Tomato Transcriptional Responses to a Foliar and a Vascular Fungal Pathogen Are Distinct

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Plant activation of host defense against pathogenic microbes requires significant host transcriptional reprogramming. In this study, we compared transcriptional changes in tomato during compatible and incompatible interactions with the foliar fungal pathogen *Cladosporium fulvum* and the vascular fungal pathogen *Verticillium dahliae*. Although both pathogens colonize different host tissues, they display distinct commonalities in their infection strategy; both pathogens penetrate natural openings and grow strictly extracellular. Furthermore, resistance against both pathogens is conveyed by the same class of resistance proteins, the receptor-like proteins. For each individual pathogen, the expression profile of the compatible and incompatible interaction largely overlaps. However, when comparing between the two pathogens, the *C. fulvum*-induced transcriptional changes show little overlap with those induced by *V. dahliae*. Moreover, within the subset of genes that are regulated by both pathogens, many genes show inverse regulation. With pathway reconstruction, networks of tomato genes implicated in photorespiration, hypoxia, and glycoylate metabolism were identified that are repressed upon infection with *C. fulvum* and induced by *V. dahliae*. Similarly, auxin signaling is differentially affected by the two pathogens. Thus, differentially regulated pathways were identified with novel strategies that allowed the use of state-of-the-art tools, even though tomato is not a genetic model organism.

Plants are continuously exposed to microbial pathogens that aim to parasitize them. In response to this threat, plants have evolved various defense mechanisms to protect themselves against microbial attack. These mechanisms comprise constitutive defense barriers and defenses that are activated upon detection of a potential invader. Regardless of whether or not a pathogen is arrested in growth, microbial attack generally leads to significant host transcriptional reprogramming. A few decades ago, the first pathogenesis-related (PR) genes were identified that are induced by pathogen attack and of which the corresponding gene products have been associated with pathogen defense (van Loon and van Strien 1999). Apart from basal

defense, PR proteins also play a role in race-specific disease resistance (Thomma et al. 2001; Thordal-Christensen 2003), demonstrating that both types of defense responses at least partly employ the same defense genes. More recently, transcriptional reprogramming has been monitored at a genome-wide scale in a growing number of plant species with the use of microarrays (Quirino and Bent 2003; van Baarlen et al. 2008; Wise et al. 2007). Plant microarray data generally demonstrate that, in the interactions of plant pathogens with their hosts, hundreds of genes are activated and repressed. Most microarray analyses are initiated for gene discovery in order to associate novel genes with effective host defense responses or as a lead to select appropriate candidate genes for functional analysis (Wan et al. 2002; Wise et al. 2007). Alternatively, microarray analyses can be used to profile the transcriptional activity of a plant tissue, providing global insight into host cell biology (Bolton et al. 2008a; van Baarlen et al. 2008; van Esse et al. 2008). Such an approach facilitates visualization of underlying biological processes (van Baarlen et al. 2008). The shared expression patterns of characterized plant genes and genes with unknown functions may suggest biological functions of the “unknown” genes. Furthermore, expression-profile comparison at the level of cellular pathways may lead to novel hypotheses concerning the biology of the interaction which, after experimental verification, can provide further insight into decisive elements that have roles in cellular processes (van Baarlen et al. 2008). Although still in its infancy in plants, this strategy has been exploited successfully in several other biological systems (Kobayashi et al. 2003; van Baarlen et al. 2008).

It has been suggested that a plant is resistant or susceptible to a specific pathogen depending on the speed and rate at which the same host defense molecules are produced, suggesting that resistance is based on quantitative rather than qualitative differences in host defense (Eulgem et al. 2004; Navarro et al. 2004; Tao et al. 2003; Thilmony et al. 2006). However, others have observed significantly divergent gene expression not only between compatible and incompatible interactions (Caldo et al. 2004) but also between incompatible interactions of the same pathogen–host combination that are mediated by different resistance (R) proteins (Adams-Philips et al. 2008).

