chain-termination inhibitors. Proceedings of the National Academy of Sciences U.S.A. **74**, 5463-5467.

Schauer, S.E., Jacobsen, S.E., Meinke, D.W. and Ray, A. (2002)**.** *DICER-LIKE1:* blind men and elephants in *Arabidopsis* development. Trends in Plant Science **7**, 487–491.

Schiff, C.L., Wilson, I.W. and Somerville, S.C. (2001). Polygenic powdery mildew disease resistance in Arabidopsis thaliana: quantitative trait analysis of the accession Warschau-1. Plant Pathology **50**, 690-701.

Schmid, K.J., Rosleff Sörensen, T., Stracke, R., Törjék, O., Altmann, T., Mitchell-Olds, T. and Weisshaar, B. (2003). Large-Scale Identification and Analysis of Genome-Wide Single-Nucleotide Polymorphisms for Mapping in *Arabidopsis thaliana*. Genome Research **13** (6a), 1250-1257.

Schmid, K.J., Torjek, O., Meyer, R., Schmuths, H., Hoffmann, M.H. and Altmann, T. (2006). Evidence for a large-scale population structure of *Arabidopsis thaliana* from genome-wide single nucleotide polymorphism markers. Theor. Appl. Genet. **112**, 1104–1114.

Schulze-Lefert, P. and Panstruga, R. (2003). Establishment of biotrophy by parasitic fungy and reprogramming of host cells for disease resistance. Annual Review of Phytopathology **41**, 641-667

Schulze-Lefert, P. and Vogel, J. (2000). Closing the ranks to attack by powdery mildew.Trends Plant Sci. **5**(8), 343-348.

Seiffert, U. and Schweizer, P. (2005). A pattern recognition tool for quantitative analysis of in planta hyphal growth of powdery mildew fungi. Journal of Molecular Plant Microbe Interactions (MPMI) **18**, 906-912.

Sharbel, T.F., Haubold, B. and Mitchell-Olds, T. (2000). Genetic isolation by distance in Arabidopsis thaliana: biogeography and postglacial colonization of Europe. Molecular Ecology **9** (12), 2109-2118.

Shirasu, K. and Schulze-Lefert, P. (2000) Regulators of cell death in disease resistance. Plant Molecular Biology **44**, 371-385.

Sidorenko, L.V. and Peterson, T. (2001). Transgene-induced silencing identifies sequences invoved in the establishment of paramutation of the maize *p1* gene. The Plant Cell **13**, 319–335.

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

Stahl, E.A. and Bishop, J.G. (2000) Plant-pathogen arms race at the molecular level. Curr. Opin. Plant Biol. **3,** 2999-29304.

Staskawicz, B.J., Ausubel, F.M., Baker, B.J., Ellis, J.G. and Jones, J.D. (1995). Molecular genetics of plant disease resistance. Science **268**(5211, 661-667.

Stein, M., Dittgen, J., Sánchez-Rodríguez, C., Hou, B.-H., Molina, A., Schulze-Lefert, P., Lipka, V. and Somerville, S. (2006). *Arabidopsis* PEN3/PDR8, an ATP Binding Cassette Transporter, Contributes to Nonhost Resistance to Inappropriate Pathogens That Enter by Direct Penetration. Plant Cell **18**, 731-746.

Stenoien, H. K., Fenster, C. B., Tonteri, A. and Savolainen, O. (2005) Genetic variability in natural populations of *Arabidopsis thaliana* in northern Europe. Mol. Ecol. **14**, 137-148.

Stokes, T.L. and Richards, E.J. (2002). Induced instability of two *Arabidopsis* constitutive pathogen-response alleles PNAS **99**, 7792-7796.

Stokes, T.L. and Richards, E.J. (2002). Induced instability of two *Arabidopsis* constitutive pathogen-response alleles. PNAS **99**, 7792-7796.

Stone, B.A., and Clarke, A.E. (1992). Chemistry and Biology of (1,3)- β-D-Glucans. (Victoria, Australia: La Trobe University Press).

Stone, B.A., Evans, N.A., Bonig, I., and Clarke, A.E. (1985). The application of Sirofluor, a chemically defined fluorochrome from aniline blue for the histochemical detection of callose. Protoplasma **122**, 191–195.

Syed, N. H. & Chen, Z. J. (2004). Molecular marker genotypes, heterozygosity and genetic interactions explain heterosis in *Arabidopsis thaliana*. Heredity **94**, 295–304.

Szabo, L.J. and Bushnell, W.R. (2001). Hidden robbers: the role of fungal haustoria in parasitism of plants. Proc Natl Acad Sci U S A. **98**(14), 7654-7655.

Taberlet, P., Fumagalli, L., Wustsaucy, A.G. and Cosson, J.F. (1998). Comparative Phylogeography and Postglacial Colonization Routes in Europe. Molecular Ecology **7**, 453–464.

