Genome-Wide Expression Profiling Arabidopsis at the Stage of *Golovinomyces cichoracearum* Haustorium Formation[1][W][OA]

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Compatibility between plants and obligate biotrophic fungi requires fungal mechanisms for efficiently obtaining nutrients and counteracting plant defenses under conditions that are expected to induce changes in the host transcriptome. A key step in the proliferation of biotrophic fungi is haustorium differentiation. Here we analyzed global gene expression patterns in *Arabidopsis thaliana* leaves during the formation of haustoria by *Golovinomyces cichoracearum*. At this time, the endogenous levels of salicylic acid (SA) and jasmonic acid (JA) were found to be enhanced. The responses of wild-type, *npr1-1*, and *jar1-1* plants were used to categorize the sensitivity of gene expression changes to NPR1 and JAR1, which are components of the SA and JA signaling pathways, respectively. We found that the infection process was the major source of variation, with 70 genes identified as having similarly altered expression patterns regardless of plant genotype. In addition, principal component analysis (PCA) identified genes responding both to infection and to lack of functional JAR1 (17 genes) or NPR1 (18 genes), indicating that the JA and SA signaling pathways function as secondary sources of variation. Participation of these genes in the SA or JA pathways had not been described previously. We found that some of these genes may be sensitive to the balance between the SA and JA pathways, representing novel markers for the elucidation of cross-talk points between these signaling cascades. Conserved putative regulatory motifs were found in the promoter regions of each subset of genes. Collectively, our results indicate that gene expression changes in response to infection by obligate biotrophic fungi may support fungal nutrition by promoting alterations in host metabolism. In addition, these studies provide novel markers for the characterization of defense pathways and susceptibility features under this infection condition.

Obligate fungal biotrophs must establish compatible interactions with their hosts to survive. These organisms have evolved unique strategies to extract nutrients from infected living cells, including the assembly of specialized structures located in intimate contact with host cells. However, it is still unclear how these parasites are able to avoid plant defense activation (Panstruga, 2003). Past research efforts have typically focused on the study of mechanisms underlying fungal nutrition (Voegele, 2006), providing relatively less information regarding host conditions leading to the establishment of compatibility (for review, see Huckelhöven, 2005; O'Connell and Panstruga, 2006). Recently, high-throughput transcriptome analyses have helped define the responses triggered in plant tissues upon recognition of these fungal pathogens. These studies were performed under conditions promoting activation of defenses involving either host resistance (Caldo et al., 2004; Eckey et al., 2004; Eulgem et al., 2004; Zierold et al., 2005; Michel et al., 2006) or nonhost resistance (Zimmerli et al., 2004; Bruggmann et al., 2005). Under conditions leading to compatibility, basic gene expression changes have been examined in *Vicia faba* (Wirsel et al., 2001), barley (*Hordeum vulgare*; Gjetting et al., 2004), and *Brassica oleracea* (Casimiro et al., 2006). Except for these reports, however, few studies have examined plant defense programs and global host metabolic features leading to the establishment of compatibility.

The interaction between Arabidopsis (*Arabidopsis thaliana*) and *Golovinomyces cichoracearum* (formerly *Erysiphe cichoracearum*) is an ideal system for exploring

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the compatibility between plants and obligate biotrophs. *G. cichoracearum* UCSC1 causes powdery mildew disease on some *Brassicaceae* and *Cucurbitaceae* species (Adam et al., 1999; Braun et al., 2002), and can establish either incompatible or compatible interactions with different Arabidopsis accessions (Adam and Somerville, 1996). Resistance against different *Golovinomyces* species is mediated by monogenic traits (Xiao et al., 1997, 2001; Adam et al., 1999) such as that conferred by the *RPW8* locus, which encodes a small novel protein conferring resistance against a wide range of powdery mildews (Xiao et al., 2001, 2005). In contrast, the conditions promoting compatibility have been less well characterized.

The progressive differentiation of fungal infection structures is believed to require diverse host target molecules that are collectively called susceptibility factors (Heath, 2000; Parniske, 2000; Schulze-Lefert and Panstruga, 2003). Researchers have hypothesized that the absence of one or more of these molecules would lead to a durable and recessively inherited resistance against all pathogen species. Forward genetic studies on the Arabidopsis-*G. cichoracearum* interaction led to the isolation of six recessive loci necessary for successful fungal proliferation (*pmr1–pmr6*), four of which have been cloned to date. *PMR2* is an ortholog of the *MLO* gene from barley, which encodes a modulator of plant defenses and cell death (Büschges et al., 1997; Panstruga and Schulze-Lefert, 2003; Consonni et al., 2006). *PMR4* encodes a callose synthase and lack of this gene product mediates resistance through deregulation of the salicylic acid (SA) pathway (Nishimura et al., 2003). *PMR5* encodes a protein of unknown function whereas *PMR6* encodes a pectate lyase-like protein and plants lacking *PMR5* or *PMR6* display alterations in cell wall composition (Vogel et al., 2002, 2004). Most of these *PMR g*enes are constitutively expressed and are expected to constitute susceptibility components. The only host gene identified to date that may encode a susceptibility factor and becomes induced by fungal interaction is *EDR1*, which encodes a MAPKKK acting as a negative regulator of the SA and ethylene (ET) pathways (Frye and Innes, 1998; Frye et al., 2001; Tang et al., 2005a). Two other host products affecting resistance to *G. cichoracearum* are those encoded by *EDR2* and *EDR3*. EDR2 is a novel protein expressed in all tissues and organs that may regulate defenses through lipid signaling (Tang et al., 2005b; Vorwerk et al., 2007), whereas EDR3 belongs to the dynamin-related protein family and is at least partially located in mitochondria (Tang et al., 2006). Further studies will be required to identify other nonconstitutive plant genes involved in the establishment of compatibility with *G. cichoracearum*.

During interactions with virulent pathogens, plants accumulate defense gene transcripts in a transient (Tao et al., 2003) or delayed manner (Frye and Innes, 1998). The major pathogen-inducible defense pathways are regulated by SA, jasmonic acid (JA) and ET, or complex networks interconnecting these defense pathways (Kunkel and Brooks, 2002; Glazebrook et al., 2003). Previous studies have partially analyzed the participation of the SA and JA/ET pathways in compatible interactions between Arabidopsis and *G. cichoracearum*. Exogenous JA or the SA analog BTH were found to protect susceptible plants against fungal infection (Maleck et al., 2002; Zimmerli et al., 2004; Glazebrook, 2005). Plants with mutations in the SA or JA signaling pathways (e.g. *pad4*, *eds5*, *eds14*, *eds15*, *sid2*, *npr1-3*, *NahG*, and *coi-1*) are hypersusceptible to *G. cichoracearum* and its closest relative *Golovinomyces orontii* (formerly *Erysiphe orontii*; Reuber et al., 1998; Dewdney et al., 2000; Ellis et al., 2002a, 2002b). In addition, plant mutants with constitutive or inducible activation of the SA- (*pmr4*, *edr1*) or JA/ET- (*wrky70*, *cev-1*) dependent defenses are resistant to *G. cichoracearum* (Frye and Innes, 1998; Ellis and Turner, 2001; Nishimura et al., 2003; Li et al., 2006). However, it is still unknown whether under compatible infection conditions these defense pathways are either not activated or inactivated by pathogen effectors. The use of defense pathway markers such as NPR1 and JAR1 would help to elucidate this point. NPR1 is an ankyrin-repeat protein that stimulates plant defenses through the SA-dependent pathway by interaction with transcription factors. This interaction is impaired in *npr1-1* (nonexpressor of pathogenesis related genes) mutant plants (Cao et al., 1997; Fan and Dong, 2002; Rairdan and Delaney, 2002). JAR1 is involved in the JA adenylation that is apparently required for JA function and the *jar1-1* (JA resistant 1) mutation reduces the impact of the jasmonate-dependent defense pathway (Staswick et al., 1992, 1998, 2002), which becomes activated upon interaction with *G. cichoracearum* (Zimmerli et al., 2004), making *jar1-1* plants useful to reveal responses associated with jasmonate-dependent pathway in this susceptible state.

The haustorium formation constitutes a relevant step for analysis of Arabidopsis gene expression changes during compatible interactions with *G. cichoracearum*. The haustorium is a structure that develops in the extracellular space of host epidermal cells, and expands to increase the surface contact with the invaded cell following invagination of a specialized hypha. Haustorial structures are only built by obligate biotrophs and their formation is considered a selective advantage for survival because they function as feeding organs for transporting sugar, amino acids, and water to the fungal tissues (Adam et al., 1999; Szabo and Bushnell, 2001), and they are likely to potentiate fungal virulence by helping release effectors to the host cell (Szabo and Bushnell, 2001; Panstruga, 2003; Ellis et al., 2006). In this sense, cells that already support the establishment of haustoria due to prior infection by compatible isolates of Erysiphales, become more susceptible to subsequent infections by nonhost fungi. Over the past decade, numerous studies have reported on the structural and physiological features of haustorial development, mostly regarding differentiation and nutrient transport (O'Connell and

Panstruga, 2006). However, the molecular characteristics of host cell responses at this particular infection step are not well understood.

Here, we sought to describe qualitative and quantitative changes in the global gene expression profiles of susceptible Arabidopsis plants supporting the development of *G. cichoracearum* haustoria. We analyzed the features of compatibility at this infection stage, and further evaluated the contribution of the SA- and JA/ET-dependent defense signaling pathways in the pathogen-induced responses by comparing responses in infected wild-type, *npr1-1*, and *jar1-1* plants. Our findings collectively contribute to knowledge regarding early host cell alterations generated in response to attack by this virulent obligate biotrophic fungus.

RESULTS

Experimental System

We used microarray analysis to characterize global transcriptional changes occurring in Arabidopsis leaf tissues infected with *G. cichoracearum* at early stages of haustorium formation. This step involves the generation of the fungal feeding organ and is critical for pathogen proliferation. To test the impact of NPR1- and JAR1-defense signaling pathways at this infection step, we examined gene expression changes in wild-type, *npr1-1*, and *jar1-1* plants treated with the virulent fungal isolate UCSC1 (Adam et al., 1999). We collected samples from infected tissues 18 h postinfection (hpi) because our observation of fungus-treated tissues indicated that the number of haustoria detected at 18 hpi was approximately 90% of that found at 24 hpi (Fig. 1A), when the *G. cichoracearum* haustorium is fully developed in Arabidopsis (Adam and Somerville, 1996; Zimmerli et al., 2004). At 18 and 24 hpi, the abundance of primary hyphae, secondary hyphae, and haustoria did not significantly differ among the three tested genotypes (Fig. 1, A and B). At 96 hpi, conidiation was slightly lower in *npr1-1* plants than in *jar1-1* plants (Fig. 1C). However, from 6 to 8 d postinfection (dpi) onward, *npr1-1* and *jar1-1* plants displayed the same or even higher levels of susceptibility than wild-type plants (data not shown).