Until recently, the vast amount of microarray data was generated for the model plant species *Arabidopsis*, and most transcriptomics studies upon pathogen attack relied on inoculations with the bacterium *Pseudomonas syringae* (Maleck et al. 2000; Scheideler et al. 2002), the fungus *Alternaria brassicicola* (Schenk et al. 2000; van Wees et al. 2003), and the oomycete *Hyaloperonospora parasitica* (Eulgem et al. 2004; Maleck et al. 2000). However, high-density microarrays are currently

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available for a growing set of crop species (Wise et al. 2007), including the Solanaceous crop plant tomato (van Esse et al. 2007).

In this study, we performed global transcriptional profiling to compare transcriptional changes in tomato during compatible and incompatible interactions with the fungal pathogens *Cladosporium fulvum* and *Verticillium dahliae*. Both pathogens have fundamentally different infection strategies because *C. fulvum* is a foliar pathogen that causes leaf mold on its sole host tomato (Thomma et al. 2005), whereas *V. dahliae* is a soilborne vascular pathogen of over 200 host plants (Fradin and Thomma 2006). Despite these differences in their infection strategies, both pathogens share a number of characteristics because they invade their host through natural openings and grow strictly extracellular without the formation of feeding structures such as haustoria (Fradin and Thomma 2006; Thomma et al. 2005). Furthermore, in incompatible interactions with tomato, recognition of these pathogens is mediated by plasma membrane-anchored extracellular receptor proteins that belong to the receptor-like protein class of resistance proteins (Fradin and Thomma 2006; Jones et al. 1994; Kawchuk et al. 2001; Thomma et al. 2005). The aim of this study was to compare global transcriptional profiles in response to host attack by *C. fulvum* and *V. dahliae* in order to identify differences and similarities in compatible and incompatible interactions with a foliar and a vascular fungal pathogen.

RESULTS

Transcriptional changes in tomato upon *C. fulvum* inoculation.

We have previously shown that, in a compatible interaction, the fungus colonizes the apoplast around leaf mesophyll cells and, by 10 days postinoculation (DPI), clear symptoms of disease are visible as chlorosis and conidiophores that emerge from stomata on the lower side of the leaves (Bolton et al. 2008b). After 10 DPI, leaf tissues start to necrotize. Using real-time polymerase chain reaction (PCR) to quantify fungal biomass in the plant tissue, it is evident that fungal biomass gradually increases until the fungus is extensively sporulating (Bolton et al. 2008b). Tomato genes that were differentially regulated (more than twofold change with a P value ≤ 0.001) upon inoculation with *C. fulvum* were identified at 3, 7, and 10 DPI using samples harvested at 0 DPI as the reference samples to which the samples from all other timepoints were compared (Fig. 1A). Fungal presence in the samples of the compatible interaction was verified with real-time PCR (Supplementary Fig. 1A). In the compatible interaction, the number of differentially induced genes amounted to 144, 1,093, and 3,360 genes at 3, 7, and 10 DPI, respectively (Fig. 1A). A small subset of only 32 genes was found to be differentially regulated throughout the three timepoints (Fig. 1A; Supplementary Table 1). In the incompatible interaction, 118, 1,227 and 3,318 genes were differentially regulated at 3, 7, and 10 DPI, respectively (Fig. 1B). Thus, although the fungus is arrested soon after tissue penetration in the incompatible interaction, the number of differentially regulated genes is comparable with that in the compatible interaction. Moreover, a similarly low number of genes ($n = 31$) was differentially regulated at the three timepoints throughout the incompatible interaction (Fig. 1B).

Often, similar gene sets are induced during compatible and incompatible plant-pathogen interactions, albeit that the regulation occurs with different rates and amplitudes (Eulgem et al. 2004; Navarro et al. 2004; Tao et al. 2003; Thilmony et al. 2006). To account for temporal variation, the differentially regulated genes monitored at the different timepoints were pooled to one gene set of 3,500 genes for the compatible and one set of 3,573 genes for the incompatible interaction (Fig. 1A and B). Roughly two-thirds of the regulated genes overlapped between both interactions (Fig. 2A). Subsequently, hierarchical clustering (HCL) (Eisen et al. 1998) was performed to cluster genes based on similarity in expression patterns, revealing that most overlapping genes displayed similar expression patterns over time in the compatible and incompatible interaction (Fig. 2B).