Thomma, B.P., Penninckx, I.A., Broekaert, W.F. and Cammue, B.P. (2001). The complexity of disease signaling in *Arabidopsis*. Curr Opin Immunol. **13**(1), 63-68.

Thordal-Christensen, H., Zhang, Z., Wei, Y., Collinge, D.B. (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.

Tian, D., Araki, H., Stahl, E., Bergelson, J., and Kreitman, M. (2002). Signature of balancing selection in *Arabidopsis*. Proc. Natl. Acad. Sci. USA **99**, 11525–11530.

Tian, D., Traw, M.B., Chen, J.Q., Kreitman, M. and Bergelson, J. (2003). Fitness costs of *R*-gene-mediated resistance in *Arabidopsis thaliana*. Nature **423**, 74–77.

Tiwari, K. R., Penner, G. A. and Warkentin, T. D. (1997). Inheritance of powdery mildew resistance in pea. Can. J. Plant Sci. **77**, 307-310.

Todokoro ,S., Terauchi, R. and Kawano, S. (1995). Microsatellite polymorphisms in natural populations of *Arabidopsis thaliana* in Japan. Japanese Journal of Genetics **70**, 543–554.

Törjék O., Berger D., Meyer R.C., Müssig C., Schmid K.J., Rosleff Sörensen T., Weisshaar B., Mitchell-Olds T. and Altmann T. (2003). Establishment of a high-efficiency SNP-based framework marker set for *Arabidopsis* Plant Journal **36** (1), 122-140.

Tsuchihashi, Z. and Dracopoli, N.C. (2002). Progress in high throughput SNP genotyping methods. Pharmacogenomics J. **2**(2):103-310.

Umemoto, N., Kakitani, M., Iwamatsu, A., Yoshikawa, M., Yamaoka, N. and Ishida, I. (1997). The structure and function of a soybean beta-glucan-elicitor-binding protein. Proc Natl Acad Sci USA **94,** 1029–1034.

Underhill, D.M. and Ozinsky, A. (2002). Toll-like receptors: key mediators of microbe detection. Curr Opin Immunol **14,** 103–110.

Ungerer, M.C., Halldorsdottir, S.S., Purugganan, M.D. and Mackay, T.F.C. (2003). Genotype–environment interactions at quantitative trait loci affecting inflorescence development in *Arabidopsis thaliana*. Genetics **165**, 353–365.

Van Damme, M., Andel, A., Huibers, R.P., Panstruga, R., Weisbeek, P.J., Van den Ackerveken, G. (2005). Identification of *Arabidopsis* loci required for susceptibility to the downy mildew pathogen *Hyaloperonospora parasitica*. Mol Plant Microbe Interact. **18**(6), 583-592.

Van der Biezen, E. A. and Jones, J. (1998) Plant disease resistance proteins and the gene-for-gene concept. Trends Biochem. Sci. **23**, 454-456.

Van der Hoorn, R.A.L., Roth, R. and de Wit, P.J.G.M. (2001). Identification of distinct specificity determinants in resistance protein Cf-4 allows construction of a Cf-9 mutant that confers recognition of avirulence protein AVR4. Plant Cell **13**, 273-285.

Van Loon, L.C., Rep, M. and Pieterse C.M.J. (2006). Significance of Inducible Defense-related Proteins in Infected Plants. Annual Review of Phytopathology **44 (**Epub ahead of print).

Van Wees, S.C. and Glazebrook, J. (2003). Loss of non-host resistance of *Arabidopsis* NahG to *Pseudomonas syringae* pv. *phaseolicola* is due to degradation products of salicylic acid. Plant J. 33(4), 733-742.

Vandaelen, R., Vanderlee, T., Diergaarde, P., Groenendijk, J., Topsch, S., Vos, P., Veit, S., Wörle, J.M., Nürnberger, T., Koch, W. and Seitz, H.U. (2001). A novel protein elicitor (PaNie) from *Pythium aphanidermatum* induces multiple defense responses in carrot, *Arabidopsis*, and tobacco. Plant Physiol **127,** 832–841.

Vogel, J., and Somerville, S. (2000). Isolation and characterization of powdery mildew-resistant *Arabidopsis* mutants. PNA**S 97,** 1897-1902.

Vogel, J., Raab, T., Schiff, C., and Somerville, S. (2002). *PMR6*, a pectate lyase-like gene required for powdery mildew susceptibility in Arabidopsis. Plant Cell **14,** 2095-2106.

Vogel, J.P., Raab, T.K., Somerville, C.R. and Somerville, S.C. (2004). Mutations in *PMR5* result in powdery mildew resistance and altered cell wall composition. The Plant Journal **40**(6), 968-978.

Wang S., Basten, C.J. and Zeng, Z.-B. (2006). Windows QTL Cartographer 2.5. Department of Statistics, North Carolina State University, Raleigh, NC.