We performed four independent replicates for each plant genotype and isolated samples from uninfected (T0) and infected (T18) leaf tissues to obtain a set of 24 samples (four replicates of three genotypes, each under two infection states) for cDNA microarray analysis.

cDNA Microarray Analysis

mRNA was isolated from the 24 samples and used to synthesize labeled cDNAs, which were hybridized into 24 microarrays as indicated in Supplemental Table S1 and "Materials and Methods". Initially, we analyzed the technical and biological variability of the observed gene expression changes by determining the Pearson's correlation coefficient (r) for data obtained at T18 and

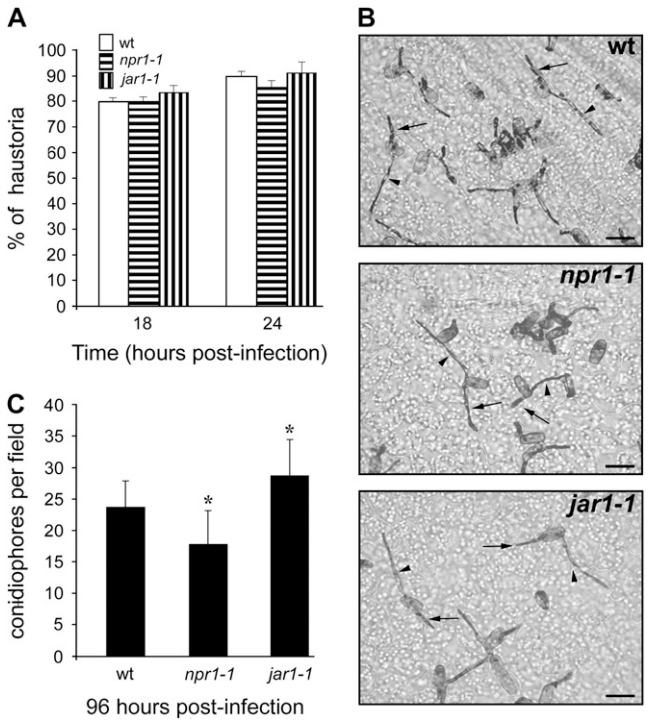

Figure 1. *G. cichoracearum* development on susceptible wild-type (wt), *npr1-1*, and *jar1-1* mutant Arabidopsis plants. A, Abundance of haustoria on the plant epidermal cells at 18 and 24 hpi. Percent values are relative to germinated conidia and represent the average ± SD of 15 infected leaves per plant. Similar results were obtained in three independent experiments. B, Bright field images illustrating fungal development at 18 hpi on representative plants of each genotype. Arrows indicate secondary germ tubes, and arrowheads indicate haustoria formed from primary germ tubes. Scale bars, 10 μm. C, Abundance of mature conidiophores per field at 96 hpi assessed by microscopic observation (200×). Values represent the average ± SD of 10 infected leaves per plant. Similar results were obtained in three independent experiments. The asterisk (*) indicates statistically significant differences at $P < 0.001$.

T0, in plants from each genotype across the four replicates. Low correlation was found among overall responses (r = 0.12–0.19; average 0.16) suggesting that the large majority of genes were insensitive to infection. Differentially expressed genes (discussed in detail in the next section) were defined as those showing an absolute standardized difference in expression between T18 and T0 ≥2.5 SD from zero in at least one replicate. When these differentially expressed genes were assessed for correlation, we observed a high level of reproducibility of responses among the four independent replicates (r = 0.49–0.60; average 0.54). Based on these results, we used the whole dataset for the following studies.

Although our cytological observations did not suggest that either of the mutants showed enhanced susceptibility at 18 hpi (Fig. 1), we tested for genotype-specific differences at the molecular level by calculating the r-values obtained for all combinations of paired samples. For each gene, the mean values from four replicates of normalized data were considered and

Table I. *Pearson correlation coefficients calculated over all the intensity data from each slide*

The Pearson correlation coefficients were calculated after obtaining the fold-change average ratios for T0/CR (time 0 hpi versus common reference) and T18/CR (time 18 hpi versus common reference) for each of the indicated dataset pairs. The numbers in the body of the table correspond to the *r*-values. The first three columns/bottom three rows indicate genes showing lack of correlation for expression levels at T0 versus T18 among the three plant genotypes. The first three columns/top three rows, and last three columns/bottom three rows indicate significant positive correlations of gene expression levels among the three genotypes in uninfected (T0, top left corner) or infected (T18, bottom right corner) samples ($P < 0.0001$).

	Wild Type (T0)	*npr1-1* (T0)	*jar1-1* (T0)	Wild Type (T18)	*npr1-1* (T18)	*jar1-1* (T18)
Wild type (T0)	1.00	–	–	–	–	–
npr1-1 (T0)	0.62	1.00	–	–	–	–
jar1-1 (T0)	0.49	0.40	1.00	–	–	–
Wild type (T18)	−0.01	−0.04	0.002	1.00	–	–
npr1-1 (T18)	0.09	0.07	−0.06	0.63	1.00	–
jar1-1 (T18)	0.11	0.09	0.09	0.63	0.70	1.00

either all data contained in the slides (10,268 ESTs; Table I), or only pathogen-regulated genes (936 ESTs; Table II) were included in the test. Significant correlations ($P < 0.0001$) were observed for all comparisons between T0 samples of different genotypes (Tables I and II) indicating that the *npr1-1* and *jar1-1* mutations did not introduce major qualitative differences in host basal gene expression. In contrast, fungal infection triggered major changes in the gene expression profiles of plants of all three genotypes. The expression levels of most genes were not altered in a coordinated way, as indicated by low or null correlations for the T0 to T18 comparisons (Table I). However, the T0 and T18 samples were fairly strongly negatively correlated when the analysis was performed only with genes displaying significant up- or down-regulation (first three columns/bottom three rows, Table II). Interestingly, significant correlations were found for all pairs of T18 samples (last three columns/bottom three rows, Tables I and II) indicating that lack of NPR1 or JAR1 did not significantly affect the large majority of the gene expression changes observed at 18 hpi.

Arabidopsis Genes Responding to *G. cichoracearum* Independently of the NPR1- or JAR1-Mediated Signaling Pathways

Plant genes showing infection-mediated changes independent of the NPR1- and JAR1-transduction pathways could conceivably modulate basal defenses or features of host compatibility (e.g. cell death inhibition, defense inhibition, fungal nutrition). In an effort to identify such genes, we sought genes showing expression changes in the same direction (induction or repression) in infected plants of all three genotypes. We applied a multivariate ordination approach (PCA), to a matrix of standardized mean differences between T0 and T18 for each plant type. The results from this analysis indicated that the first principal component (PC1), which expressed the effect of infection, was the dominant source of variability (76%). We then selected the 1% of genes showing the highest differences between T18 and T0 samples, yielding 144 genes (67 induced and 77 repressed; Supplemental Table S3). To increase the confidence in detection of gene expression changes, we applied two other statistical analyses, significant analysis of microarrays (SAM) and ANOVA, and selected genes that consistently showed expression changes in all three analyses. SAM identified 217 differentially expressed genes (116 induced and 101 repressed; Supplemental Table S4) whereas ANOVA identified 223 such genes (117 induced and 106 repressed; Supplemental Table S5).

The results obtained from the three statistical methods are shown in Figure 2. Similar fractions of differentially expressed genes were selected by each method, 281 genes were selected by at least one method (145 induced, 136 repressed), and 70 genes were selected by all three methods (36 induced, 34 repressed). Northern-blot assays were performed to

Table II. *Pearson correlation coefficients among the infection-responsive genes*

The uncentered Pearson correlation was used to calculate correlation coefficients among the 936 genes showing differential expression between 0 hpi (T0) and 18 hpi (T18) in all three plant genotypes. The first three columns/bottom three rows indicate genes having a significant negative correlation between gene expression levels at T0 versus T18 for the three genotypes, showing consistent changes on gene expression (either induction or repression). The first three columns/top three rows and last three columns/bottom three rows indicate high significant positive correlations of gene expression levels among the three genotypes in uninfected (T0, top left corner) or infected (T18, bottom right corner; $P < 0.0001$).

	Wild Type (T0)	*npr1-1* (T0)	*jar1-1* (T0)	Wild Type (T18)	*npr1-1* (T18)	*jar1-1* (T18)
Wild type (T0)	1.00	–	–	–	–	–
npr1-1 (T0)	0.85	1.00	–	–	–	–
jar1-1 (T0)	0.77	0.71	1.00	–	–	–
Wild type (T18)	−0.68	−0.69	−0.61	1.00	–	–
npr1-1 (T18)	−0.64	−0.65	−0.65	0.84	1.00	–
jar1-1 (T18)	−0.64	−0.64	−0.64	0.84	0.84	1.00

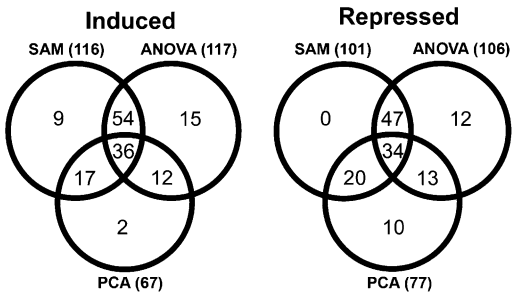

Figure 2. Venn diagrams showing the number of Arabidopsis genes differentially regulated during *G. cichoracearum* haustoria development. The total number of genes identified by each statistical analysis is given in brackets.

evaluate the expression of nine of the 70 selected genes in naïve and infected tissues of wild-type, *npr1-1*, and *jar1-1* plants. The northern-blot results confirmed the expression patterns predicted by the microarray data, in all cases validating the selection criteria used in our microarray analysis (four examples are shown; see Fig. 4A).

The identities, average fold changes, and functional categories assigned to the 70 selected genes (according to the Munich Information Center for Protein Sequences [MIPS] criteria; http://mips.gsf.de/proj/thal/db/index.html) are listed in Table III. The largest group of genes included those affecting basal metabolism (12 induced, 12 repressed), with other notable groups including genes affecting transcription and signal transduction (seven induced, 11 repressed), energy generation (five induced genes), cellular transport (three induced, one repressed), and defense (two induced, three repressed).

Host Genes Sensitive to NPR1- and JAR1-Mediated Signaling

We looked for genes sensitive to infection through the NPR1- or JAR1-dependent pathways, by seeking those genes showing differential responses in wild-type plants versus *npr1-1* or *jar1-1* mutants. We applied PCA-based analysis to discriminate and further subtract the influence of PC1 (infection). We found that the ordination space created by the PC2 and PC3 together represented 24% of the total variability (see ''Materials and Methods''). The genes ordered in this space are presented in Figure 3A, which shows the approximately 1% of ESTs furthest from the origin. Among them, PC2 distinguishes *jar1-1* with a positive coefficient from wild-type and *npr1-1* with negative contributions. Genes induced during infection in wild-type and *npr1-1* plants but not in *jar1-1* plants, appear on the negative side of the PC2 axis. PC3 distinguishes *npr1-1* from wild type and *jar1-1*. Genes induced in wild-type and *jar1-1* plants but not in *npr1-1* plants are located on the negative side of the axis. This analysis allowed the selection of 74 genes. To determine whether these genes shared common expression patterns, we performed cluster analysis. Four gene clusters were obtained based on their common expression profiles (Groups 1–4, Fig. 3B). The identity and functional classification of genes from these four groups are shown in Table IV. Group 1 includes 22 genes showing infection-induced activation in wild-type plants, slight repression in *jar1-1* plants, and no change in *npr1-1* plants (Fig. 3B). Group 2 includes 17 genes repressed by infection in wild-type plants and slightly induced in infected *jar1-1* and *npr1-1* plants. Group 3 contains 17 genes displaying differences in infected *jar1-1* versus wild-type or *npr1-1* plants and Group 4 contains 18 genes showing differences in infected *npr1-1* versus wild-type or *jar1-1* plants. Thus, Groups 3 and 4 seemed to include genes responding to JAR1- and NPR1-dependent signaling at the time of haustorium formation, as supported by the presence of *PDF1.1*, which is known to be sensitive to JA (Glazebrook et al., 2003 and references therein) in Group 3, and that of *ICS1* (isochorismate synthase), which is sensitive to SA (Wildermuth et al., 2001) in Group 4.

