Active defence responses associated with non-host resistance of Arabidopsis thaliana to the oomycete pathogen Phytophthora infestans

EDGAR HUITEMA1, VIVIANNE G. A. A. VLEESHOUWERS2,†, DAVID M. FRANCIS3 AND SOPHIE KAMOUN1, *

1Department of Plant Pathology, The Ohio State University, Ohio Agricultural Research and Development Center, Wooster, Ohio 44691, USA, 2Plant Research International, PO Box 16, 6700 AA, Wageningen, the Netherlands, 3Department of Horticulture and Crop Science, Ohio Agricultural Research and Development Center, Wooster, Ohio 44691, USA

SUMMARY

The molecular basis of non-host resistance, or species-specific resistance, remains one of the major unknowns in the study of plant–microbe interactions. In this paper, we describe the characterization of a non-host pathosystem involving the model plant Arabidopsis thaliana and the economically important and destructive oomycete pathogen Phytophthora infestans. Cytological investigations into the early stages of this interaction revealed the germination of P. infestans cysts on Arabidopsis leaves, direct penetration of epidermal cells, formation of infection vesicles and occasionally secondary hyphae, followed by a typical hypersensitive response. P. infestans biomass dynamics during infection of Arabidopsis was monitored using kinetic PCR, revealing an increase in biomass during the first 24 h after inoculation, followed by a decrease in the later stages. Transgenic reporter lines and RNA blot analyses were used to characterize the defence responses induced following P. infestans infection. Significant induction of PDF1.2 was observed at 48 h after inoculation, whereas elevated levels of PR gene expression were detected three days after inoculation. To further characterize this defence response, DNA microarray analyses were carried out to determine the expression profiles for c. 11 000 Arabidopsis cDNAs 16 h after infection. These analyses revealed a significant overlap between Arabidopsis non-host response and other defence-related treatments described in the literature. In particular, non-host response to P. infestans was clearly associated with activation of the jasmonate pathway. The described Arabidopsis–P. infestans pathosystem offers excellent prospects for improving our understanding of non-host resistance.

INTRODUCTION

Plants are challenged by numerous pathogens throughout their life cycles and yet are able to fend off most infections. Indeed, in interactions between plants and microbial pathogens, resistance is the rule and disease the exception. This phenomenon is known as non-host resistance or species-specific resistance and is thought to explain why a pathogen can cause disease in particular plant species but not in others. Understanding the molecular basis of non-host resistance remains one of the elusive quests in the study of plant–microbe interactions. Pre-formed barriers and compounds such as saponins are ubiquitous in plants and play an important role in non-host resistance to filamentous fungi (Morrissey and Osbourn, 1999; Osbourn, 1996). However, most contemporary models of non-host resistance evoke a complex overlay of specific resistance and nonspecific defence responses (Gomez-Gomez and Boller, 2002; Heath, 2000; Kamoun et al., 1999; Kamoun, 2001; Nurnberger and Brunner, 2002). Specific resistance has been extensively studied in host pathosystems and typically follows Flor’s gene-for-gene model. In this model, resistance is determined by the simultaneous expression of a pathogen avirulence (Avr) gene with the corresponding plant resistance (R) gene, leading to the hypersensitive response (HR), a general defence response of plants that includes apoptotic cell death (Dangl and Jones, 1998; Flor, 1971; Staskawicz et al., 1995). The extent to which the gene-for-gene model can be expanded to non-host interactions remains unclear. However, we and others have speculated that in many pathosystems non-host resistance can be explained by the occurrence of an arsenal of R genes that recognize multiple or essential Avr genes (Heath, 2000; Kamoun et al., 1998; Kamoun et al., 1999; Kamoun, 2001; Staskawicz et al., 1995).

The oomycetes represent a diverse and phylogenetically unique branch of eukaryotic microbes that includes many important pathogens of plants (Baldauf et al., 2000; Margulis and Schwartz, 2000; Sogin and Silberman, 1998). The most notorious
oomycetes are Phytophthora species, arguably the most devastating pathogens of dicot plants (Erwin and Ribeiro, 1996; Kamoun, 2000; Kamoun, 2003). For example, Phytophthora infestans causes late blight, a devastating disease that results in multibillion-dollar losses in potato and tomato production (Fry and Goodwin, 1997a,b; Garelik, 2002; Smart and Fry, 2001). Most plants, such as weeds and various crops, are resistant to P. infestans, and grow unimpaired in or next to fields with a high incidence of late blight (Colon et al., 1992; Kamoun et al., 1999). Understanding the molecular basis of non-host resistance to P. infestans will provide insight into a key molecular process and will offer novel perspectives for engineering durable late blight resistance in crop plants.

A first insight into the basis of non-host resistance to Phytophthora infestans came through detailed cytological analyses. Microscope observations revealed penetration of epidermal cells by P. infestans in all examined interactions, including those with plant species unrelated to the solanaceous hosts (Gross et al., 1993; Kamoun et al., 1998; Kamoun et al., 1999; Naton et al., 1996; Schmelzer et al., 1995; Vleeshouwers et al., 2000). Fully resistant plants, such as the non-hosts Solanum nigrum, parsley and tobacco display a typical localized HR at all infection sites (Colon et al., 1992; Kamoun et al., 1998, 1999; Naton et al., 1996; Schmelzer et al., 1995; Vleeshouwers et al., 2000). The HR can be highly localized to a single epidermal cell or can affect a group of cells surrounding the penetrating hyphae, depending on the interaction examined (Kamoun et al., 1998; Vleeshouwers et al., 2000). The view that has emerged from these studies is that the HR, perhaps mediated by R genes, is associated with all known forms of genetic resistance to P. infestans including non-host resistance (Kamoun et al., 1999; Kamoun, 2001).