For tomato, a whole-genome sequence is not yet available, and gene annotation and information is still limited. Because many tomato genes are still of unknown function, obtaining insight into the underlying biology of microarray experiments is difficult. A starting point to explore poorly characterized genes and predict plant protein function is the use of Gene Ontology (GO) annotation (The Gene Ontology Consortium 2000), a controlled formal vocabulary that consists of general terms for gene and protein annotation in any organism, making comparisons across taxa possible (van Baarlen et al. 2008). Therefore, we decided to perform an orthology prediction for all 22,721 gene transcripts that can be probed by the GeneChip, and were able to assign GO annotations to 6,730 of these transcripts. These were used to identify the major differentially regulated biological processes (Table 1), showing that most differentially regulated genes belong to the categories transport, metabolism, and phosphorylation. Interestingly, despite the observation that only two-thirds of the regulated genes appeared to overlap for both interactions, no significant differ-

Fig. 1. Differentially regulated tomato genes during compatible and incompatible interactions with *Cladosporium fulvum* and *Verticillium dahliae*. **A**, Venn diagram displaying overlap in differentially regulated genes between different time points of the compatible *C. fulvum*-tomato interaction. **B**, Venn diagram displaying overlap in differentially regulated genes between different time points of the incompatible *C. fulvum*-tomato interaction. **C**, Venn diagram displaying overlap in differentially regulated genes between different time points of the compatible *V. dahliae*-tomato interaction in roots and in foliage. **D**, Venn diagram displaying overlap in differentially regulated genes between different time points of the incompatible *V. dahliae*-tomato interaction in roots and in foliage.

ences were observed in differentially regulated biological processes between the compatible and the incompatible interaction (Table 1).

Transcriptional changes in tomato upon *V. dahliae* inoculation.

As for the foliar pathogen *C. fulvum*, transcriptional changes were monitored in tomato upon inoculation with the vascular pathogen *V. dahliae* (more than twofold change with a P value ≤ 0.001). Because *V. dahliae* is a soilborne pathogen, samples were taken of foliage (leaves and stems) and of roots at 3 and 7 DPI. Compared with inoculation with *C. fulvum*, display of typical symptoms of disease occurred faster upon inoculation with *V. dahliae*; by 7 DPI, clear stunting of plants and chlorosis was observed. At 10 DPI, disease symptoms were very severe and necrosis was observed. Therefore, this timepoint was not included in the assay. Fungal presence in the samples of the compatible interaction was verified with real-time PCR, showing a continuous increase in fungal biomass in the roots whereas, in foliage, the biomass increased by 3 DPI and decreased by 7 DPI. This agrees with previous observations of peaks of *Verticillium* spp. growth at 2 to 4 days and 12 to 15 DPI, succeeded by intermittent periods of fungal elimination (Heinz et al. 1998). *V. dahliae* inoculation requires uprooting

of the plants followed by root-dip inoculation. This method is likely to introduce transcriptional changes that are not related to pathogen attack; therefore, mock-inoculated plants harvested at 3 DPI were taken as the reference to which all other samples were compared. In the compatible interaction, 0 and 2 genes were found to be differentially regulated at 3 DPI in foliage and roots, respectively, whereas, at 7 DPI, 518 and 1,188 genes were differentially regulated in those tissues (Fig. 1C and D). In the incompatible interaction, 3 and 22 genes were differentially regulated at 3 DPI while 389 and 130 genes were differentially regulated at 7 DPI in the foliage and roots, respectively (Fig. 1C and D). Remarkably, in roots, the number of differentially expressed genes in the compatible interaction was almost 10-fold the number of differentials in the incompatible interaction. Thus, in contrast to the *C. fulvum*-induced transcriptional changes, the *V. dahliae*-induced transcriptional changes concern significantly more genes in the compatible interaction than in the incompatible interaction.