Northern-blot analysis was then used to confirm the behavior of 10 genes from Groups 3 and 4 (five from each group) in naïve and fungus-infected tissues from wild-type and mutant plants. We chose genes having identical basal expression levels in naïve plants of all three genotypes and differences in the transcript abundance in one or the other infected mutants (Fig. 4B; Supplemental Table S2), because they may respond to signals generated upon infection. Consistent with our microarray data, these genes failed to show infection-mediated induction or repression in one of the mutants. The responses of four from these 10 genes are shown in Figure 4B.

These studies allowed us to identify genes apparently sensitive to the JA or SA pathways that require JAR or NPR1 for infection-mediated activation such as *At4g17090* (glycosyl hydrolase like; GH14) and *At3g16640* (tumor-related gene; TURP), respectively (Fig. 4B).

Genes Modulated by Balances between the JA and SA Pathways

Next, we analyzed possible causes for the differential expression of genes in Groups 3 and 4 in a given mutant. There seemed to be two possibilities. First, virulent *G. cichoracearum* could stimulate gene expression changes through the NPR1- and JAR1-dependent pathways. In this case, the absence of gene activation in one mutant could be due to lack of activation functions sensitive to this pathway. Alternatively, because both pathways display mutual antagonistic interactions (Schenk et al., 2000; Kloek et al., 2001; Glazebrook et al., 2003; Spoel et al., 2003), the absence of gene activation could be due to active signaling by the opposite pathway. In this case, repression or lack of induction of genes from Group 3 in infected *jar1-1* plants could be due to exacerbation of SA-sensitive responses, whereas down-regulation of genes from

Table III. *Arabidopsis genes differentially regulated during the early interaction with G. cichoracearum*

LS, Light-sensitive genes according to Smith et al. (2004). Bold type indicates genes that are induced or repressed by the large majority of other biotic stresses (e.g. bacterial pathogens, nonhost bacteria, and bacterial and fungal elicitors including LPS and flg22, fungal necrotrophs and biotrophs, according to data obtained from www.bbc.botany.u-toronto.ca). Underlined type indicates genes that appear to be specifically induced or repressed by *G. cichoracearum* or *G. orontii*.

AGI No.	EST No.	Gene Product[a]	Subcellular Localization[b]	Functional Category[b]	Fold Change[c]
Induced					
At4g33120	F1A4T7	Cyclopropane-fatty-acyl-phospholipid synthase-like protein	Cytosol	Metabolism	2.37
At2g30140[LS]	189L18T7	UDP-glucoronosyl and UDP-glucosyl transferase family protein. UGT87A1/A2	Endomembranes	Metabolism	1.90
At4g22720	186N2T7	Glycoprotease M22 family protein, similar to sialylglycoprotease	Endomembranes	Metabolism	2.33
At3g02870	241A20T7	Inositol-1(or 4)-monophosphatase 3, (IMP3) putative	Endomembranes	Metabolism	2.49
At3g16520[LS]	119P15T7	UDP-glucoronosyl/UDP-glucosyl transferase family protein. UGT88A1	Endomembranes	Metabolism	1.97
At4g29950[LS]	H10A12T7	Microtubule-associated protein	Mitochondrion	Metabolism	2.39
At2g34500	122P6XP	Cytochrome P450 family protein, C-22 sterol desaturase CYP710A1	Mitochondrion	Metabolism	2.69
At1g73480[LS]	179H9XP	Hydrolase, α/β-fold family protein	Chloroplast	Metabolism	2.09
At4g28680	193C4T7	Tyr decarboxylase, putative	Cytosol	Metabolism	3.11
At5g40760	184F4T7	Glc-6-P 1-dehydrogenase/G6PD (ACG9)	Cytosol	Metabolism	2.16
At5g67030[LS]	133D24T7	Zeaxanthin epoxidase (ZEP; ABA1)	Chloroplast	Metabolism	3.24
At1g18460[LS]	G2G11T7	Lipase family protein	Cytosol	Metabolism	2.22
At2g05070	118I1T7 119A21T7 92F2T7	Chlorophyll *a*/*b*-binding protein/LHCII type II (LHCB2.2)	Chloroplast	Energy	8.95
At1g44446[LS]	103D24XP	Chlorophyll *a* oxygenase (CAO)	Chloroplast	Energy	6.80
At3g08940[LS]	45E2T7	Chlorophyll *a*/*b*-binding protein (LHCB4.2; CP29)	Chloroplast	Energy	4.09
At1g70290 At1g23880	CI0021	Trehalose-6-P synthase, putative, similar to trehalose-P synthase/trehalose 6P phosphatase (TPS6 and TPS8)	Endomembranes	Energy	5.63
At2g34430	110O24T7	Chlorophyll *a*/*b*-binding protein/LHCII type I (LHB1B1)	Chloroplast	Energy	3.53
At5g45820[LS]	92F7T7	CBL-interacting protein kinase 20 (CIPK20)	Cytosol	Signal transduction	3.91
At2g42590	153H8T7	14-3-3 protein GF14 (GRF9)	Chloroplast/nucleus	Signal transduction	1.49
At3g17510[LS]	192B23T7	CBL-interacting protein kinase 1 (CIPK1)	Cytosol	Signal transduction	2.19
At1g04530[LS]	E1H4T7	Expressed protein, with HAT motif	Unknown	RNA processing	3.64
At3g53420[LS]	134N2T7	Plasma membrane intrinsic protein 2A (PIP2A)/aquaporin PIP2.1	Plasma membrane	Cellular transport	2.11
At3g52400	86D5T7	Syntaxin protein of plants SYP122	Plasma membrane	Cellular transport	2.92
At5g25520	K2B12T7	PHD finger protein related, transcription elongation factor-related	Nucleus	Transcription	2.67
At3g59060[LS]	224K3T7	Basic helix-loop-helix (bHLH) family protein	Nucleus	Transcription	1.94
At4g11970	179L10T7	YT521-B-like family protein, RNA splicing-related protein	Cytosol	Transcription	2.66
At2g25080[LS]	121P8T7	Phospholipid hydroperoxide glutathione peroxidase, PHGPx (GPX1)	Chloroplast	Defense	2.16
At3g56940[LS]	202D1T7	Putative ZIP protein (AT103)	Chloroplast	Defense	3.83
At5g44580	96O24T7	Expressed protein	Endomembranes	Unknown	2.64
At3g54500[LS]	158J22T7	Expressed protein	Unknown	Unknown	2.66
At1g54390	192A3T7	PHD finger protein-related	Intracellular /unknown	RNA processing	2.12
At1g11200	122I2XP	Expressed protein	Endomembranes	Unknown	2.39
At4g01880	124K8T7	Expressed protein	Chloroplast	Unknown	2.54
At4g21850	G3C11T7	Met sulfoxide reductase domain-containing protein/SeIR	Unknown	Unknown	2.24
At2g30520[LS]	G8G6T7	Signal transducer of phototropic response (RPT2)	Nucleus	Unknown	3.65
At5g19190	186F13T7	Expressed protein	Unknown	Unknown	2.17

(*Table continues on following page.*)

Table III. (*Continued from previous page.*)

AGI No.	EST No.	Gene Product[a]	Subcellular Localization[b]	Functional Category[b]	Fold Change[c]
Repressed					
At5g23660[LS]	42B2T7	Nodulin MtN3 family protein	Endomembranes	Metabolism	−2.21
At2g33830[LS] (two sequences)	K4F7TP	Dormancy/auxin-associated family protein	Cytosol/nucleus	Metabolism	−2.83
At2g25610	174O23T7	H+-transporting two-sector ATPase, C-subunit family protein	Vacuole membrane	Metabolism	−1.84
At5g26570[LS]	151D20XP	Similar to starch excess protein (SEX1) chloroplastidic phosphoglucan water dikinase (PWD)	Chloroplast	Metabolism	−5.99
At1g27630	G8D10T7	Cyclin family protein	Nucleus	Metabolism	−3.27
At3g10410[LS]	114J9T7	Ser carboxypeptidase III, putative	Endomembranes	Metabolism	−2.18
At2g36390[LS]	82F10T7	1,4-α-glucan branching enzyme/starch branching enzyme class II (SBE2-1)	Chloroplast	Metabolism	−1.91
At2g29630[LS]	173E13T7	Thiamine biosynthesis family protein/thiC	Chloroplast	Metabolism	−4.03
At1g18170	113C9XP	Immunophilin/FKBP-type peptidyl-prolyl cis-trans-isomerase family protein (PPiase; rotamase)	Chloroplast	Metabolism	−1.91
At3g19960	H3A1T7	Myosin (ATM)	Phragmoplast	Metabolism	−2.28
At1g79440[LS]	93P2T7	Mitochondrial succinic semialdehyde dehydrogenase (ALDH5F1)	Mitochondrial Matrix	Metabolism	−2.45
At2g28900[LS]	88L10XP	Similar to mitochondrial import inner membrane translocase subunit Tim17/Tim22/Tim23 family protein	Mitochondrial inner membrane	Signal transduction	−3.66
At3g59350[LS]	172P5T7 H3D6T7	Ser/Thr protein kinase, putative, similar to Pto kinase interactor 1 (Pti1)	Cytosol	Signal transduction	−5.09
At4g32551	192K6T7	WD-40 repeat family protein, similar to LEUNIG; β-transducin-like protein	Nucleus	Signal transduction	−3.27
At3g24450	E11B5T7	Copper-binding family protein	Endomembranes	Signal transduction	−14.36
At4g25160[LS]	173N1T7	Protein kinase family protein	Chloroplast/cytosol	Signal transduction	−3.19
At5g61380[LS]	H1G8T7	ABI3-interacting protein 1 (AIP1), identical to pseudoresponse regulator 1	Nucleus	Signal transduction	−12.2
At1g56300[LS]	E6H4T7	DNAJ heat shock N-terminal domain-containing protein	Mitochondrion	Cellular transport	−2.13
At5g63780[LS]	F4B7T7	Zinc finger (C3HC4-type RING finger) family protein	Chloroplast/nucleus	Transcription	−2.93
At3g53460[LS]	91L18T7	Ribonucleoprotein (29-kD), chloroplast/RNA-binding protein cp 29	Chloroplast	Transcription	−3.07
At1g12760	187A11T7	Similar to zinc finger (C3HC4-type RING finger) family protein, RING finger protein 12	Chloroplast/nucleus	Transcription	−3.45
At1g78630	149D9T7	Ribosomal protein L13 family protein	Chloroplast	Transcription	−2.31
At3g07650[LS]	151B13XP	CONSTANS gene family	Nucleus	Transcription	−2.15
At5g20630[LS]	E8E6T7	Germin-like protein (GER3)	Extracellular matrix	Defense	−6.05
At4g30660[LS]	231P23T7	Low-temperature and salt-responsive protein, putative, similar to LTI6A	Endomembranes	Defense	−2.08
At1g06460[LS]	186C19T7	Small heat shock family protein/hsp20 (31.2 kD) family protein	Mitochondrion	Defense	−2.45
At5g05740	115N4T7	S2P-like putative metalloprotease, homolog of EGY1	Chloroplast	Unknown	−1.98
At4g26670[LS]	143E9T7	Mitochondrial import inner membrane translocase subunit Tim17/Tim22/Tim23 family protein, weak similarity to subunit TIM17 A	Mitochondrion	Unknown	−4.19
At3g47860[LS]	103E18T7	Expressed protein	Chloroplast	Unknown	−4.31
At2g40080[LS]	118E14T7	ELF4-like protein	Unknown	Unknown	−12.03
At5g14920[LS]	193O8T7	GA-regulated protein 1 precursor	Endomembranes	Unknown	−2.83
At2g36320	E11A4T7	Expressed protein, zinc finger (AN1-like) family protein	Nucleus	Unknown	−5.64
At3g63160[LS]	206G8T7	Expressed protein	Chloroplast	Unknown	−6.89