Wang, G.-L., Ruan, D.-L., Song, W.-Y., Sideris, S. and Chen, L. (1998). *Xa21D* encodes a receptor-like molecule with a leucine-rich repeat domain that determines race-specific recognition and is subject to adaptive evolution. Plant Cell **10,** 765-779.

Waterhouse, P.M. (2006). Defense and counterdefense in the plant world. Nat Genet. **38**(2), 138-139.

Wildermuth, M.C., Dewdney, J., Wu, G., and Ausubel, F.M. **(2001).** Isochorismate synthase is required to synthesize salicylic acid for plant defence. Nature **414**(6863), 562-565.

Wilson, I.W., Schiff, C.L., Hughes, D.E. and Somerville, S.C. (2001). Quantitative Trait Loci Analysis of Powdery Mildew Disease Resistance in the *Arabidopsis thaliana* Accession Kashmir-1. Genetics **158**, 1301-1309.

Wolter M, Hollricher, K., Salamini F, Schulze-Lefert P. (1993). The *mlo* resistance alleles to powdery mildew infection in barley trigger a developmentally controlled defence mimic phenotype. Mol Gen Genet. **239,** 122-128.

Wright, S.I. and Gaut, B.S. (2005). Molecular population genetics and the search for adaptive evolution in plants. Mol Biol Evol. 22(3), 506-519.

Wulff, B.B.H., Thomas, C.M., Smoker, M., Grant, M. and Jones, J.D.G. (2001). Domain swapping and gene shuffling identify sequences required for induction of an Avr-dependent hypersensitive response by the tomato Cf-4 and Cf-9 proteins. Plant Cell **13**, 255-272.

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

Xiao, S., Calis, O., Patrick, E., Zhang, G., Charoenwattana, P., Muskett, P., Parker, J.E. and Turner, J.G. (2005). The atypical resistance gene, *RPW8*, ecruits components of basal defence for powdery mildew resistance in *Arabidopsis*. Plant J. **42**(1), 95-110.

Xiao, S., Charoenwattana, P., Holcombe, L. and Turner, J.G. (2003). The *Arabidopsis* genes *RPW8.1* and *RPW8.2* confer induced resistance to powdery mildew diseases in tobacco. Mol Plant Microbe Interact. **16**(4), 289-94.

Xiao, S., Ellwood, S., Calis, O., Patrick, E., Li, T., Coleman, M. and Turner, J.G. (2001). Broad-spectrum mildew resistance in *Arabidopsis thaliana* mediated by *RPW8*. Science. **291**(5501), 118-120.

Xiao, S., Ellwood, S., Findlay, K., Oliver, R.P. and Turner, J.G. (1997). Characterization of three loci controlling resistance of *Arabidopsis thaliana* accession Ms-0 to two powdery mildew diseases. Plant J. **12**(4), 757-768.

Xiao, S., Emerson, B., Ratanasut, K., Patrick, E., O'Neill, C., Bancroft, I. and Turner, J.G. (2004). Origin and maintenance of a broad-spectrum disease resistance locus in *Arabidopsis*. Mol Biol Evol. **21**(9), 1661-72.

Xiey, Z., Johansen, L.K., Gustafson, A.M., Kasschau, K.D., Lellis, A.D., et al. (2004). Genetic and Functional Diversification of Small RNA Pathways in Plants. PLoS Biol **2**(5): e104

Xin, Z., Velten, J.P., Oliver, M.J. and Burke, J.J. (2003). High-Throughput DNA Extraction Method Suitable for PCR. BioTechniques 34 (4), 820-826.

Yang, B., Sugio, A. and White, F.F. (2006). *Os8N3* is a host disease-susceptibility gene for bacterial blight of rice. Proc Natl Acad Sci USA **103**(27), 10503-10508.

Yang, B., Zhu, W., Johnson, L.B. and White, F.F. (2000). The virulence factor AvrXa7 of *Xanthomonas oryzae* pv. *oryzae* is a type III secretion pathway-dependent nuclear-localized double-stranded DNA-binding protein. Proc. Natl. Acad. Sci. USA **97**, 9807–9812.

Yang, Y., Shah, J. and Klessig, D.F. (1997). Signal perception and transduction in plant defense responses. Genes Dev **11,** 1621–1639.

Yi, H. Riddle, N.C., Stokes, T.L., Woo, H.-R. and Richards, E.J. (2004). Induced and Natural Epigenetic Variation. Cold Spring Harbor Symposia on Quantitative Biology 2004 **69**(1), 155-160.

Yu, G.L., Katagiri, F. and Ausubel, F.M. (1993). *Arabidopsis* mutations at the *RPS2* locus result in loss of resistance to *Pseudomonas syringae* strains expressing the avirulence gene *avrRpt2*.. Mol Plant Microbe Interact. **6**(4), 434-443.