As for the *C. fulvum*-tomato analyses, temporal variation was countered by pooling the transcriptional changes monitored in samples harvested at 3 and 7 DPI for each interaction. Overall, in the foliage, 280 genes were identified in the overlap between the total of 518 and 389 differentially regulated genes in the compatible and incompatible interaction, respectively

Fig. 2. Comparison of differentially regulated tomato gene sets during interactions with *Cladosporium fulvum* and *Verticillium dahliae*. **A**, Venn diagrams displaying specificity and overlap in differentially regulated gene sets between compatible (c) and incompatible (i) interactions with tomato. **B**, Expression profiles of differentially regulated genes in the compatible (c) and incompatible (i) *C. fulvum*-tomato interaction at 3, 7, and 10 days postinoculation (DPI), respectively. **C**, Expression profiles of differentially regulated genes in foliage and roots in the compatible (c) and incompatible (i) *V. dahliae*-tomato interaction at 7 DPI in foliage and roots. **D**, Venn diagrams displaying specificity and overlap in differentially regulated gene sets of the compatible and incompatible *V. dahliae*-tomato interaction between foliage (f) and roots (r). **E**, Expression profiles of differentially regulated genes in the compatible and incompatible *V. dahliae*-tomato interaction between foliage (f) and roots (r).

(Fig. 2A). In roots, 94 genes were identified in the overlap between the 1,188 and 147 differentially regulated genes of the compatible and incompatible interactions, respectively (Fig. 2A). As for *C. fulvum*, HCL showed that most overlapping genes displayed similar expression patterns over time in the compatible and incompatible interactions (Fig. 2C). The assessment of GO categories for the *V. dahliae*-regulated genes showed only minor differences between the compatible and incompatible interactions (Table 1).

Comparison of transcriptional changes in foliage and roots upon *V. dahliae* inoculation.

V. dahliae is a soilborne pathogen that penetrates the roots and travels through the vascular system toward the green tissues. When comparing the transcriptional changes in roots and foliage, a rather small overlap was observed (Fig. 2D). For the compatible interaction, 59 genes overlapped whereas, for the incompatible interaction, only 16 genes overlapped between the differentially regulated gene sets for roots and foliage (Fig. 2D; Supplementary Table 2). In the overlapping gene set of the compatible interaction, the *PR* genes encoding P69B, PR-5, and hevein-like protein, a homolog of the *Arabidopsis* monosaccharide symporter *STP6* gene (Scholz-Starke et al. 2003), and several aquaporin δ -TIP genes that are implied in osmotic housekeeping (Daniels et al. 1996) were induced in both tissue types, while the TAS14 dehydrin gene was strongly repressed. Of the 59 genes, 13 genes displayed an inverse expression pattern between roots and foliage (Fig. 2E), among which was a homologue of the tobacco nitrate reductase *nir-3* gene (Kronenberger et al. 1993) that was induced in foliage and repressed in roots. Highly induced foliage-specific genes upon *V. dahliae* inoculation included photosynthesis genes, aquaporin δ -TIP genes (Daniels et al. 1996), genes implicated in salt tolerance (Nagaoka and Takano 2003), an ammonium transporter gene, and a nitrate transporter gene. Interestingly, root-specific induced genes included the *PR* genes *PR1a*, *PR-5x*, and *P96F*, but also the *Pip1* and *Rcr3* genes that are induced upon infection with *C. fulvum* and *Phytophthora infestans* (Krüger et al. 2002; Tian et al. 2007; van Esse et al. 2008). Other root-specific induced genes were the inorganic phosphate transporter gene *LePT2*, the iron transporter gene *LeIRT1* (Eckhardt et al. 2001), and an *Arabidopsis* copper transporter gene homolog. In the incompatible interaction, the overlap between the transcriptional changes of the roots and foliage consisted of only 16 genes with similar expression patterns (Fig. 2D and E) that included the *PR* gene *PR-5x* and chitinase genes. Genes involved in proteolysis and transcription were induced in foliage but not in roots, as was the case for the pathogen-inducible *Rcr3*, *Pip1*, and *P69B* genes. In roots, the iron transporter gene

LeIRT1 (Eckhardt et al. 2001) was induced. Overall, it can be concluded that the transcriptional changes in root and foliar tissues of tomato upon *V. dahliae* inoculation are highly distinct.

Transcriptional changes upon *C. fulvum* and *V. dahliae* inoculation differ.