[a]Genes showing the same trend of induction or repression among the three tested genotypes (Col-0, *npr-1*, and *jar-1*). [b]Subcellular localizations and functional categories were extracted from the TAIR Web site (www.arabidopsis.org). [c]Average of the fold change between uninfected (0 hpi) and 18 hpi samples of the three genotypes.

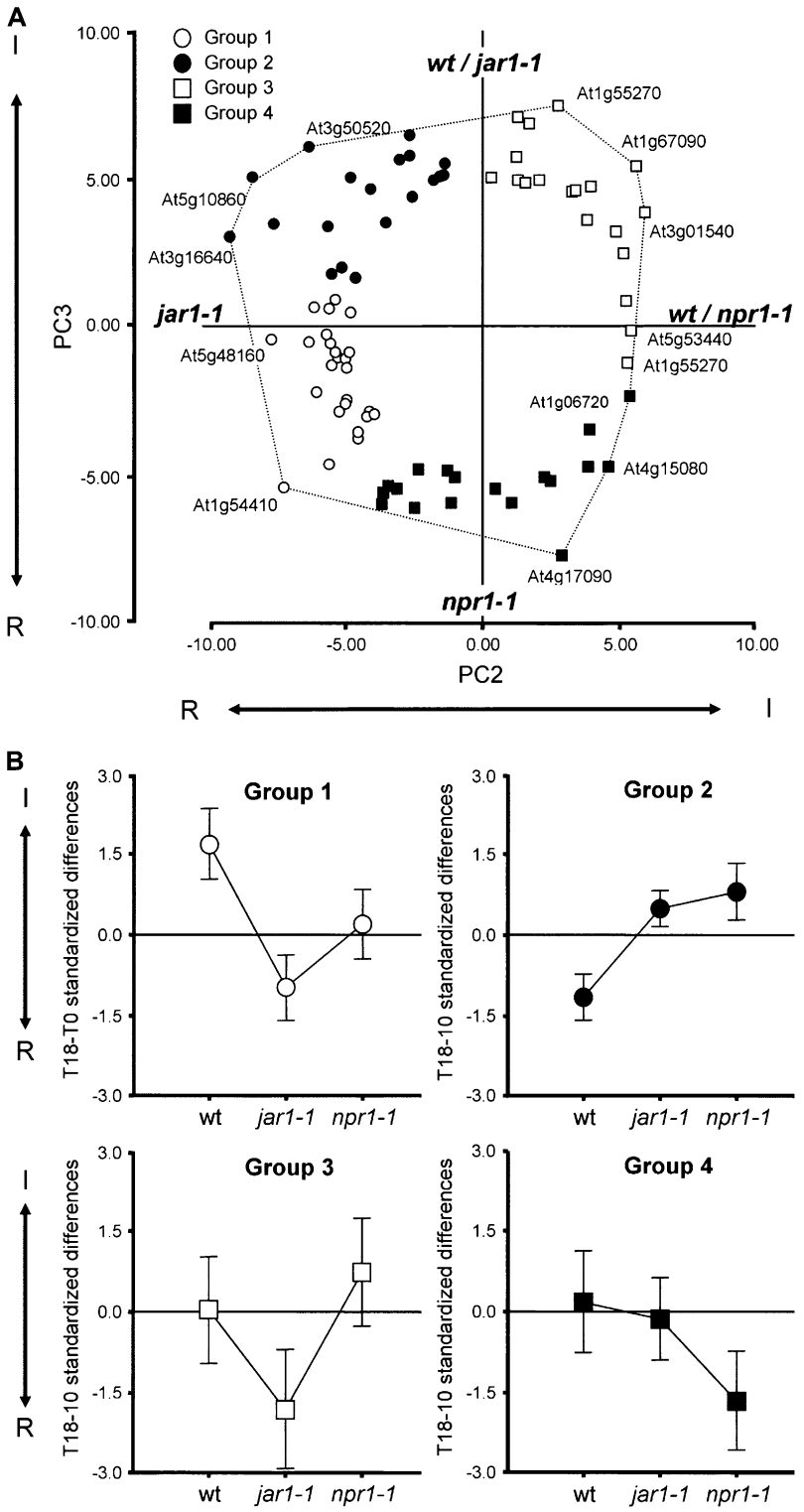

Figure 3. Graphical output of the PCA indicating the spatial ordination of genes influenced by infection and genotype variables. A, Four groups of genes are defined based on their different behaviors in infected wild-type and *npr1-1* versus *jar1-1* plants (principal component 2 [PC2]), or infected wild-type and *jar1-1* versus *npr1-1* (principal component 3 [PC3]). Genes are distributed according to fold-change values, indicated in an arbitrary scale. Genes with higher fold-change values are located on the periphery. Genes with distance to the axis intersection <5 were omitted. Infection-mediated repression (R) or induction (I) are indicated on the *x* and *y* axes. B, Average fold-change values for each group defined in A are indicated based on their behavior in each plant genotype. Values correspond to average ± SD of T0 versus T18 using the standardized differences of all genes within a cluster. Groups 3 and 4 include genes dependent only on JAR1 or NPR1, respectively.

Group 4 in infected *npr1-1* plants could be caused by enhanced flux through the JA pathway.

To test the latter possibility, we evaluated the sensitivity of five randomly selected genes from Group 3 to exogenous SA (1 and 5 mM), and that of five randomly selected genes from Group 4 to JA (0.5% and 1.0% v/v; Fig. 5A). Our results revealed that SA repressed the expression of four of the tested genes from Group 3 (*At3g16640* [*TURP*], *At3g50520* [*PGM* {similar to phosphoglycerate mutase}], *At5g18120* [*DI* {disulfide isomerase-like protein}], and *At2g10410* [*UNK* {unknown gene}]), whereas *At5g10860* (*CBS*

Table IV. *Grouping of genes via PCA and clustering*

Group 1 AGI No.	Gene Product	Functional Category
At1g54410	Dehydrin family protein	Cellular transport
At5g48160	Expressed protein, auxin-related protein	Transcription regulation
At1g65980	Peroxiredoxin type 2, putative	Defense, virulence, cell rescue
At2g33830	Dormancy/auxin associated family	Signaling
At3g08940	Chlorophyll *a*/*b*-binding protein (LHCB4.2)	Energy
At4g31180	Aspartyl-tRNA synthetase, putative	Protein synthesis
At3g23600	Similar to dienelactone hydrolase family protein	Hydrolase activity
At5g15880	Expressed protein	Unknown
At4g34630	Expressed protein	Unknown
At1g15340	MBD10, methyl-CpG-binding domain-containing protein	Protein degradation
At3g14050	RelA/SpoT protein, putative (RSH2), nearly identical to RelA/SpoT homolog RSH2	Metabolism
At5g21170	5′-AMP-activated protein kinase β-2 subunit, putative (AMPK β-2 chain)	Signal transduction
At2g21240	Expressed protein	Unknown
At1g28330	Dormancy-associated protein, putative (DRM1)	Metabolism
At2g05380	Gly-rich protein (GRP3S)	Unknown
At4g32470	Ubiquinol-cytochrome C reductase complex 14-kD protein, putative	Energy
At3g43720	Protease inhibitor/seed storage/lipid transfer protein (LTP) family protein	Cellular transport, defense
At2g24360	Ser/Thr/Tyr kinase, putative	Signal transduction
At2g34420	Chlorophyll *a*/*b*-binding protein/LHCII type I (LHB1B2)	Energy
At4g35100	Plasma membrane intrinsic protein (aquaporin)	Cellular transport
At5g02500	Heat shock cognate 70-kD protein 1 (HSC70-1; HSP70-1)	Cellular rescue, defense, and virulence
At2g40940	Ethylene receptor, subfamily 1. ERS1	Cellular rescue, defense, and virulence
Group 2 AGI No.	Gene Product	Functional Category
At1g55270	Kelch repeat-containing F-box family protein	Protein degradation
At1g67090	Ribulose bisphosphate carboxylase small chain 1A	Energy
At1g21550	Calcium-binding protein, putative	Signal transduction
At5g11030	Aberrant lateral root formation 4, ALF4	Morphogenesis
At3g01540	DEAD box RNA helicase (DRH1)	Transcription
At5g01520	Zinc finger (C3HC4-type RING finger) family protein, similar to MTD2	Unknown
At3g02420	Expressed protein	Unknown
At3g19390	Cys proteinase, putative	Protein degradation
At5g44120	12S seed storage protein (CRA1)	Metabolism
At5g63840	α-Glucosidase, putative	Metabolism
At1g30510	Ferredoxin-NADP(+) reductase, putative	Cellular transport
At4g21720	Expressed protein	Unknown
At1g07350	Transformer Ser/Arg-rich ribonucleoprotein, putative	Transcription
At4g39540	Shikimate kinase family protein	Metabolism
At1g35320	Expressed protein	Unknown
At3g03380	DegP protease, putative	Protein degradation
At4g21860	Met sulfoxide reductase domain-containing protein	Unknown
Group 3 AGI No.	Gene Product	Functional Category
At5g10860	CBS-domain-containing protein	Transcription
At3g50520	Phosphoglycerate/bisphosphoglycerate mutase family protein	Energy
At5g18120	Protein disulfide isomerase-like (PDIL) protein, thioredoxin (TRX) superfamily	Unknown
At2g10410	Sadhu noncoding retrotransposon family	Unknown
At3g59360	Ser/Thr protein kinase, putative	Signal transduction
At1g25210	UDP-3-*O*-acyl *N*-acetylglycosamine deacetylase family	Metabolism
At1g75830	Plant defensin-fusion protein, putative (PDF1.1)	Defense
At3g16240	δ-Tonoplast integral protein (δ-TIP)	Cellular transport
At5g45300	Glycosyl hydrolase family 14 protein like	Energy
At5g15230	GA-regulated protein 4 (GASA4)	Metabolism, hormone response (GA)
At1g69460	Transmembrane protein Tmp21 precursor	Cellular transport
At2g23350	Polyadenylate-binding protein, putative	Transduction