Some of the Phytophthora molecules that trigger the HR or other defence responses in non-host plants are known. Species-specific elicitors have been described in P. infestans and other Phytophthora species and can trigger defence responses in non-host plants. For example, an extracellular transglutaminase that is conserved in P. infestans and other Phytophthora species induces defence responses in the non-host parsley (Brunner et al., 2002; Nurnberger and Brunner, 2002). Members of the INF elicitin family induce the HR and related biochemical changes specifically in Nicotiana (Kamoun et al., 1997, 1998; Sasabe et al., 2000). P. infestans strains deficient in the elicitin INF1 induce disease lesions on Nicotiana benthamiana, suggesting that INF1 functions as an Avr factor that conditions resistance in this species (Kamoun et al., 1998). Using gene silencing, Peart et al. (2002) recently showed that the response of N. benthamiana to INF1 was dependent on the ubiquitin ligase-associated protein SGT1, which is also required for non-host resistance to bacterial plant pathogens. The N. benthamiana pathosystem holds great promise for dissecting elicitor response and resistance to P. infestans, since this plant is amenable to high-throughput functional assays using virus-induced gene silencing (VIGS) (Baulcombe, 1999). Nevertheless, the N. benthamiana–P. infestans interaction does not qualify as a strict non-host pathosystem since some wild-type isolates of P. infestans were recently found to infect this plant (F. Govers, personal communication; C. Smart & W.E. Fry, personal communication) (Kamoun, 2001).

We elected to employ Arabidopsis thaliana as a model for understanding non-host resistance to oomycete pathogens. Several biotrophic oomycetes, such as Peronospora parasitica and Albugo candida, are known to infect Arabidopsis (Holub et al., 1995; Parker et al., 1996; Rehmany et al., 2000; Reignault et al., 1996). Cabbage isolates of Phytophthora brassicae (previously known as Phytophthora porri) (Roetschi et al., 2001) and several isolates of Phytophthora cinnamoni (Robinson & Cahill, 2003) can also infect Arabidopsis, and these pathosystems are expected to facilitate the study of host infection by Phytophthora. However, most Phytophthora species, such as P. infestans, and the root pathogen Phytophthora sojae cannot infect Arabidopsis suggesting that this plant forms an untapped source of resistance to Phytophthora (Kamoun et al., 1999; Kamoun, 2001; Takemoto et al., 2003). Considering the impressive set of functional genomic resources that are available, Arabidopsis offers good prospects for dissecting the complex interactions that take place between a non-host plant and an oomycete pathogen and forms both an alternative and a complementary system to ongoing work on the resistance of Nicotiana to P. infestans. In this study, we describe the characterization of a non-host pathosystem involving Arabidopsis and an economically important Phytophthora species. Using cytological and molecular analyses, as well as microarray gene expression profiling, we obtained an overview of the active defence responses associated with the non-host resistance of A. thaliana to P. infestans.

RESULTS

Interaction between Arabidopsis and P. infestans

To characterize the interaction between Arabidopsis and P. infestans, we performed repeated inoculations of Arabidopsis with P. infestans zoospores. We tested numerous inoculation parameters, including Arabidopsis leaves at the seedling or rosette stage, multiple combinations of Arabidopsis ecotypes and P. infestans strains, detached vs. attached leaves, and drop vs. spray inoculations. In all treatments, late blight lesions and sporulation were never observed, whereas infection of the host plant tomato was observed under most of the conditions tested. Normally, no macroscopic symptoms could be detected on Arabidopsis, but occasionally, discrete necrotic spots typical of the HR could be observed at the inoculation site, particularly when highly concentrated zoospore solutions were used.
Cellular responses of *Arabidopsis* to *P. infestans*

To determine the cellular responses of *Arabidopsis* to *P. infestans*, we performed microscope examinations of leaves inoculated at the rosette stage with droplets of zoospores. These analyses revealed the penetration of *Arabidopsis* epidermal cells in multiple independent infection sites. Cyst germination, penetration of epidermal cells and the formation of infection vesicles occurred as early as 46 h after inoculation, and in some cases was followed by the formation of a short secondary hyphae (Fig. 1). Penetrated epidermal cells displayed features typical of the HR, including granulated cell cytoplasm, thickened cell walls, condensed nuclei near the penetration site and autofluorescence under UV light (Fig. 1). These responses were typically limited to the penetrated epidermal cell.

**Phytophthora infestans** biomass dynamics during infection of *Arabidopsis*

We used kinetic PCR technology to examine changes in *P. infestans* biomass during the interaction with *Arabidopsis* and the host plant tomato (Fig. 2). Primers specific to highly repetitive sequences (> ×10,000) in the *P. infestans* genome were previously used to quantify relative levels of *P. infestans* DNA in infected plant tissue, and were found to reflect an accurate and sensitive estimate of the *P. infestans* biomass (Judelson and Tooley, 2000). We performed kinetic PCR on DNA extracted from discs excised from *Arabidopsis* leaves infected with droplets of *P. infestans* zoospores at successive time points (0, 16, 24, 48 and 72 h after inoculation). Control treatments included inoculated leaves from tomato (host), and inoculum incubated in water in the absence of plant tissue (no-host). In both *Arabidopsis* and tomato, significant increases in the *P. infestans* biomass were observed in the initial 16 h. The *Phytophthora infestans* biomass continued to increase over the 3-day period on tomato, whereas it steadily declined on *Arabidopsis* to reach the lowest level at 72 h after inoculation (Fig. 2). In contrast, no notable changes in biomass were observed over the 3-day period for inoculum...
incubated in the absence of plant tissue (Fig. 2). Statistical analyses using ANOVA were performed for each time point and suggested that the changes in biomass observed on Arabidopsis are statistically significant ($P = 0.0011$). Based on a protected mean separation, biomass increased significantly during the initial stages of Arabidopsis infection followed by a significant decrease in the later stages of the interaction ($P < 0.05$). Independent repetitions of the time course and the kinetic PCR experiments demonstrated that these biomass changes are reproducible (data not shown), however, the extent of the decrease in biomass observed at the later stages varied between experiments.

**Local induction of PR1 and BGL2 genes during P. infestans infection**

We used two transgenic Arabidopsis lines carrying fusions between the PR1 and BGL2 promoters to the β-glucuronidase (GUS) reporter gene (Cao et al., 1997; Manners et al., 1998; Stone et al., 2000) to examine the expression of these defence genes during P. infestans infection. The transgenic lines were inoculated at the rosette stage with either P. infestans zoospores or water droplets (mock treatment) and the leaves were excised and stained with X-Gluc at successive time points after inoculation. In both lines, elevated levels of PR-gene expression was detected 3 days after inoculation, as GUS staining around the inoculation sites (Fig. 3). No GUS expression was detected around mock-inoculated sites. Similar results were obtained from a series of independent experiments. Occasionally, light GUS staining was observed at some sites as early as 2 days after inoculation, but in most cases GUS staining was only observed 3 days or later after inoculation.