Although *C. fulvum* and *V. dahliae* colonize different host tissues, both pathogens have several commonalities in their infection strategies because they only penetrate natural openings and grow strictly extracellular without the formation of haustoria. However, during compatible interactions with tomato, the major differentially regulated biological processes by these pathogens differed (Table 1). Interestingly, *C. fulvum*-induced transcriptional changes in foliar tissues more resembled *V. dahliae*-induced transcriptional changes in root tissues than those monitored in foliar tissues (Table 1). Nevertheless, the percentages of genes related to transport and proteolysis (the class that also contains many *PR* genes) were larger in roots during the compatible interaction with *V. dahliae* than with *C. fulvum*, whereas the percentage of genes related to metabolism was larger in the compatible interaction with *C. fulvum* than in *V. dahliae*-infected roots.

For both *C. fulvum* and *V. dahliae*, resistance in incompatible interactions is conveyed by extracellular transmembrane receptors that belong to the class of receptor-like proteins (Fradin and Thomma 2006; Thomma et al. 2005). It may be anticipated that pathogen receptors that belong to the same class converge into the same signal transduction cascade that mediates a defense response. In such cases, the transcriptional changes are expected to largely overlap. Nevertheless, overall, considerable differences are observed in the major regulated biological processes in both incompatible interactions (Table 1).

To study the *C. fulvum*- and *V. dahliae*-induced transcriptional changes in more detail, we compared the 3,500 *C. fulvum*-regulated genes with the 1,647 *V. dahliae*-regulated genes in the roots and foliage for both compatible interactions, showing that the overlap contained only 454 genes (Fig. 3A; Supplementary Table 3). Similarly, both incompatible interactions were compared, showing only 172 overlapping genes (Fig. 3A). When the differentially *C. fulvum*-regulated genes at 10 DPI were removed from the analysis, the overlap was even smaller, with 160 and 74 genes for the compatible and incompatible interactions, respectively (Supplementary Fig. 2). The *C. fulvum*-specific genes in both the compatible and incompatible interaction included many glutathione S-transferase genes which are implicated in attenuation of the oxidative burst (Lamb and Dixon 1997) (Supplementary Table 4). Furthermore, many WRKY transcription factor genes were specifically regulated in the *C. fulvum*-tomato interaction (12 in

Table 1. Differentially regulated biological processes (Gene Ontology [GO] process) identified in the diverse interactions of tomato with *Cladosporium fulvum* and *Verticillium dahliae*

GO process	Differentially expressed genes (%) ^a					
	<i>C. fulvum</i> foliage		<i>V. dahliae</i> foliage		<i>V. dahliae</i> roots	
	Compatible	Incompatible	Compatible	Incompatible	Compatible	Incompatible
Biosynthesis	11	11	9	10	9	36
Metabolism	29	29	23	24	22	21
Phosphorylation	13	12	7	5	11	7
Photosynthesis	4	3	6	4	2	0
Proteolysis	7	8	7	9	10	0
Response to stress	4	4	9	7	3	2
Transcription	4	5	5	2	4	0
Transport	26	25	29	33	35	25
Signal transduction	1	1	2	2	2	2
Cell wall	2	2	3	4	2	7

^a Values refer to the percentage (of the total number) of differentially expressed genes for which a GO annotation could be assigned.

the compatible and 18 in the incompatible interaction), whereas no regulation of WRKY transcription factor genes was monitored in the interactions with *V. dahliae*. Similarly, no apical meristem (NAM)-like proteins were found to be specifically induced in the *C. fulvum*–tomato interaction (6 in the compatible and 10 in the incompatible interaction). Unique *V. dahliae*-regulated genes in both the compatible and incompatible interaction included genes that encode osmotic housekeeping factors, such as δ -tonoplast intrinsic aquaporins (Kjellbom et al. 1999), salt tolerance-like proteins, and a homologue of the SOS2 salt tolerance protein (Liu et al. 2000). In addition, an inorganic phosphate transporter (Daram et al. 1998) and iron-regulated transporter 1 (Eckhardt et al. 2001) were highly induced in root tissue.