(*Table continues on following page.*)

Table IV. (*Continued from previous page.*)

Group 3 AGI No.	Gene Product	Functional Category
At1g74670	GA-responsive protein, putative	Transcription
At3g21720	Isocitrate lyase, putative	Energy
At1g31580	Identical to ORF1	Unknown
At1g51400	Photosystem II 5-kD protein	Energy
Group 4 AGI No.	**Gene Product**	**Functional Category**
At4g17090	β-Amylase (CT-BMY) glycoside hydrolase, family 14 B	Metabolism
At2g25490	F-box protein, ubiquitin/proteasome-dependent proteolysis of EIN3	Metabolism, defense, and hormone signaling
At1g74710	Isochorismate synthase 1 (ICS1)	Defense
At2g36830	Tonoplast intrinsic protein, γ-TIP, or MIP water channel	Cellular transport
At3g06050	Mitochondrial matrix localized peroxiredoxin.	Cellular rescue, defense
At4g15080	Zinc finger (DHHC type) family	Unknown
At3g43670	Copper amine oxidase, putative	Metabolism
At2g22660	Similar to Gly-rich protein	Unknown
At3g27770	Expressed protein	Unknown
At1g21550	Calcium-binding protein, putative	Signal transduction
At1g06720	Expressed protein	Transcription
At1g51830	Leu-rich repeat protein kinase	Signal transduction
At1g49750	Leu-rich repeat family protein	Signal transduction
At5g14920	Similar to GA-regulated protein 1 precursor GASA3	Metabolism, defense
At1g10660	Expressed protein	Unknown
At1g65980	Peroxiredoxin type 2, putative	Defense
At3g09770	Zinc finger (C3HC4-type RING finger) family protein	Metabolism, defense
At5g47560	Tonoplast malate/fumarate transporter	Cellular rescue and transport

[CBS-domain-containing protein]) was insensitive to SA (Fig. 5A). Similarly, JA inhibited four of the tested genes from Group 4 (*At4g17090* [*GH14*], *At2g25490* [*F-box* {F-box-containing protein FB26} or EIN3-binding F-box protein 1 {EBF1}], *At3g06050* [*AHR* {alkyl hydroperoxide reductase like}], and *At2g36830* [*MIP* {major intrinsic protein aquaporin}]), but not *At1g74710* [*ICS1* {isochorismate synthase 1}]).

To further characterize the expression patterns of the subset of genes from Groups 3 and 4 that are sensitive to both SA and JA, we analyzed the behavior of *TURP* (Group 3) and *GH14* (Group 4) in response to other treatments. Because infection-mediated induction of *TURP* requires functional JAR1 to be induced by fungal treatment (Fig. 4B), we tested whether this gene could be induced by treatment of wild-type tissues with exogenous JA. As shown in Figure 5B, JA had no effect on the basal expression levels of *TURP*. We thus hypothesized a possible mechanism for *TURP* regulation (Fig. 5A) wherein *TURP* induction in fungus-infected wild-type tissues is signaled through JAR1 via release of SA-mediated gene repression. To evaluate this possibility, we monitored the expression level of *TURP* in wild-type plants exposed to an avirulent race of *Pseudomonas syringae* pv *tomato*, which strongly stimulates the SA pathway. As expected, *TURP* was repressed in this condition (Fig. 5C). In addition, *TURP* was also repressed by bacterial treatment of *jar1-1* plants but not *npr1-1* plants. These results may indicate that either SA-mediated *TURP* repression requires functional NPR1, or that the enhanced JA levels found in *npr1-1* plants (Spoel et al., 2003) released the proposed SA-mediated repression.

The Group 4 gene *GH14* displayed behavior equivalent to that described for *TURP*, but involving SA and JA in opposite roles. Our results revealed that *GH14* was not induced by exogenous SA (Fig. 5B) and electronic-northern (e-northern) data reported by others (Botany Department, University of Toronto; http://bbc.botany.utoronto.ca/) indicated that this gene was repressed under conditions that strongly stimulate the JA pathway (infection by *Botrytis cinerea* and *Phythophthora infestans*). Interestingly, we found that treatment with avirulent bacteria did not modify *GH14* expression in wild-type plants, whereas this treatment induced the gene in *jar1-1* plants and slightly repressed its expression in *npr1-1* plants. These results are consistent with a regulatory mechanism involving negative control of *GH14* by SA, which may in turn release JA-mediated gene repression (Fig. 5A).

Conserved cis-Elements among Genes from the Same Category

Gene expression profile studies allow genes to be clustered based on similar expression patterns in response to the same treatment. Because it is likely that some genes within a cluster will contain analogous regulatory information, we looked for common cis-

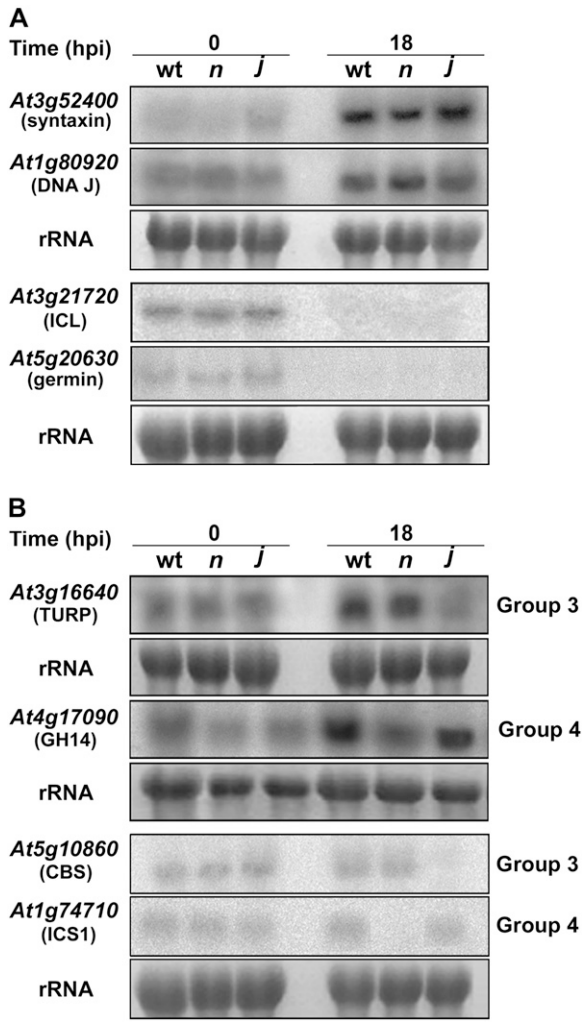

Figure 4. Validation of microarray expression data by northern-blot experiments. Total RNA (10 μg/line) isolated from healthy or infected tissues from wild-type (wt), *npr1-1* (*n*), or *jar1-1* (*j*) plants, sampled at the indicated hpi, was analyzed using cDNA probes for the indicated genes. A, Induction (top part) or repression (bottom part) of genes corresponding to the Venn diagram shown in Figure 2 corroborated the results obtained from our microarray assays. B, Expression pattern analysis of genes from Groups 3 and 4, those that could not be induced by infection in *jar1-1* or *npr1-1* plants (top) and those that were repressed under the same conditions (bottom).

regulatory elements common to the identified gene clusters. We were unable to find sequences recognized by transcription factors associated with previously reported responses to pathogens, such as binding motifs for ERF/AP2, R2R3MYB, TGAbZIP, Whirly, WRKY, ORCA, EIN3/EIL, MYB1, and ABI3 (PlantCARE database). However, we did identify a few cis-elements previously described as being involved in diverse defense responses (Table V). Among them, the GAAGAAGAA motif was present in the promoters of all the genes showing infection-mediated expression, regardless of the plant genotype. This motif is recognized by the TL1 element, which provides NPR1-dependent regulation for genes encoding proteins involved in the secretory pathway during defense responses (Wang et al., 2005). Another motif, the GTCCA sequence, was abundant in the promoters of genes showing infection-mediated repression, regardless of plant genotype. This motif was previously described as being overrepresented in the promoters of genes repressed by chitin oligomers (Zhang et al., 2002). In the promoters of genes from Groups 3 and 4, the [A/T]GTGACG motif was highly represented. This motif was previously identified in JA-responsive gene promoters from barley (http://intra.psb.ugent.be:8080/PlantCARE). Interestingly, this sequence forms the core of the *as-1* element found in the promoter of genes responding in a SA-dependent, NPR1-independent fashion (Uquillas et al., 2004). We additionally found several apparently novel motifs that have not yet been described as targets of known transcription factors (according to searches in the PlantCARE, TRANSFAC, and PLACE databases). Among them, the TATGTG and the TTTTTCTTCTTC motifs were conserved in the promoters of genes that were induced or repressed by fungal infection, respectively, regardless of genotype. The promoters of members of Group 3 contained the CCCACC motif that is identical to the consensus sequence for the animal transcription factor KLF6 (Kruppel-like factor 6), which is a tumor suppressor gene. Another putative regulatory sequence, GAAGTGATAG, was also abundant in the promoters of Group 3 genes, whereas the majority of Group 4 genes contained the motifs, ACAAGAAAAAAA and GTTATA, in their promoter sequences.

DISCUSSION

The Arabidopsis Transcriptome at the Time of *G. cichoracearum* Haustorium Formation

We herein explored the molecular features of plant susceptibility to infections caused by obligate biotrophic fungi by examining changes in the Arabidopsis transcriptome during a compatible interaction with *G. cichoracearum*. Our studies were performed at the stage of haustorium formation because this is a critical stage of reproduction in these parasites (Mendgen and Hahn, 2002; Schulze-Lefert and Panstruga, 2003). To further characterize the pathogen-induced gene expression changes, we categorized them with respect to their sensitivity to the SA- and JA-dependent pathways.

In our studies, RNA was extracted from whole infected leaf tissues. Considering that only epidermal cells come into contact with the fungus, the responses of infected cells may be diluted by those of uninfected cells, leading to modest changes in overall gene expression levels. To overcome this limitation and increase the confidence for detection of true expression changes, we evaluated the fold-change data by combining supervised (SAM and ANOVA) and unsupervised (PCA) statistical analyses and selected only genes showing changes in all three methods. Thus, we expected to rescue a subset of genes having the most robust expression changes following infection.