**Induction of PDF1.2 during P. infestans infection**

We assayed the expression of known defence genes during infection of Arabidopsis by P. infestans using Northern blot time-course analyses. Rosette leaves of Arabidopsis plants were sprayed with either water or P. infestans zoospore suspensions, and used for RNA extraction at 0, 16, 24, 48 and 72 h after inoculation. Northern blot hybridizations were performed with the defence-response genes PDF1.2, BGL2 and the constitutive gene UBQ5 (Fig. 4). A significant induction of PDF1.2 was observed at 48 h after inoculation. No induction of BGL2 was observed under Northern blot conditions over the 3-day period. No changes in transcript levels were observed in mock-inoculated plants. Independent replications of the time course suggested that the induction of PDF1.2 is significant and reproducible (data not shown), however, the timing of PDF1.2 induction varied between 16 and 48 h depending on the experiment.

---

**Fig. 3** Induction of Arabidopsis (A) PR1::GUS and (B) BGL2::GUS expression by Phytophthora infestans. Transgenic lines were drop-inoculated with P. infestans zoospore suspensions. Leaves were harvested and stained with X-Gluc 3 days after inoculation with a droplet of P. infestans zoospores. Local expression of GUS was detected in both transgenic lines, 3 days after inoculation. Mock-inoculated sites did not show any detectable staining (left side of leaf in panel A).

**Fig. 4** Time course Northern blot analysis of genes expressed in Arabidopsis rosette leaves 0, 16, 24, 48 and 72 h after inoculation with Phytophthora infestans zoospores or mock inoculation with water. The probes corresponded to PDF1.2, a marker gene for the jasmonic acid pathway, and BGL2, a marker gene for the salicylic acid-mediated defence pathway. As a loading control, a probe for the constitutive ubiquitin 5 (UBQ5) gene was used.
DNA microarray gene-expression profiling of Arabidopsis non-host response to P. infestans

To further investigate Arabidopsis non-host response to P. infestans, we used DNA microarray analyses to determine expression profiles for c. 11 000 Arabidopsis cDNAs using the Arabidopsis Functional Genomics Consortium (AFGC) Microarray Facility (Wisman and Ohlrogge, 2000). In two hybridization experiments, RNA populations derived from mock-treated and P. infestans-infected leaves were compared against each other using a dye-swap approach. Normalized data were subjected to regression analysis and subsequent outlier detection. Lists of outliers extracted from both hybridization data sets were compared to each other and a list containing an overlapping set of cDNAs was generated. A total of 89 cDNAs were identified that fall outside a 99% confidence interval (CI) in both hybridizations (supplementary data at http://www.oardc.ohio-state.edu/phytophthora/supp.htm). The cDNA identifiers were used to retrieve their respective predicted loci using the TAIR annotation database (http://www.arabidopsis.org) resulting in 54 annotated and non-redundant Arabidopsis genes (Table 1). A total of 11 genes were represented by multiple cDNAs (range 2–12) that were identified independently as being differentially expressed in both experiments. This suggests that the experiments and analyses we employed are reliable and robust.

Of the 54 Arabidopsis genes identified as differentially expressed during the non-host interaction with P. infestans, 52 were up-regulated and 2 were down-regulated. The 54 genes were classified into 7 functional categories that included physiological states related to metabolism, cell wall modification, development, as well as defence and stress responses. Genes that had known functions but that could not be placed in a particular functional category, were compiled in a separate class (Table 1).

Validation of DNA microarray analysis using Northern Blot analysis

We validated the analysis of the microarray data by a regression approach using Northern Blot hybridizations (Fig. 5). A Northern blot containing the RNA samples that were used in the microarray experiment was hybridized with probes from two genes, At1g21400 and At5g25350, that were selected as differentially expressed (Table 1), and two genes, At2g40000 and At3g43740, that were not. In addition, probes for PDF1.2 and the constitutive gene UBQ5 were included as controls (Fig. 5). The signals obtained with the various probes were quantified using a phosphor imager, normalized to the UBQ5 signal, and used to calculate induction ratios. The induction levels obtained by Northern blot and microarray hybridization correlated well (Fig. 5). At1g21400 and At5g25350 were at least twofold induced in the Northern blot experiment vs. 3–5-fold in the microarray hybridizations. In contrast, At2g40000 and At3g43740 showed no significant differential expression by Northern blot hybridization.

We also validated the microarray data by Northern Blot analysis using RNA isolated from an independently performed experiment (biological replicate). In this experiment, At1g21400 and At5g25350 were induced 2.8- and 2.4-fold relative to the UBQ5 gene (data not shown). Overall, these results suggest that the microarray experiment and data processing by regression analyses are reliable.

Comparison of Arabidopsis non-host response to P. infestans to other defence-related treatments

Transcriptional changes observed during P. infestans infection were compared with those reported in two recent microarray analyses of defence-related treatments (Maleck et al., 2000; Schenk et al., 2000). We used rBLASTN searches to compare the data set of differentially expressed genes from our study to those
Table 1  Arabidopsis gene loci and number of representative cDNAs found differentially expressed during Phytophthora infection by microarray analysis. 11 000 cDNAs were used as targets for labelled cDNA from the two treatments. Genes were selected or considered significantly differentially expressed when the data points fell out of a 99% confidence interval during regression analysis in two separate comparisons (slides I and II). Genes that were found in previous studies (Maleck et al., 2000; Schenk et al., 2000) are indicated.