K-Means clustering (Soukas et al. 2000) was performed on the overlapping genes (Fig. 3B) to group genes with a similar expression pattern. Between the *C. fulvum*- and *V. dahliae*-induced transcriptional changes, we identified 51 induced and 17 repressed genes in both compatible interactions. Similarly, 35 and 2 genes were induced and repressed, respectively, in both incompatible interactions. As may be expected, *PR* genes

Fig. 3. *Cladosporium fulvum* and *Verticillium dahliae* induce largely different transcriptional changes. **A**, Venn diagrams displaying specificity and overlap in compatible and incompatible tomato interactions with *C. fulvum* (*Cf*) and *V. dahliae* (*Vd*). **B**, Expression profiles of differentially regulated genes in compatible and incompatible tomato interactions with *C. fulvum*-infected tomato (C) and *V. dahliae*-infected foliar (Vf) and root (Vr) tissues at 7 days postinoculation.

were induced in all interactions. HCL on the subsets of overlapping genes for the compatible but also for the incompatible interaction showed that most of the differentially regulated genes in *V. dahliae*-infected roots tissues displayed a regulation similar to that in *C. fulvum*-infected leaves, whereas the same genes showed an inverse regulation in the *V. dahliae*-infected foliage (Fig. 3B).

Overall, our results show that the transcriptional response of tomato toward both pathogens is completely different. Within the 454 differentially regulated genes that were shared between both compatible pathogen interactions, 164 genes displayed an inverse expression pattern, consisting of 62 genes that were induced by *C. fulvum* and repressed by *V. dahliae* and 102 genes that were repressed by *C. fulvum* and induced by *V. dahliae* (Fig. 3). A similar situation was true for the incompatible interactions, for which 72 of the 172 shared differentials displayed inverse regulation (Fig. 3). Remarkably, of the 102 inversely regulated genes in the compatible interactions, 16 genes could be implied in photosynthesis. To further investigate the effect of both pathogens on photosynthesis, HCL was performed on the 41 photosynthesis genes represented on the GeneChip by plotting the response of these 41 genes to *C. fulvum* and *V. dahliae* infection at the various timepoints (Fig. 4; Supplementary Table 5). Strikingly, most of these 41 genes were inversely regulated upon inoculation with the two pathogens. This strongly suggests that, upon *C. fulvum* infection, photosynthesis is repressed, while *V. dahliae* infection induces photosynthesis genes in both the compatible and incompatible interactions (Fig. 4).

Over-representation analysis identifies regulated cellular processes.

Over-representation analysis (ORA) can detect over- or under-representation of gene categories in a data set when compared with a reference data set while correcting for chance (Backes et al. 2007). A powerful web-based tool to perform ORA is GeneTrail (Backes et al. 2007), which is able to use

Fig. 4. *Cladosporium fulvum* and *Verticillium dahliae* have inverse effects on photosynthesis. Transcriptional regulation of the 41 photosynthesis genes that are represented on the tomato GeneChip in the interactions of tomato with *C. fulvum* and *V. dahliae* at various time points. The different lanes represent the compatible tomato interaction with *C. fulvum* at 3, 7, and 10 days postinoculation (DPI) (C3, C7, and C10, respectively), the incompatible interaction with *C. fulvum* at the same time points (I3, I7, and I10, respectively), the compatible and incompatible tomato interaction with *V. dahliae* in foliage at 7 DPI (Cf and If, respectively), and the compatible and incompatible tomato interaction with *V. dahliae* in roots at 7 DPI (Cr and Ir, respectively).

Arabidopsis Reference Sequence (RefSeq) identifiers as input. Therefore, all tomato genes that are monitored with the GeneChip were BLASTed against the National Center for Biotechnology Information (NCBI) RefSeq database (Pruitt et al. 2005) to identify the closest *Arabidopsis* homologue. For the ORA, we only considered tomato genes for which an *Arabidopsis* homologue with significant similarity could be identified (E value $\leq 1 \times 10^{-6}$). Therefore, only 15,835 of the 22,721 genes that are monitored with the GeneChip were used in the ORA. As expected, ORA on repressed genes in the *C. fulvum*–tomato interactions (false discovery rate <0.01) identified photosynthetic processes (Table 2; Supplementary Table 6). Interestingly, for the compatible interaction, the ORA suggests that the major defense response to *V. dahliae* occurs in roots whereas, in the foliage, the over-represented gene categories concern housekeeping and metabolism. Due to the low number

of differentially regulated genes in roots during the incompatible interaction, no overrepresented categories are identified.