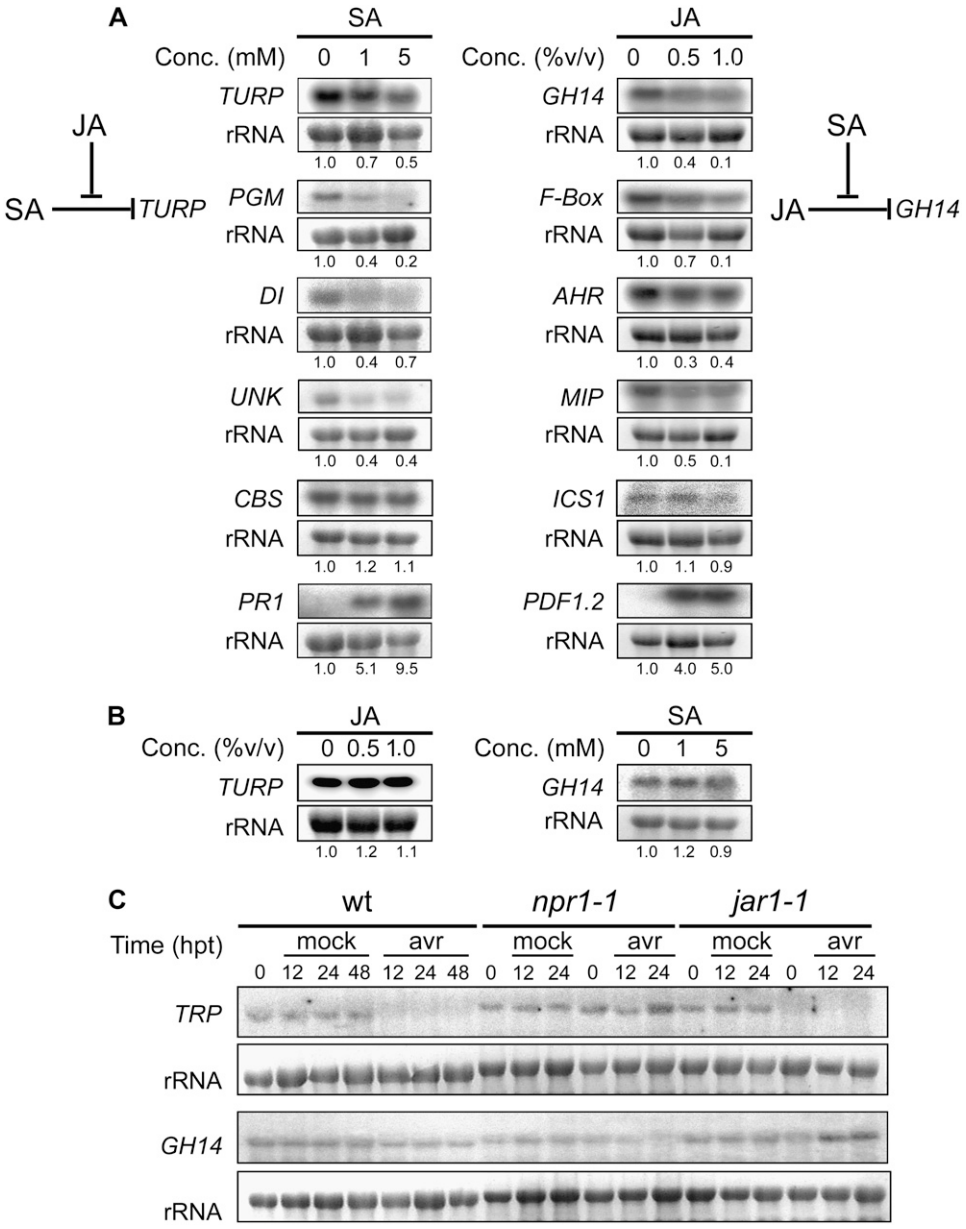

Figure 5. Northern-blot experiments showing the sensitivity of genes from Groups 3 and 4 to exogenous SA or JA. A and B, RNA was extracted from wild-type plants treated with two different concentrations of SA or JA at 24 h posttreatment (hpt). C, RNA was isolated from wild-type, *npr1-1*, or *jar1-1* plants infiltrated with *Pseudomonas syringe* pv *tomato* DC3000 harboring the *avrRpm1* gene (5×10^6 cfu/mL; avr) or 10 mM $MgCl_2$ (mock). The following genes were analyzed by northern blotting: *CBS*, CBS-domain-containing protein (*At5g10860*); *TURP*, tumor-related protein (*At3g16640*); *PGM*, phosphoglycerate-mutase-like protein (*At3g50520*); *DI*, disulfide isomerase-like protein (*At5g18120*); *ICS1*, isochorismate synthase 1 (*At1g74710*); *GH14*, glycosyl hydrolase family 14 (*At4g17090*); *F-Box*, FBL6/EBF1 F-box-domain-containing protein (*At2g25490*); *AHR*, alkyl hydroperoxide-reductase-like protein (*At3g06050*); *MIP*, major intrinsic protein aquaporin (*At2g36830*). Hybridization with rRNA probes was used as a loading control. Probes for the genes *PR1* and *PDF1.2* were used as controls for the effects of SA and JA.

To confirm our findings, we randomly chose 10 genes from this subset and subjected them to northern-blot analysis, corroborating our microarray results (Fig. 4).

Changes Independent from NPR1 and JAR1

Seventy NPR1- and JAR1-insensitive infection-responsive genes were identified based on their identical behaviors in infected wild-type, *jar1-1*, and *npr1-1* plants. Using a free e-northern facility (Botany Department, University of Toronto; http://bbc.botany.utoronto.ca/), we evaluated how these genes responded to other biotic stresses including virulent and avirulent bacteria, necrotrophic and biotrophic fungi, nonhost pathogens, and bacterial and fungal elicitors. We found that nearly half of the genes altered during the interaction with *G. cichoracearum* were also affected in a similar direction and to a similar extent by at least one other pathogen or treatment. A subset of eight induced and nine repressed genes responded similarly to the majority of the stresses. Among them, the induced genes included those encoding UDP-glycosyl transferase (*At2g30140*); microtubule-associated protein (*At4g29950*), cytochrome P450 (*At2g34500*), Glc-6-P dehydrogenase (*At5g40760*), syntaxin SYP122 (*At3g52400*), and three unknown proteins (*At4g21850*, *At4g01880*, and *At1g11200*). The subset of repressed genes included those encoding starch branching enzyme (*At2g36390*), germin-like protein AtGER3 (*At5g20630*), thiamine biosynthesis protein (*At2g29630*), pyruvate dikinase (*At5g26570*), peptidylprolyl isomerase (*At1g18170*), ribosomal protein L13 (*At1g78630*), membrane channel related protein (*At2g28900*), low-

Table V. *Conservation of putative regulatory elements in the promoters of coregulated Arabidopsis genes altered during G. cichoracearum infection*

Results of promoter sequence analysis for genes that were induced or repressed in all three infected genotypes (36 and 34 genes, respectively), as well as genes belonging to Groups 3 (17 genes) and 4 (18 genes), showing differences in SA- or JA-dependent responses, respectively.

	Motif[a]	No.[b]	P <[c]
Induced	gaagaaga[d]	36/36	10^{-5}
	tatgtg[e]	26/36	10^{-4}
Repressed	ttttttcttcttc[d]	33/34	10^{-5}
	gtccaa[e]	20/34	10^{-3}
Group 3	gaagatgatag[d]	16/17	10^{-6}
	tgtgacg[e]	14/17	10^{-4}
	cccacc[d,e]	13/17	10^{-4}
Group 4	acaagaaaaaaa[d]	17/18	10^{-6}
	gttata[e]	16/18	10^{-3}
	agtgacg[e]	11/18	10^{-3}

[a]Motifs overrepresented in the promoter sequences of the genes from each group. [b]Number of sequences with the indicated motif versus the total number of sequences in the group. [c]*P*-values for the binomial distribution. [d]Motifs found using the MEME algorithm. [e]Motifs found using the Motif finder and/or AlignACE programs.

temperature and salt-responsive protein LTI6A (*At4g30660*), and an unknown protein (*At5g05740*). Interestingly, only seven of the examined genes were specifically regulated by the biotrophic fungi *G. orontii* (http://bbc.botany.utoronto.ca) and *G. cichoracearum* (this work). Among them, five were induced (chlorophyll *a* oxygenase [*At1g44446*], putative peroxisomal transport protein [*At1g04530*], aquaporin PIP2A [*At3g53420*], glutathione peroxidase 1 [*At2g25080*], and a signal transducer of phototropic response [*At2g30520*]), whereas two were repressed (nodulin from MtN3 family [*At5g23660*] and ABI3 interacting protein 1 [*At5g63780*]). Additional studies will be required to assess if these genes, which appear to be specifically modified by *G. orontii* and *G. cichoracearum*, are involved in the regulation of plant defenses and/or susceptibility conditions.

Plant susceptibility factors are thought to be involved in controlling the early stages of interactions with obligate parasites (Schulze-Lefert and Vogel, 2000; Mendgen and Hahn, 2002; Panstruga, 2003). In Arabidopsis, a few genes capable of enhancing *G. cichoracearum* pathogenesis have been described. Among them, some of the *PMR* genes (*PMR2*, *PMR5*, and *PMR6*) function independently of the SA- and JA-dependent defense pathways. As expected, these *PMR* genes were not detected in our experiments because they have constitutive expression. Similarly, the *EDR1* gene encoding a MAPKKK was not selected by our assays because it becomes induced late in the infection process (Frye et al., 2001). We believe that some of the genes identified in this work are likely to encode susceptibility factors and that some of them could be sensitive to NPR1 and/or JAR1. In other pathosystems, pathogen-mediated regulation of genes encoding susceptibility factors such as *NHO1* (Kang et al., 2003) and *Bax-inhibitor 1* (Sanchez et al., 2000) have been shown to depend on the JA-signaling cascade.

To examine overall alterations in the host that were specifically stimulated by *G. cichoracearum*, we disregarded the 17 genes affected by the majority of other biotic stresses. Among the 53 remaining genes, the highest fold-change levels involved genes regulating host energy generation. The changes in these genes suggest a net accumulation of chlorophyll binding proteins (*At2g05070*, *At3g08940*, and *At2g34430*) and chlorophyll *a* oxygenase (*At1g44446*) in infected tissues. Because epidermal cells colonized by the fungus lack chloroplasts, these genes must be induced in nearby uninfected cells, probably neighboring mesophyllic tissues. Previous studies have shown that expression of photosynthetic genes is stimulated by *G. orontii* infection (http://bbc.botany.utoronto.ca/) but is severely repressed by nonhost mildews at 18 hpi (Zimmerli et al., 2004). A local increase of photosynthetic functions is in agreement with the phenomenon of "green islands" produced in compatible interactions between other *Erysiphales* and their hosts. In this case, the infected tissues increase their photosynthetic rate subsequently altering the source-sink balance (Truernit et al., 1996).