<table>
<thead>
<tr>
<th>Putative function</th>
<th>Locus ID</th>
<th>Number of cDNAs</th>
<th>Slide I</th>
<th>Slide II</th>
<th>Identified by</th>
<th>Maleck et al.</th>
<th>Schenk et al.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stress/defence</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>leucine-rich repeats containing protein</td>
<td>At5g25350</td>
<td>1</td>
<td>3.2</td>
<td>4.5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>catalase 3</td>
<td>At1g20620</td>
<td>11</td>
<td>2.2</td>
<td>3.0</td>
<td>yes</td>
<td>yes</td>
<td></td>
</tr>
<tr>
<td>peroxidase, putative ATP2a</td>
<td>At2g37130</td>
<td>2</td>
<td>2.8</td>
<td>4.3</td>
<td>yes</td>
<td>yes</td>
<td></td>
</tr>
<tr>
<td>glutathione transferase</td>
<td>At2g30860</td>
<td>1</td>
<td>2.4</td>
<td>2.6</td>
<td>yes</td>
<td>yes</td>
<td></td>
</tr>
<tr>
<td>glutathione transferase, putative</td>
<td>At4g02520</td>
<td>1</td>
<td>2.2</td>
<td>2.7</td>
<td>yes</td>
<td>yes</td>
<td></td>
</tr>
<tr>
<td>family II lipase EXL3</td>
<td>At1g75900</td>
<td>1</td>
<td>2.3</td>
<td>2.6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>lipoxygenase AtLOX2</td>
<td>At3g45140</td>
<td>1</td>
<td>−1.8</td>
<td>−2.3</td>
<td>yes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>thaumatin-like protein</td>
<td>At1g75030</td>
<td>1</td>
<td>−1.3</td>
<td>−1.3</td>
<td>yes</td>
<td>yes</td>
<td></td>
</tr>
<tr>
<td>Cell wall modification</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>xylosidase (glycosyl hydrolase family 3)</td>
<td>At5g49360</td>
<td>3</td>
<td>2.4</td>
<td>3.1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>xylosidase (glycosyl hydrolase family 3)</td>
<td>At5g64570</td>
<td>1</td>
<td>2.9</td>
<td>4.1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B glycosyl hydrolase family 35</td>
<td>At5g6870</td>
<td>1</td>
<td>2.5</td>
<td>4.7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>galactosidase (glycosyl hydrolase family 35)</td>
<td>At3g13750</td>
<td>2</td>
<td>2.1</td>
<td>2.7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Development</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ethylene response sensor ERS</td>
<td>At2g40940</td>
<td>1</td>
<td>2.5</td>
<td>3.6</td>
<td>yes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>senescence-associated protein SEN1</td>
<td>At4g35770</td>
<td>5</td>
<td>2.2</td>
<td>3.0</td>
<td>yes</td>
<td>yes</td>
<td></td>
</tr>
<tr>
<td>dormancy-associated protein, putative</td>
<td>At1g28330</td>
<td>2</td>
<td>2.4</td>
<td>2.7</td>
<td>yes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>auxin-regulated protein</td>
<td>At2g33830</td>
<td>3</td>
<td>2.4</td>
<td>2.8</td>
<td>yes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>cytochrome P450 CYP83B1, indole glucosinolate synthase</td>
<td>At4g31500</td>
<td>2</td>
<td>2.9</td>
<td>4.2</td>
<td>yes</td>
<td>yes</td>
<td></td>
</tr>
<tr>
<td>late embryogenesis abundant protein</td>
<td>At4g02380</td>
<td>1</td>
<td>5.0</td>
<td>6.2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>nodulin-like protein</td>
<td>At5g14120</td>
<td>1</td>
<td>2.0</td>
<td>3.1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Metabolism</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>branched-chain amino transferase</td>
<td>At1g10070</td>
<td>1</td>
<td>3.4</td>
<td>3.5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>branched-chain alpha keto-acid dehydrogenase</td>
<td>At1g21400</td>
<td>1</td>
<td>3.7</td>
<td>3.6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10-formyltetrahydrofolate synthetase</td>
<td>At1g50480</td>
<td>1</td>
<td>2.3</td>
<td>2.8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>acetylactate synthase, putative</td>
<td>At2g31810</td>
<td>1</td>
<td>2.8</td>
<td>2.7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>phytochelatin synthase APCS1</td>
<td>At5g44070</td>
<td>1</td>
<td>2.2</td>
<td>3.1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>formate dehydrogenase FDH</td>
<td>At5g14780</td>
<td>1</td>
<td>2.1</td>
<td>3.0</td>
<td>yes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>aldehyde dehydrogenase homolog</td>
<td>At1g54100</td>
<td>1</td>
<td>2.2</td>
<td>2.9</td>
<td>yes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>putative triosephosphate isomerase</td>
<td>At2g21170</td>
<td>1</td>
<td>2.1</td>
<td>2.2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>molybdenum sulphurylase</td>
<td>At5g55130</td>
<td>1</td>
<td>2.5</td>
<td>2.1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>glucose transporter</td>
<td>At1g11260</td>
<td>2</td>
<td>2.5</td>
<td>2.2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Transcription</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AP2 domain protein RAP2.3</td>
<td>At3g16770</td>
<td>3</td>
<td>2.21</td>
<td>3.34</td>
<td>yes</td>
<td>yes</td>
<td></td>
</tr>
<tr>
<td>probable transcription regulator protein</td>
<td>At3g48530</td>
<td>1</td>
<td>2.66</td>
<td>3.19</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>glycine-rich RNA binding protein AtGRP7</td>
<td>At2g21660</td>
<td>12</td>
<td>2.39</td>
<td>2.79</td>
<td>yes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>putative patatin</td>
<td>At2g26560</td>
<td>1</td>
<td>2.86</td>
<td>5.37</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>expressed protein, similar to ubiquitin</td>
<td>At1g26270</td>
<td>1</td>
<td>2.08</td>
<td>2.86</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>villin 3 fragment</td>
<td>At3g57410</td>
<td>1</td>
<td>2.7</td>
<td>2.8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>putative myosin heavy chain</td>
<td>At2g32240</td>
<td>1</td>
<td>3.5</td>
<td>4.7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>elongation factor 1-alpha</td>
<td>At1g07940</td>
<td>1</td>
<td>2.5</td>
<td>2.5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>60S ribosomal protein L7A</td>
<td>At3g62870</td>
<td>1</td>
<td>1.9</td>
<td>3.4</td>
<td>yes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unknown function</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>unknown ORF</td>
<td>At1g31580</td>
<td>1</td>
<td>2.09</td>
<td>2.60</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>expressed protein</td>
<td>At5g57655</td>
<td>1</td>
<td>1.92</td>
<td>3.12</td>
<td>yes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>expressed protein</td>
<td>At2g36320</td>
<td>1</td>
<td>2.10</td>
<td>3.41</td>
<td>yes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>expressed protein</td>
<td>At3g01290</td>
<td>1</td>
<td>2.66</td>
<td>4.24</td>
<td>yes</td>
<td>yes</td>
<td></td>
</tr>
</tbody>
</table>
Nonhost resistance of Arabidopsis to P. infestans