Based on gene expression data (Fig. 3B), the compatible *C. fulvum*–tomato interaction most resembled the compatible *V. dahliae*–tomato interaction in roots. ORA of these data sets showed that categories such as “defense response, incompatible interaction”, “defense response against fungus”, and “response to biotic stimulus” are indeed over-represented in both gene sets (Tables 2 and 3). Nevertheless, many of the genes that are activated in these categories are different between the two interactions (Supplementary Table 7). However, most of the over-represented categories are unique for either the *C. fulvum*– or *V. dahliae*–tomato interaction. Remarkably, two categories that are over-represented in the *C. fulvum*–tomato interaction but not in the *V. dahliae*–tomato interaction are “defense response to bacterium” and “response against bacterium” con-

Table 2. Over-representation analysis of differentially regulated genes during the compatible *Cladosporium fulvum*–tomato interaction

Gene Ontology subcategory ^a	P value ^b
<i>C. fulvum</i> –tomato induced categories	
Response to other organism	1.40E-10
Response to biotic stimulus	5.70E-10
Multi-organism process	1.50E-09
Defense response	7.10E-07
Response to bacterium	1.40E-06
Immune system process	2.60E-06
Immune response	2.60E-06
Response to stimulus	8.20E-06
Defense response to bacterium	1.20E-05
Defense response, incompatible interaction	1.20E-05
Innate immune response	1.20E-05
Cellular catabolic process	2.00E-05
Catabolic process	7.90E-05
Response to wounding	8.10E-05
Response to fungus	1.00E-04
Response to SA stimulus	3.30E-04
Toxin catabolic process	3.30E-04
Toxin metabolic process	3.30E-04
SA-mediated signaling pathway	3.30E-04
SAR, SA-mediated signaling pathway	7.20E-04
Defense response to fungus	8.90E-04
Biopolymer modification	8.90E-04
Response to chemical stimulus	1.10E-03
Response to external stimulus	1.10E-03
JA- and ethylene-dependent systemic resistance	1.10E-03
Protein modification process	1.20E-03
JA-mediated signaling pathway	1.40E-03
Response to JA stimulus	1.50E-03
SAR	2.50E-03
Proteolysis	2.70E-03
Response to toxin	2.70E-03
Response to stress	3.10E-03
Integral to plasma membrane	3.10E-03
Potassium ion transport	3.30E-03
Endomembrane system	3.70E-03
Post-translational protein modification	3.80E-03
Protein amino acid phosphorylation	4.50E-03
Intrinsic to plasma membrane	5.60E-03
Glutathione transferase activity	7.00E-03
Catalytic activity	7.60E-03
<i>C. fulvum</i> –tomato repressed categories	
Chloroplast	4.00E-108
Plastid part	7.10E-67
Cytoplasmic part	2.50E-64
Chloroplast part	2.20E-60
Cytoplasm	2.80E-60
Thylakoid	4.60E-52

(continued in next column)

^a SA = salicylic acid, SAR = systemic acquired resistance, and JA = jasmonic acid.

^b False discovery rate.

Table 2. (continued from preceding column)