A large set of genes (23) modulating host metabolism showed important alterations upon infection and some of them are compatible with conditions favoring fungal nutrition. Concerning sugar metabolism, we found strong activation of genes encoding for trehalose-6-P synthase (*At1g70290* and *At1g23880*), which importantly affects carbon assimilation in plants (Eastmond and Graham, 2003). Other general pathogen-induced transcriptional changes, such as up-regulation of the gene encoding Glc-6-P dehydrogenase (*At5g40760*) and repression of the gene encoding the starch branching enzyme (*At2g36390*), may help increase the availability of simple sugars at infection sites. Interestingly, at 18 hpi we did not detect changes associated with carbon assimilation, such as up-regulation of sugar transporters found to occur in this interaction several days after infection (Fotopoulos et al., 2003). Conversely, by SAM and ANOVA tests we did detect at this time of infection modest induction of *At3g11900*, encoding an amino acid transporter (data not shown). These results are in agreement with the notion that transportation of nutrients from plant cells to fungal cells through the haustorium is active at 18 hpi.

The naïve and infected samples analyzed in this study were excised at two different day times, corresponding to 3 (T18) and 9 (T0) hours after light onset (12 and 6 PM, respectively). We evaluated if the 70 genes selected as sensitive to *G. cichoracearum* displayed diurnal changes according to data reported by Smith et al. (2004). Genes *At4g33120*, *At2g05070*, *At2g34430*, *At5g19190*, and *At1g12760* could not be analyzed in this way because they were absent in these studies. We observed that 25 of these genes were insensitive to light whereas the reminding 40 displayed expression differences at 2 to 4 and 8 h after light onset (samples equivalent to T18 and T0,

respectively). The 40 light-sensitive genes were indicated in Table III. Most of these genes changed in the same trend (induction, repression) in the Smith et al. (2004) experiment and in ours. However, genes such as *At5g45820*, *At4g26670*, *At3g07650*, *At2g40080*, and *At1g56300*, displayed quantitative or kinetic differences between both experiments (not shown), suggesting that pathogens may alter the light-mediated regulation of these genes. It is important to note that because the Smith et al. (2004) study used plants grown under different light/dark cycles from our study, as well as samples isolated at slightly different daytimes, the exclusive effect of light on the selected genes, under the infection condition here evaluated, cannot be deduced from direct comparison of both sets of data. Additional experiments will be required to determine how light and pathogens independently affect these genes.

The high abundance of light-sensitive genes among those selected in Table III was not unexpected. Light influences host cellular processes, such as the photosynthetic activity, antioxidant defenses, carbohydrate catabolism among others, and it is likely that these processes may be affected by fungal infection. Genes sensitive to both, light and pathogens, have been characterized in several species including those encoding for ascorbate peroxidase from rice (*Oryza sativa*; Agrawal et al., 2003), lipoxygenase from maize (*Zea mays*; Nemchenko et al., 2006), and *DEA1* from tomato (*Solanum lycopersicum*; Weyman et al., 2006).

Haustorial development constitutes a key stage for fungal propagation involving the intimate contact between the invader and the host. In the interaction here analyzed, about 90% of the infection attempts are successful. Thus, it is expected that gene expression changes occurring at this time reflect the establishment of susceptibility, as well as the activation of PAMP-triggered immunity (PTI), which proved to be insufficient to limit fungal propagation. Suppression of PTI by fungal effectors may also take place under this condition, as reported for compatible interactions with powdery mildews where PTI suppression may facilitate the formation of haustoria (Caldo et al., 2006).

As mentioned before, SAM, ANOVA, and PCA tests detected 70 genes responding to *G. cichoracearum*. As expected, this group included genes sensitive to several other biotic stresses (17 genes indicated in Table III) that may participate in PTI modulation. Activation of a gene encoding for phospholipid hydroperoxide glutathione peroxidase (*At2g25080*), which may display chloroplastic antioxidant activities, was also detected. In addition, all three tests pointed out a down-regulation of genes encoding germin-like protein AtGER3 (*At5g20630*), low-temperature and salt-responsive protein LTI6A (*At4g30660*), and heat shock protein Hsp20α (*At1g06460*). This finding supports the notion that fungal obligate biotrophs may avoid host defenses (Mendgen and Hahn, 2002; O'Connell and Panstruga, 2006). It is important to note that selection of expression changes by combination of SAM, ANOVA, and PCA tests may be a reliable way of identifying robust alterations, although it may not be useful to detect mild expression changes occurring under this condition. The activation of *PAL1* (*At2g37040*), *CAD4* (*At3g19450*), and *NHO1* (*At1g80460*), genes perceived by SAM test (Supplemental Table S4) but not by all three methods, may illustrate the latter kind of responses.

Collectively, robust expression changes of genes insensitive to NPR1 and JAR1 during the interaction with *G. cichoracearum* at 18 hpi suggested that compatibility conditions are already established for fungal propagation at this time. We found changes compatible with activation of defenses including PTI as well as suppression of host defenses. We also found changes that seemed to indicate that the pathogen plays an active role in forcing the host's metabolism to enhance photoassimilation and amino acid levels at the infection sites. Furthermore, our results suggest that photosynthesis may be increased in uninfected cells located near the penetration sites, thus providing substrates for fungal feeding through the haustoria.

Genes Sensitive to SA and JA

Cytological observations indicated that under our experimental conditions, the initial steps of *G. cichoracearum* proliferation proceeded similarly in *jar1-1*, *npr1-1*, and wild-type plants. Similar abundance of haustoria and infection structures developed at 18 to 24 hpi in the mutant and wild-type plants. We did observe a slight reduction in the number of conidiophores in *npr1-1* plants versus *jar1-1* plants at 96 hpi, although these differences were not sustained by 6 to 8 dpi. This finding may suggest that the increased JA levels found in naïve *npr1-1* plants (Spoel et al., 2003) may help delay the initial stages of fungal propagation.

SA, JA, and ET accumulate in response to different pathogens, leading to specific changes in gene expression. Under several infection conditions, the SA and JA/ET pathways display mutual repression mechanisms (Schenk et al., 2000; Kloek et al., 2001; Glazebrook et al., 2003; Spoel et al., 2003; Bostock, 2005). These pathways regulate host responses in a variety of ways depending on the timing and magnitude of JA/ET and SA accumulation, as well as cross-talk between these pathways and with other yet-unknown regulatory mechanisms (De Vos et al., 2005). For interactions between Arabidopsis and *G. cichoracearum*, SA-dependent responses are known to be induced at 4 dpi (Frye and Innes, 1998; for review, see Glazebrook, 2005), but these responses have not previously been analyzed during the early stages of infection. Other studies have shown that early activation of *PDF1.1*, *PDF1.2*, and *PDF1.3* in this system takes place at 18 hpi and decreases by 24 hpi (Zimmerli et al., 2004).

We sought to characterize JAR1- and NPR1-dependent responses at the time of haustoria formation. Analysis of the bi- and tridimensional spaces generated by the PCs in a PCA identified four groups of genes with common behaviors, including a group of JAR1-sensitive genes that were repressed or not induced in infected *jar1-1* plants (Group 3) and a group of NPR1-

sensitive genes that were repressed or not induced in infected *npr1-1* plants (Group 4). The identification of these two groups indicated that there were some changes in NPR1 and JAR1 signaling in the host transcriptome at 18 hpi. Consistent with this observation, measurements of JA and SA levels at 18, 24, and 48 hpi showed that levels for both hormones were elevated in infected leaves. In this period, these hormones undergo up to 5-fold increase above their basal levels (Supplemental Fig. S1). We used e-northern analysis (http://bbc.botany.utoronto.ca/) to evaluate how genes from Groups 3 (17 genes) and 4 (18 genes) responded to other biotic stresses. Eight genes from Group 3 were repressed by avirulent bacteria suggesting that SA plays a role in their repression. In contrast, four other genes from this group displayed the opposite response (induction by avirulent bacteria). We further found that 10 genes from Group 4 were repressed by necrotrophic pathogens known to induce the JA pathway, whereas only one of them was induced by these pathogens. These results support the belief that Groups 3 and 4 include genes sensitive to SA and JA, respectively. Among the genes categorized in these two groups, only *PDF1.1* (Group 3) and *ICS1* (Group 4) are classical markers of the JA and SA pathways, respectively, whereas the remainder constitute additional targets of these signaling cascades. In the future, these genes may contribute to the characterization of cross-talk points between the JA and SA signaling cascades in other plant-pathogen interactions. In addition, genes from Group 3 may be used to evaluate the extent and timing of JA-pathway induction in this interaction, along with the basis for its eventual down-regulation. These findings, along with a previous study showing that constitutive activation of the JA/ET pathway occurring in the *cev1* mutant leads to enhanced resistance to *G. cichoracearum* (Ellis et al., 2002), seem to suggest that this pathway signals effective defenses against the fungi, but becomes repressed by fungal virulence products in infected wild-type plants.

The enhancement of SA and JA levels detected at 18 hpi in this compatible interaction (Supplemental Fig. S1) is consistent with the interplay of the SA- and JA-dependent defenses at this stage of infection. Some of the genes from Groups 3 and 4 were found to be sensitive to both SA and JA, suggesting that their expression levels might be determined by the balance between these pathways. To examine this hypothesis, we characterized the transcriptional features of one gene from each group, *TURP* (Group 3) and *GH14* (Group 4). Our results suggested that the expression of *TURP* may be controlled by two negative regulatory steps involving both SA and JA, as shown in Figure 5A. Interestingly, in wild-type tissues, *TURP* is induced upon fungal infection but repressed upon treatment with avirulent bacteria. The reason for this differential response is not yet known but it could be related to differences in the balance between the JA and SA pathways and the timing of accumulation of these components under each condition. In this sense, our findings suggest that the JA-stimulated responses in plants treated with avirulent bacteria may not be as robust as the SA-mediated responses. This is in agreement with a previous report that activation of *VSP2* and *PDF1.2* was not observed in these tissues, even though they contained increased JA levels (De Vos et al., 2005). Alternatively, other factors specifically controlling defenses against virulent fungi or avirulent bacteria could account for the observed differences. Similarly, the *GH14* gene from Group 4 appears to be controlled by two negative regulatory steps involving JA and SA, as shown in Figure 5A. Treatment with avirulent bacteria did not alter the expression of *GH14* in wild-type plants, whereas it induced the gene in *jar1-1* plants and slightly repressed its expression in *npr1-1* plants. Accumulation of JA is expected to occur beginning at 3 hpi in *Pseudomonas*-treated plants (De Vos et al., 2005). Under this condition *GH14* may be down-regulated by JA because infected *jar1-1* mutants show activation of this gene (Fig. 5C). The repression of *GH14* found in infected *npr1-1* plants could be caused by enhanced JA levels in the mutant (Spoel et al., 2003) or by the requirement of NPR1 for execution of the SA-mediated gene repression.

Another member of Group 4, the gene encoding ICS1, has been extensively characterized in the context of pathogenesis (Dewdney et al., 2000; Wildermuth et al., 2001). We here found that basal *ICS1* expression is completely abolished upon infection. Because only epidermal cells are infected by the fungus, this result may suggest that *ICS1* expression could be regulated at the systemic level. Our data showed that *ICS1* is insensitive to exogenous JA and is down-regulated in infected *npr1-1* plants. The latter observation is inconsistent with previous reports showing that *ICS1* is negatively regulated by NPR1 in *G. cichoracearum*-infected plants at 7 dpi (Wildermuth et al., 2001). This disparity may be due to the difference in the analyzed time points or it may indicate that an NPR1-sensitive repressor of *ICS1* functions at the late stages of infection.