<table>
<thead>
<tr>
<th>Putative function</th>
<th>Locus ID</th>
<th>Number of cDNAs</th>
<th>Slide I</th>
<th>Slide II</th>
<th>Identified by</th>
</tr>
</thead>
<tbody>
<tr>
<td>expressed protein</td>
<td>At3g15450</td>
<td>1</td>
<td>1.93</td>
<td>2.58</td>
<td>yes</td>
</tr>
<tr>
<td>expressed protein</td>
<td>At1g21680</td>
<td>1</td>
<td>2.05</td>
<td>3.34</td>
<td></td>
</tr>
<tr>
<td>unknown protein</td>
<td>At2g30600</td>
<td>1</td>
<td>2.04</td>
<td>2.44</td>
<td></td>
</tr>
<tr>
<td>unknown protein</td>
<td>At1g73960</td>
<td>1</td>
<td>2.63</td>
<td>3.87</td>
<td></td>
</tr>
<tr>
<td>unknown protein</td>
<td>At1g78110</td>
<td>1</td>
<td>2.08</td>
<td>2.44</td>
<td></td>
</tr>
<tr>
<td>conserved hypothetical protein</td>
<td>At3g24860</td>
<td>1</td>
<td>2.05</td>
<td>3.56</td>
<td></td>
</tr>
<tr>
<td>hypothetical protein</td>
<td>At4g16110</td>
<td>1</td>
<td>1.03</td>
<td>1.38</td>
<td>yes</td>
</tr>
<tr>
<td>putative protein; hypothetical protein</td>
<td>At3g49590</td>
<td>1</td>
<td>2.07</td>
<td>2.62</td>
<td></td>
</tr>
<tr>
<td>putative protein</td>
<td>At4g17900</td>
<td>1</td>
<td>2.47</td>
<td>3.42</td>
<td></td>
</tr>
<tr>
<td>putative protein</td>
<td>At4g24690</td>
<td>1</td>
<td>2.05</td>
<td>3.56</td>
<td></td>
</tr>
<tr>
<td>putative protein</td>
<td>At5g53160</td>
<td>1</td>
<td>2.15</td>
<td>4.32</td>
<td></td>
</tr>
</tbody>
</table>

Published previously (see Experimental procedures). Of the 54 Arabidopsis genes represented in our data set, 15 matched cDNAs identified in the study performed by Maleck et al. (2000) and 15 cDNAs identified by Schenk et al. (2000) (Table 1). The expression ratios of the respective genes were extracted from the data sets provided with the two studies and used for building two data matrices. Cluster analysis of the two matrices generated an overview of relatedness between the various treatments and P. infestans infection (Fig. 6). Patterns of defence responses induced by MeJA treatment were found to be more similar to responses induced by P. infestans infection, whereas ethylene, SA and Alternaria inducing conditions resulted in less similar defence-induction profiles (Fig. 6A). Similar comparisons to the SAR-related treatments described by Maleck et al. (2000) were made. Cluster analysis revealed a notable resemblance of our expression data to gene expression profiles in cim11 mutant genotypes, as well as gene expression changes 48 h after treatment with the salicylate analogue benzothiadiazole (BTH). In addition, P. infestans...
defence responses shared some similarity to those induced by
nim1 over-expression, compatible and incompatible Peronospora
parasitica interactions, Pseudomonas syringae (AvrRpt2) infection,
and early response after BTH treatment (4 h) (Fig. 6B). Experimental
treatments involving plant genotypes containing the nahG
gene showed least similarity, together with cim6 and cim7 and various double-mutant genotypes.

DISCUSSION

We performed the cytological and molecular characterization of
a non-host pathosystem involving the model crucifer plant A.
thaliana and the destructive and economically important oomycete pathogen P. infestans. Our cytological observations confirm
previous work by Vleeshouwers et al. (2000), which showed that
the interaction of P. infestans with non-host plants, including
those that are phylogenetically distant from the solanaceous
hosts, is typically associated with the penetration of plant tissue
and the HR. However, a more detailed cytological investigation
needs to be performed to determine whether the proportion of
successful penetration events and the level of HR induction fol-
lowing penetration differ between host and non-host interac-
tions. Nevertheless, our results suggest that recognition of P.
infestans by Arabidopsis takes place and may form one important
barrier in non-host resistance. Therefore, a model that evokes an
arsenal of Arabidopsis R genes that recognize multiple or essen-
tial P. infestans Avr genes is sufficient to explain non-host resistance
in this pathosystem, but it cannot be ruled out that additional layers of nonspecific defence responses occur. With
extensive genetic and genomic resources available, the described
Arabidopsis–P. infestans pathosystem offers excellent prospects
for dissecting the complex layers that may form non-host resistance.

In addition to cytological analyses, we used kinetic PCR to
monitor relative levels of P. infestans DNA and consequent bio-
mass during infection (Fig. 2). Previously, DNA and RNA blot
hybridizations have been used to estimate the biomass of path-
genic oomycetes in plant tissue (Kamoun et al., 1998; Rairdan
et al., 2001). However, these techniques are not sensitive enough
to monitor the small changes in P. infestans biomass that are
expected to occur on non-host plants. In contrast, kinetic PCR is
highly sensitive, quantitative, objective and should prove ideal
for non-host pathosystems. To enhance the sensitivity of the
kinetic PCR quantification, we used primers corresponding to
highly repetitive (> ×10 000) sequences from the P. infestans
genome that allow amplification of as little as 10 fg of P.
infestans DNA (Judelson and Tooley, 2000). The sensitivity of
these primers is obvious since we routinely obtained quantifiable
signals from inoculation sites bearing as little as 1000 zoospores.

Phytophthora infestans exhibited dynamic changes in biomass
over a 3-day infection of Arabidopsis (Fig. 2). Over the first 16 h,
a significant increase in biomass was observed that was similar
to the increase observed on the host tomato. This early increase
may correspond to the germination of cysts, penetration of plant
epidermis, and formation of infection vesicles and short second-
ary hyphae, as determined by cytology on both Arabidopsis and
host plants. This suggests that some level of growth and nuclear
division, perhaps in the infection vesicles or secondary hyphae,
occur in P. infestans during early infection of Arabidopsis. Sub-
sequently, a gradual but significant decrease in P. infestans
biomass was observed from 24 to 72 h after inoculation of
Arabidopsis, and contrasted sharply with the steady increase
observed on tomato. This decrease may reflect death and degra-
dation of P. infestans hyphae caused by the HR and correlates
with the termination of pathogen ingress determined by cytology.
Interestingly, the dynamic changes in P. infestans biomass observed
on Arabidopsis contrasted with the constant level of biomass
observed for P. infestans cysts germinating in water in the
absence of plant tissue. These results support the interpretation
that a successful penetration of the plant epidermis rather than
surface growth is required for the biomass increase we observed
in the early stages of the interaction. Taken together, these results
indicate that P. infestans is able to successfully initiate an infec-
tion on Arabidopsis and complement the cytological analyses.