Zhou, N., Tootle, T.L., Tsui, F., Klessig, D.F., and Glazebrook, J. (1998). PAD4 functions upstream from salicylic acid to control defense responses in *Arabidopsis*. Plant Cell **10**, 1021–1030.

Zhu, N., Yang, B., Chittoor, J.M., Johnson, L.B. and White, F.F. (1998). AvrXa10 contains an acidic transcriptional activation domain in the functionally conserved C terminus, Mol. Plant Microbe Interact. **11** , 824–832.

Zimmermann, P., Hirsch-Hoffmann, M., Hennig, L. and Gruissem, W. (2004). GENEVESTIGATOR. *Arabidopsis* Microarray Database and Analysis Toolbox. Plant Physiol. 136, 2621-2632.

Zipfel, C., Robatzek, S., Navarro, L., Oakeley, E., Jones, J., Felix, G., and Boller, T. (2004). Bacterial disease resistance in *Arabidopsis* through flagellin perception. Nature **428,** 764-767.

VII. ACKNOWLEDGEMENTS

Ich möchte mich bei allen bedanken, die mich während der Anfertigung dieser Doktorarbeit unterstützt haben, insbesondere bei:

Ralph Panstruga für die Möglichkeit zur Promotion in seiner Gruppe und für eine sehr angenehme und hilfreiche Betreuung.

Prof. Dr. Paul Schulze-Lefert für die vielen Fragen und Vorschläge.

Prof. Dr. M. Hülskamp für die Übernahme des Korreferats sowie
Prof. Dr. U.-I. Flügge für die Übernahme des Prüfungsvorsitzes.

Allen Kollegen in der Abteilung, besonders aus den Gruppen Panstruga und PSL für viel Spaß im Labor und das Beantworten vieler kleiner und großer Fragen, vor allem Anja Reinstaedler, Matt Humprey und Chiara Consonni.

Dem Kochclub der Abteilung für hervorragende Mittagessen in großartiger Gesellschaft und ein paar Extrakilos (Jan Dittgen, Anil Kumar Batchu, Dorit Meyer, Christina Neu und Anja Reinstaedler).

Heidrun Häweker und Jan Dittgen für viele nette Kinoabende abseits des Labors.

Chiara Consonni, Sandra Noir, Dorit Meyer, Mariam Benjdia und Katja Schneider für sportliche Motivation.

Christian Kleinhenrich und Anett Matthäi für viel Geduld, offene Ohren und tröstende Worte.

Meiner Familie für ihre lange und liebevolle Unterstützung.

"Ich versichere, daß ich die von mir vorgelegte Dissertation selbständig angefertigt, die benutzten Quellen und Hilfsmittel vollständig angegeben und die Stellen der Arbeit - einschließlich Tabellen, Karten und Abbildungen -, die anderen Werken im Wortlaut oder dem Sinn nach entnommen sind, in jedem Einzelfall als Entlehnung kenntlich gemacht habe; daß diese Dissertation noch keiner anderen Fakultät oder Universität zur Prüfung vorgelegen hat; daß sie - abgesehen von unten angegebenen Teilpublikationen - noch nicht veröffentlicht worden ist sowie, daß ich eine solche Veröffentlichung vor Abschluss des Promotionsverfahrens nicht vornehmen werde. Die Bestimmungen dieser Promotionsordnung sind mir bekannt. Die von mir vorgelegte Dissertation ist von Prof. Dr. Paul Schulze-Lefert betreut worden."

Köln, 17. August 2006

Katharina Göllner

Katharina Göllner

Curriculum vitae

Persönliche Informationen

Geburtsdatum:	25.05.1977
Geburtsort:	Berlin
Nationalität:	Deutsch

Ausbildung

seit März 2003:	Promotionsarbeit am Max-Planck-Institut für Züchtungsforschung, Köln, AG Dr. Ralph Panstruga, Leitung Prof. Dr. Paul Schulze-Lefert. Thema: Genetic variation of powdery mildew resistance in *Arabidopsis thaliana*.
Oktober 2002	Studienabschluß als Diplom-Biologin an der Freien Universität Berlin
Feb. 2002 – Okt. 2002:	Diplomarbeit im Fachgebiet Pflanzengenetik, Freie Universität Berlin / Stanford University USA, Prof. Dr. Virginia Walbot. Thema: Insertion mutations caused by *RescueMu* elements and their molecular and phenotypical consequences in maize.
Okt. 1999 – April 2000:	Auslandsaufenthalt an der Università degli Studi di Parma, Italien
Okt. 1996 – Okt. 2002:	Studium der Biologie (Diplomstudiengang), Freie Universität Berlin
Juni 1996	Abitur am Albert-Einstein-Gymnasium, Berlin

Köln, 17. August 2006