We herein report the first use of genome-wide expression analysis to characterize Arabidopsis genes specifically regulated during *G. cichoracearum* haustorium formation. We found uncharacterized putative regulatory elements in the promoters of coregulated Arabidopsis genes altered during *G. cichoracearum* infection, functional characterization of which require further analysis. Our findings may contribute to the discovery of novel plant genes altered by the pathogen to establish successful infection, although future studies will be required to examine the participation of these gene products in pathogenic virulence strategies, including plant defense repression. Our results also suggest new cross-talk points between the SA- and JA/ET-dependent networks in plant-pathogen interactions. Collectively, this work is an important first step toward understanding the molecular mechanisms involved in the establishment of compatible interactions with obligate biotrophs.

MATERIALS AND METHODS

Plant and Fungal Materials

Arabidopsis (*Arabidopsis thaliana*) plants were grown on potting mix and maintained in clean growth chambers under a short day cycle (14-h day, 10-h night) at 22°C with 100 μE m^{-2} s^{-1} of light. Wild-type, *npr1-1*, and *jar1-1* plants are from the ecotype Columbia of Arabidopsis (Col-0) accession were obtained from the Arabidopsis Biological Resource Center. *Golovinomyces cichoracearum* isolate UCSC1 was grown on susceptible Col-0 Arabidopsis plants and on squash (*Cucurbita maxima*) 'Kuta' plants.

Plant Infections

Four-week-old wild-type, *npr1-1*, and *jar1-1* plants were inoculated with a *G. cichoracearum* UCSC1 conidia, using a settling tower as previously described (Adam et al., 1999), with high-sporulating infected squash leaves being tapped over the surface of 4-week-old Arabidopsis plants. The inoculated plants were kept in the dark under 100% humidity for 1 h and then placed into growth chambers. Fungal development was monitored by trypan blue staining and optical microscopy, as previously described (Adam et al., 1999). Leaves of healthy and infected plants isolated at 18 hpi (when haustorium development was at 90% of maximum) were collected and shock-frozen in liquid nitrogen. Four independent replicates of each experiment were collected, each on a different date.

Treatments of Plants with SA and JA

Plants were treated with SA or JA according to protocols described by Schenk et al. (2000). Briefly, 5-week-old Col-0 plants were either spayed with 1 or 5 mM SA (Sigma Aldrich) solutions or treated with methyl jasmonate (MJ; 95%; Sigma Aldrich), as follows. Aliquots of 400 μL of 0.5% or 1.0% v/v MJ ethanolic solutions were applied on cotton balls and allowed to evaporate into a 20-L sealed container holding the plants, giving maximum concentrations of 0.42 or 0.84 μmol MJ/L, respectively.

SA and JA Determination

The endogenous SA and JA levels present in naive and infected Arabidopsis leaves were determined from near 500 mg of plant samples.

Extraction and quantification processes were performed using jasmonic acid and deuterated salicylic acid as internal standards and HPLC electrospray tandem mass spectrometry technology, according to standardized procedures (Ross et al., 2004).

RNA Isolation

For microarray hybridization, total RNA was isolated from frozen leaf tissues using the TRIzol Reagent (Invitrogen) according to the protocol recommended by the Arabidopsis Functional Genomics Consortium (http://www.arabidopsis.org/info/2010_projects/comp_proj/AFGC/RevisedAFGC/site2RnaL.htm#isolation). The RNA samples were treated with RQ1 DNAse (Promega) and poly-A RNA was isolated from 300 to 400 μg of total RNA using the Oligotex Mini Prep kit (QIAGEN). For northern-blot hybridization, total RNA was isolated from rosette leaves (Alvarez et al., 1998) and 10 μg of RNA was blotted and hybridized with the appropriate EST probes.

Microarray Hybridization

Labeled cDNAs were synthesized from each sample and hybridized onto 24 microarrays as described in Supplemental Table S1, where the access number of each slide is provided. Four biological replicates were performed and for each experiment two sets of cDNAs were hybridized onto 11.5 K arrays and another two onto 15 K arrays. For each of the four replicates, six slides (three genotypes under two conditions) were hybridized with a common reference consisting on pooled samples from wild-type, *npr1-1*, and *jar1-1* cDNAs, as described in Supplemental Table S1. The common reference was labeled with Cy3, whereas Cy5 was used to label either the T0 or T18 samples on the same slides. The normalized values corresponding to Cy5/Cy3 spot intensity ratios from each slide are shown in Supplemental Table S2. The raw intensity data and all array details can be accessed through http://genome.www5.stanford.edu. Microarray hybridization was performed using the CIW-MSU joint array protocol (http://www.arabidopsis.org/info/2010_projects/comp_proj/AFGC/RevisedAFGC/AFGC_Protocols_Dec_2001L.pdf) as described previously (Ramonell et al., 2002).

Acquisition, Transformation, and Processing of Microarray Data

The hybridization signal for each channel (Cy3 and Cy5) was read using a ScanArray Lite scanner (Packard Biochip Technologies) and spot signals were analyzed using the Genepix 3.0 software (Axon Instruments). Grids were predefined and manually adjusted to ensure optimal spot recognition, and spots with dust or locally high background were flagged as bad. ESTs spots that were not flagged and had a regression correlation greater than 0.6 were selected for further analysis (Gollub et al., 2003). The raw data were deposited into the Stanford University microarray database (http://genome-www5.stanford.edu/microarray/SMD; Sherlock et al., 2001) under the identifiers given in Supplemental Table S1.

The resulting values were consolidated in spreadsheet files (Excel; Microsoft), and imported into the GeneSpring 6.0 microarray analysis software (SiliconGenetics). Further normalizations of the values were performed per spot (data channel divided by control channel both previously corrected by background) and per chip. We applied within-sample normalization to correct for intensity dependencies using LOWESS adjustments of the relationship between expression level (M) and intensity (A). We also performed between-sample normalization by dividing the expression levels of each array by their 50th percentile). Log_2 transformations of the normalized fold-change ratios (Cy5 channel/Cy3 channel) were then analyzed as described below and in the Results section.

Data Analysis

SAM analysis was performed using SAM version 1.21 obtained from www-stat.stanford.edu/~tibs/SAM (Tusher et al., 2001). ANOVA, PCA, and cluster analysis were all performed using the InfoStat statistical software (www.infostat.com.ar; InfoStat Group, 2004; National University of Córdoba, Argentina).

For SAM analysis, when a two-class unpaired SAM test with 1,000 permutations was applied to our data (11,250 spotted ESTs) for comparison of T0 to T18 samples, 486 ESTs were selected as significant (367 induced and 161 repressed) showing 1.7-fold differential expression, threshold of fold-change value is 0.71, false significant-median number is 2.11, and false discovery rate is 0.43%. To increase the stringency of selection, we selected only ESTs exhibiting a T0 to T18 fold-change ratio ≤ 0.5 or ≥ 2 on at least two of the three genotypes. This analysis yielded 217 ESTs, (116 induced and 101 repressed genes; Supplemental Table S4).

ANOVA analysis was used to evaluate whether the changes observed in average gene expression were due to the infection response, the plant genotype, or an interaction between these two factors. The original matrix of 11,250 EST spots was reduced to 10,286 when those ESTs having more than two missing data points in each treatment were discarded. We then estimated the mean expression value from the 2 to 4 data points available for each EST. Our ANOVA results corroborated our finding that the observed gene expression changes were primarily due to infection, with several genes yielding P-values <0.0001. In contrast, the plant genotype and the interaction effects had smaller contributions to the observed changes (Supplemental Table S5). Selection based on P-values with a cutoff of 1/1,000 (one false positive gene per 1,000 analyzed) identified 225 differentially expressed genes (227 ESTs), 117 induced and 106 repressed by infection (Supplemental Table S5).

To generate an ordination space in which it was easer to visualize the relationship between genes and treatments and to select differentially expressed genes, PCA was applied to the matrix of standardized mean differences of the expression values at T0 and T18. The technical details of the implementation of PCA in the context of this application are presented in Supplemental Data Analysis S1. Then, PCA was used not to reduce the number of dimensions of the problem, but rather to look for new axes that might allow us to discriminate ESTs showing consistent and different responses to infection in wild-type, *npr1-1*, and *jar1-1* plants. The eigenvalues and eigenvectors of the spectral decomposition of the matrix D, which were used to interpret the PCA analysis, are presented in Supplemental Table S6. Interpreting the meaning of principal components according to eigenvector

coefficients (factor loading) and its relevance according to its eigenvalues revealed that the PC1 expressed the global effect of the infection, which is the dominant source of variability (76%). Furthermore, the extreme values of PC1 correspond to genes that were under- and overexpressed with respect to the uninfected condition in plants of all three genotypes. The genes repressed during infection were located on the positive side of the axes, whereas induced genes were located at the negative extremes. We selected the 1% of genes located furthest to the right (induced) and left (repressed) with respect to the center of the ordination space (indicating no change). We chose 1% to select a manageable number of genes and to reduce the possibility of false positives. This analysis yielded 144 ESTs: 67 induced and 77 repressed (Supplemental Table S3).

Cluster analysis was performed by applying the k-centroid algorithm on the matrix of standardized differences. The number of EST clusters was determined by plotting a measure of heterogeneity within clusters along with the number of clusters. We then used the slope change criteria on this graphical representation of the reduction of heterogeneity as a function of the number of clusters (data not shown) to select the appropriate number of groups of genes with different patterns of differences between treatments.

Conserved cis-Regulatory Elements in the Identified Gene Clusters

To identify conserved cis-elements, the sequences 1,000 bp upstream of the ATG of the promoter regions of the gene groups identified in this work were analyzed using the MEME (http://meme.sdsc.edu/meme/website/meme.html), Motif-finder (www.arabidopsis.org), CREDO (http://mips.gsf.de/proj/regulomips/), and POBO (http://ekhidna.biocenter.helsinki.fi:9801/pobo) programs. We looked for conserved motives not exclusively present in genes sensitive to light. We therefore analyzed all genes within a group to further select those motives only present in both, light-sensitive and light-insensitive genes. The elements reported in Table V satisfied this criterion. Note that only 16 out of the 20 genes containing the "GTCCAA" motif are light sensitive, and that Groups 3 and 4 contain four and six light-sensitive genes, respectively (not shown).

Supplemental Data

The following materials are available in the online version of this article.

Supplemental Figure S1. Accumulation of JA and SA in *G. cichoracearum*-infected tissues.

Supplemental Table S1. Experimental design of the microarray slide hybridization.

Supplemental Table S2. Normalized gene expression data for all 24 samples.

Supplemental Table S3. Genes responding to infection selected by PCA.

Supplemental Table S4. Genes responding to infection selected by SAM analysis.

Supplemental Table S5. Genes responding to infection selected by ANOVA analysis.

Supplemental Table S6. Eigenvalues of the spectral decomposition of the standardized differences (T0 versus T18) matrix between uninfected (0 hpi) and infected (18 hpi) samples on the expression of 10,286 genes.

Supplemental Data Analysis S1.

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