To gain a first insight into the molecular aspects of Arabidopsis
non-host response to P. infestans, we examined changes in the
expression of defence genes using Northern blot hybridizations
with probes for PDF1.2 and BGL2 (Fig. 4), and the transgenic
lines PR1::GUS and BGL2::GUS (Fig. 3). Although no induction of
PR1 or BGL2 was detected by Northern blot analyses, a localized
expression of both genes was detected around inoculation sites
in the transgenic reporter lines beginning 3 days after inocula-
tion. Since we found PR1 and BGL2 to be locally induced at very
late stages of the interaction, the discrepancy between the two
methods may point to a difference in sensitivity. Considering that
most of the cells in the inoculated leaves are not infected, a dilution
effect may have reduced the sensitivity of the Northern analysis.

PDF1.2 is a marker for the jasmonate (JA)/ethylene (ET)-
mediated defence-response pathways, and its up-regulation has
been associated with numerous pathogen or defence-related treatments (Glazebrook, 2001). On the other hand, PR1 and BGL2 are
marker genes for the salicylate (SA)-mediated defence pathway
that is typically induced following infection by necrotizing path-
genoms or the HR, and during systemic acquired resistance (SAR)
(Glazebrook, 2001; Ryals et al., 1996). Taken together, our data
suggest the sequential induction of the JA/ET pathway followed
by the SA pathway during non-host response of Arabidopsis to
P. infestans. Studies in other Arabidopsis pathosystems suggest
significant cross-talk and the co-regulation of both SA and JA/ET
mediated defence pathways (Clarke et al., 2000; Ellis et al., 2002;
Glazebrook, 2001; Schenk et al., 2000). In addition, these path-
ways have been shown to work antagonistically as well as in
concert, to confer enhanced resistance to fungal, bacterial and
oomycete pathogens (Cohn et al., 2001; Ellis et al., 2002; van Wees et al., 2000). The direct role of JA, ET and SA signalling in non-host resistance to *P. infestans* remains to be determined. Quantitative assays using *Arabidopsis* mutant genotypes covering the various branches of known defence pathways are currently underway to address this question.

To gain a better understanding of *Arabidopsis* non-host responses to *P. infestans*, we performed DNA microarray experiments comparing the responses of *Arabidopsis* plants inoculated with *P. infestans* to their mock inoculated counterparts. Despite the harsh selection conditions imposed on the data set, a remarkably high level of redundancy was found amongst the positive cDNAs (see supplementary data at http://www.oardc.ohio-state.edu/phytophthora/supp.htm). Subsequent Northern blot analysis validated our regression analysis strategy. There was a clear correlation in induction levels between the microarray experiment and Northern blot hybridizations using two selected genes and two non-selected genes (Fig. 5). In addition, about one-third of the cDNAs that were identified in our experiments overlapped with cDNAs identified in the defence response gene expression profiling studies of Maleck et al. (2000) and Schenk et al. (2000) (Table 1).

We classified plant responses using cluster analyses of gene expression profiles across the *P. infestans* treatments and other defence related treatments (Fig. 6). *P. infestans*-induced defence responses were most similar to gene-expression changes after MeJA treatment. From the 15 genes that were used for these comparisons, 14 genes were also induced by MeJA, according to Schenk et al. (2000), suggesting that non-host defence responses to *P. infestans* are associated with activation of the JA response pathway (Fig. 6A). In contrast, the expression profiles of all other treatments, such as ethylene, *Alternaria* and SA, had less similarity to our data set. Similarly, patterns of overlap were found in the comparison of SAR-related treatments (Maleck et al., 2000) to our data set (Fig. 6B). Cluster analysis of expression profiles indicated a similarity between treatments and the occurrence of two general groups of *Arabidopsis* genes within the set of *P. infestans* up-regulated genes. Two genes were up-regulated 48 h after BTH treatment and had higher expression levels in the *cim11* (constitutive immunity) mutant background. A second and larger group of seven genes was commonly up-regulated in our treatment and during *Peronospora parasitica* compatible and incompatible interactions, BTH treatment (4 h), plants over-expressing *NIM1*, and *Pseudomonas syringae* infection. The transcript levels of the genes used in this comparison were either unchanged or lowered in all NahG plants and NahG-containing mutants (*cim11*NahG and *cim6*NahG), *cim6* and *cim7* mutant genotypes and other related treatments.

Some notable genes that are induced in the defence-related treatments examined by Schenk et al. (2000) and Maleck et al. (2000) were identified in this study (Table 1). Among these, *Rap2.3* (At3g16770), which encodes an AP2 domain transcription factor, was up-regulated during non-host resistance and many other defence responses, suggesting that it may mediate common regulatory steps in defence pathway activation or modulation. Another gene, *Cyp83B1* (At4g31500), is a member of a large family of cytochrome P450 genes, and is involved in the production of indole-glucosinolates as well as the plant hormone IAA (auxin) (Bak and Feyereisen, 2001). The expression of *Cyp83B1* and related members of this gene family was found to be elevated upon SA and MeJA treatments and were associated with an increase in indole-glucosinolates (Mikkelsen et al., 2003). Smolen and Bender (2002) identified a non-functional mutant of *Cyp83B1* that showed a lesion-mimic phenotype. Taken together, these data indicate a possible involvement of *Cyp83B1*, and perhaps indole-glucosinolates, in defence responses and possibly regulation of the HR.

Many of the *Arabidopsis* genes identified as up-regulated during non-host response to *P. infestans* could be related to cellular aspects of signalling and defence. For instance, the expression of a glycosyl hydrolase gene (At3g13750, At5g49360, At5g56870 and At5g64570), that are possibly involved in modifications of cell wall components were up-regulated two- to fivefold (Table 1). During cell stress and pathogen attack, cell wall modifications are commonly observed (Heath, 1998; Nicholson and Hammerschmidt, 1992; Vleeshouwers et al., 2000) which is also illustrated by the cell wall depositions described in Fig. 1. Therefore, cell wall alterations are likely to form a major barrier in non-host resistance. Other notable genes that were up-regulated include genes related to oxidative stress such as catalase (At1g20620), glutathione transferases (At2g30860 and At4g02520), and peroxidase (At2g37130). The occurrence of these genes during non-host HR is not surprising since the role of oxidative stress and production of active oxygen species (AOS) during the HR is well documented (Delledonne et al., 2001; Levine et al., 1994; Sasabe et al., 2000). Since the HR is observed in the *Arabidopsis*–*P. infestans* interaction, induction of these genes in concert with early defence is plausible. Overall, these data support the concept that defence responses induced by *P. infestans* involve the HR as well as JA-mediated signalling and defence.

In addition to genes in common between non-host and various host defence treatments, some genes uniquely up-regulated during the *P. infestans* interaction were identified (Table 1). The function of these genes in non-host resistance remains unclear, but they represent attractive candidates for functioning in processes unique to non-host resistance to *P. infestans*, and perhaps, other non-host pathogens.

In this study we characterized the interaction between *P. infestans* and the non-host plant *A. thaliana*. An integrated multifaceted approach has enhanced our understanding of this interaction and is helping us to devise future research strategies. Based on the diversity of molecular genetic tools and genomic...
resources available for *Arabidopsis* and *Phytophthora*, we expect this non-host pathosystem to become of key importance in studies on molecular plant–microbe interactions. Further research on this pathosystem will provide significant insight into key molecular processes regulating non-host resistance to an economically important pathogen. The knowledge gained will result in immediate biotechnological applications and will offer novel perspectives for engineering durable resistance in crop plants.

**EXPERIMENTAL PROCEDURES**

**Plant growth conditions**

*Arabidopsis* (Col-3) seeds were routinely surface sterilized in 70% EtOH for 30 s, followed by incubation in 50% bleach solution for 10 min. Seeds were then washed multiple times in dH2O before plating on to MS-Phytagar sucrose plates (1 × MS salts, 2% w/v sucrose, 0.8% w/v Phytagar). Plated seeds were incubated at 4 °C for 3–4 days prior to germination. Seven-to-10 day old seedlings were transferred to potting media and grown under controlled conditions (22 °C, 8 h photoperiod). Mature non-bolting plants at the rosette stage (4–5 weeks) were used for infection experiments.

**Phytophthora infestans** culturing and infection assays

Cultures of *P. infestans* isolate 90128 (A2 mating type, race 1.3.4.7.8.9.10.11, isolated from potato in the Netherlands in 1990), were routinely grown on rye agar medium supplemented with 2% sucrose (Caten and Jinks, 1968). Zoospores were produced by flooding 11–14-day-old cultures with dH2O, followed by incubation at 4 °C for 1–3 h. Rosette leaves of 4–5 week-old *Arabidopsis* (Col-3) plants were inoculated with 10 μL droplets of zoospore suspensions for microscopy. For the DNA microarray and Northern time course experiments, complete rosette stage plants were sprayed with zoospore suspensions. Concentrations ranging from 200 000 to 500 000 zoospores/mL were used for all experiments. Deionized water was used as a negative control in all relevant experiments.

**Microscope observations**

Leaf discs containing the inoculum were excised at various times after inoculation and examined by microscopy for plant response and growth of *P. infestans*. Lactophenol-trypsin blue staining and destaining with chloral hydrate were performed as previously described (Colon et al., 1992; Wilson and Coffey, 1980). The discs were examined using a Zeiss Axiophot microscope equipped with a high-pressure mercury vapour lamp. Autofluorescence was observed with a G365 excitation filter, FT395 interference beam splitter and LP420 barrier filters.

**GUS staining procedure**

Complete *Arabidopsis* leaves were immersed in a GUS staining solution (2 mM 5-bromo-4-chloro-3-indolyl-beta-D-glucuronic acid (X-Gluc) (Rose Scientific, Edmonton Alberta, Canada) in 0.2% Triton X-100, 50 mM NaHPO4 Buffer (pH 7.2), 2 mM potassium ferrocyanide, 2 mM potassium ferricyanide). A vacuum was applied for 10 min and was then gently released over several minutes. Leaves were incubated at 37 °C for 24 h and examined macroscopically for staining patterns.

**Kinetic PCR quantification of *P. infestans***

For kinetic (real-time) PCR quantification of *P. infestans* biomass in plant tissue, four samples were taken for every experimental treatment or time point, with each sample consisting of a pool of four leaf discs corresponding to four independent inoculation sites. Total DNA was extracted from each pool of four leaf discs using a Qiagen Plant DNA extraction kit following the manufacturer’s instructions. DNA was quantified using the picogreen ds DNA quantification kit (Molecular Probes, Eugene, OR) and checked by electrophoresis. Dilutions were made using volumes of 10 μL or larger to minimize pipeting errors. Kinetic PCR was performed on a Roche Lightcycler (Roche, Indianapolis, IN) using Lightcycler-FastStart DNA Master SYBR Green I reagents, primers J-08-3 and J-08-4 previously described by Judelson and Tooley (2000), and 2–10 ng of total DNA as a template. PCR conditions were 45 cycles of 1 s at 95 °C, 5 s at 50 °C, and 10 s at 72 °C, with a temperature transition rate of 20 °C per s. *Phytophthora infestans* relative DNA amounts were estimated using a standard curve generated using 100, 10, 1 and 0.1 pg of purified *P. infestans* total DNA. Natural log (ln) transformation was applied on the data to obtain distributions that approached normality. Subsequently, ANOVA was performed on the normalized data using both treatment and replication in the model as fixed effects using the SAS statistical software package version 8 (SAS Institute, Cary, NC). Least significant difference mean separation was used to detect significant differences between the time points assayed.

**RNA manipulations and Northern blot analysis**

RNA was extracted using the Trizol reagent (Gibco-BRL, Bethesda, MD) using the manufacturer’s directions. RNA samples were checked for purity and integrity using spectrophotometry and electrophoresis. When necessary, samples were cleaned further by adding 1 volume of phenol:chloroform:isoamyl-alcohol (125 : 24 : 1, pH 6.7) to the sample, high-speed centrifugation (15800 g, 15 min) and subsequent collection of the aqueous phase. This step was then followed by another extraction using equal volumes of chloroform. RNA was ethanol precipitated from the aqueous phase and dissolved in dH2O. Membranes for Northern blot
analysis were prepared using a modified method from McMaster and Carmichael (1977) as described in Sambrook et al. (1989). 15–20 µg of RNA per sample was used and RNA was blotted on Hybond N+ membranes (Amersham, Piscataway, NJ) following standard procedures and instructions from the manufacturer.

Hybridization probes were first prepared using Polymerase Chain Reaction (PCR) amplifications. Primers for the amplification of PDF1.2, BGL2 and UBQ5 were as described elsewhere (Glazebrook et al., 1996; Penninckx et al., 1996; Xiao et al., 2000). Primers for amplification of the selected genes for microarray validation were: At1g21400-F (5′-AACCGATGTGGAGTGAGGAAG-3′); At5g25350-F (5′-CTTCACCTCCTGATACACTCA-3′) and At5g25350-R (5′-CTTCAGATATGAGAGGAGGAGAG-3′) and At1g21400-R (5′-CTTCAGATATGAGAGGAGGAGAG-3′); At1g421400-R (5′-AACCGATGTGGAGTGAGGAAG-3′); At5g25350-F (5′-CTTCACCTCCTGATACACTCA-3′) and At5g25350-R (5′-CTTCAGATATGAGAGGAGGAGGAG-3′) and At2g40000-R (5′-GGAACAATCCCAAAACGGA-3′); At3g43740-F (GAGGAAGATGTTATCATCAG-3′) and At3g43740-R (5′-TCCATTCACGGTGGTT-3′) and At3g43740-R (5′-TCCATTCACGGTGGTT-3′).

All probes were labelled with α-32P-dCTP using a random primer labelling kit (Gibco-BRL, Bethesda, MD). All Northern blot hybridizations were carried out at 65 °C in Modified Church Buffer (0.36 M Na2HPO4, 0.14 M NaH2PO4, 1 mM EDTA and 7% primer labelling kit (Gibco-BRL, Bethesda, MD). All Northern blot and 0.5 × SD. Membranes were washed for 15 min in 1× SSC/0.5% SDS and 0.5× SSC/0.5% SDS at 65 °C, followed by 1 wash in 0.5× SSC/0.5% SDS for 1 min at room temperature. Membranes were exposed to a phosphor imager screen for 24–48 h and scanned using a Molecular Dynamics Storm 840 Phosphor Imager.

DNA microarray hybridizations

Total RNA samples were subjected to standard probe preparations and microarray hybridization procedures as described in the protocol section on the Arabidopsis Functional Genomics Consortium (AFGC) website (http://afgc.stanford.edu/afgc.html/site2.htm). Briefly, two purified mRNA samples were used for synthesis of probes labelled with either CY3 or CY5 fluorescent dye. Four separately labelled cDNA samples were generated using a dye swap to distinguish replicates of the same treatment. These were hybridized to two slides in a dye-swap experimental setup (technical replication). Hybridized slides were scanned using a Scan Array 3000 (GSI Lumonics, Billerica, MA). Two data points were obtained for each spot on one slide.

Microarray data analysis

The generated data were normalized by the default method specified by the AFGC (http://afgc.stanford.edu/afgc.html/site2.htm). All data analysis and processing steps were done using the SAS statistical software package version 8 (SAS Institute, Cary, NC, USA). For analysis purposes, normalized but ‘raw’ expression values were downloaded for both hybridized slides (technical replicates) from the AFGC web site. The natural log of every expression value was calculated to obtain a data set with distributions that approached normality. Transformed data points were then used for comparisons, applying a regression analysis/ outlier detection technique. In short, for every slide, normalized and transformed expression values of both treatments were plotted against each other (x-axis, expression values of mock treatment, y-axis, expression values of P. infestans treatment). A 99% confidence interval (CI) was generated and used to select cDNAs that correspond to data points falling outside of the 99% CI, representing significant differential expression. Lists of identifiers representing the cDNA spots that were detected were compiled. Only cDNAs identified as outliers at both slides were considered significantly differentially expressed and were used for further analysis. The cDNA identifiers were used to retrieve annotated locus names from the Arabidopsis Information Resource (TAIR) database (http://www.Arabidopsis.org). A non-redundant data set was generated and was used to construct cluster data files.

Data compilation and cluster analysis

To identify overlap between the genes identified in this study and published data sets, we first compiled files containing nucleotide sequences for the differentially regulated genes reported in the DNA microarray studies of Schenk et al. (2000) and Maleck et al. (2000). We then used amino acid sequences corresponding to the 54 differentially expressed genes (Table 1), to perform a TBLASTN search against the generated sequence files. E-value scores below 10−12 were considered significant. Data matrices were constructed by retrieving and combining expression data of the genes common in either relevant data set. Expression ratios for each gene and treatment were calculated using the inherent control of each treatment as the denominator. Two data matrices were used for cluster analysis using the Cluster analysis software package (Eisen et al., 1998). Self-Organizing Map (SOM) analysis was first used to generate classes of genes based on expression ratios across all treatments. Output files generated in this procedure were then used to order the input file for cluster analysis. Complete average linkage hierarchy clustering was used for both comparisons presented. Generated results were visualized and evaluated using TreeView (Eisen et al., 1998).

Microarray data availability

The entire data set can be freely obtained and searched at the AFGC website (http://afgc.stanford.edu/afgc.html/site2.htm). Data for the differentially regulated genes is also provided as a supplementary file (http://www.oardc.ohio-state.edu/phytophthora/supp.htm).
ACKNOWLEDGEMENTS

This work was supported by the OARDC Research Enhancement Grant Program. Salaries and research support were provided, in part, by State and Federal Funds appropriated to the Ohio Agricultural Research and Development Center, the Ohio State University. We are grateful to Caitlin Cardina, Shujing Dong, Diane Kinney and Antonino Testa for their expert technical assistance; Bert Bishop for his suggestions regarding statistical analysis; as well as Tea Meulia and the staff of the OARCD Molecular and Cellular Imaging Center for help with the kinetic PCR experiments. We are also grateful to Alan Shapiro and Brian Staskawicz for the PR1-GUS line, the Arabidopsis Biological Resource centre for the Arabidopsis lines and the Arabidopsis Functional Genomics Consortium for providing their DNA microarray service, expertise and assistance.

SUPPLEMENTARY MATERIAL

A Table has been provided, which is available at <http://www.blackwellpublishing.com/products/journals/suppmat/MPP/ MPP195/MPP195sm.htm>, and which lists identified cDNAs with their respective GenBank accession and Locus ID numbers. Redundancy represents the number of cDNAs identified from each locus. Ratios S1 and S2 were calculated using the normalized expression values from each hybridized slide.

REFERENCES


Vleeshouwers, V.G.A.A., van Dooijeweert, W., Govers, F., Kamoun, S.