Gene Ontology subcategory ^a	P value ^b
<i>C. fulvum</i> –tomato repressed categories (Continued)	
Plastid thylakoid	2.90E-51
Chloroplast thylakoid	5.90E-51
Organelle subcompartment	1.10E-50
Thylakoid part	2.80E-49
Chloroplast thylakoid membrane	1.50E-46
Plastid thylakoid membrane	1.50E-46
Intracellular membrane-bound organelle	1.80E-46
Membrane-bound organelle	1.90E-46
Thylakoid membrane	3.40E-46
Intracellular organelle	1.40E-45
Organelle	1.60E-45
Cell part	5.10E-41
Cell	5.10E-41
Intracellular part	4.60E-38
Intracellular	1.60E-33
Organelle membrane	1.00E-19
Intracellular organelle part	2.80E-18
Organelle part	3.10E-18
Chloroplast thylakoid lumen	4.00E-14
Thylakoid lumen	4.00E-14
Plastid thylakoid lumen	4.00E-14
Plastid stroma	6.10E-13
Photosynthesis	1.80E-11
Cellular biosynthetic process	3.50E-11
Membrane	8.90E-11
Biosynthetic process	2.70E-08
Catalytic activity	6.00E-08
Porphyrin biosynthetic process	6.10E-08
Tetrapyrrole biosynthetic process	6.10E-08
Plastoglobule	2.50E-07
Carboxylic acid metabolic process	9.20E-07
Metabolic process	2.20E-06
Tetrapyrrole metabolic process	2.20E-06
Porphyrin metabolic process	2.20E-06
Amino acid metabolic process	3.60E-06
Plastid envelope	4.00E-06
Chloroplast envelope	4.10E-06
Chlorophyll biosynthetic process	4.20E-06
Chloroplast stroma	8.70E-06
Photosystem	1.30E-05
Amino acid biosynthetic process	3.90E-05
Chloroplast inner membrane	7.70E-05
Nitrogen compound metabolic process	7.70E-05
Amine metabolic process	1.10E-04
Plastid inner membrane	1.20E-04
Pigment biosynthetic process	1.40E-04
Carbohydrate metabolic process	1.40E-04
Heterocycle metabolic process	1.50E-04
Chlorophyll metabolic process	2.10E-04
Cofactor metabolic process	2.10E-04
Translation	2.10E-04

(continued on next page)

taining the genes *Jar1* (Staswick et al. 1992), *Pad4* (Glazebrook et al. 1997), *Npr1* (Cao et al. 1994), *Rin4* (Mackey et al. 2002), and several *Wrky* transcription factor genes, suggesting that tomato homologues of these genes are involved in defense against *C. fulvum* but not against *V. dahliae*. Interestingly, jasmonic acid and salicylic acid responses are over-represented in the compatible *C. fulvum*–tomato interaction, whereas ethylene responses are over-represented in the compatible *V. dahliae*–tomato interaction. Water channel activity and ion-binding categories are over-represented in the *V. dahliae*–tomato interaction and not in the *C. fulvum*–tomato interaction. The most prominent ion-binding category, the manganese ion binding, contains several germin-like proteins (Table 2). Furthermore, categories involved in cytoskeletal and microtubule organization, movement, and transport are overrepresented in the *V. dahliae*–tomato interaction. Finally, the Cajal body category (GO cellular localization) is repressed in the *V. dahliae*–tomato interaction.

Table 2. (continued from preceding page)

Gene Ontology subcategory ^a	P value ^b
<i>C. fulvum</i> –tomato repressed categories (Continued)	
Nucleoid	2.20E-04
Plastid nucleoid	2.20E-04
Plastid chromosome	2.20E-04
tRNA aminoacylation for protein translation	2.60E-04
Response to light stimulus	2.60E-04
Amino acid activation	2.60E-04
tRNA aminoacylation	2.60E-04
Nitrogen compound biosynthetic process	3.10E-04
Pigment metabolic process	3.60E-04
Plastid organization and biogenesis	3.60E-04
Ligase activity, forming aminoacyl-tRNA and related compounds	3.70E-04
Ligase activity, forming carbon-oxygen bonds	3.70E-04
Aminoacyl-tRNA ligase activity	3.70E-04
Chloroplast membrane	3.70E-04
Response to radiation	4.30E-04
Cofactor biosynthetic process	4.50E-04
Photosystem II	5.50E-04
Anchored to membrane	6.60E-04
Amine biosynthetic process	6.60E-04
Plastid membrane	7.20E-04
Adenyl nucleotide binding	9.80E-04
Macromolecule biosynthetic process	1.70E-03
Amino acid and derivative metabolic process	2.00E-03
FK506 binding	2.50E-03
Macrolide binding	2.50E-03
Generation of precursor metabolites and energy	2.50E-03
Cellular component	2.50E-03
Organelle ribosome	2.50E-03
Plastid ribosome	2.50E-03
Hydrolase activity, acting on glycosyl bonds	2.50E-03
Chloroplast organization and biogenesis	2.50E-03
Unsaturated fatty acid metabolic process	2.60E-03
Unsaturated fatty acid biosynthetic process	2.60E-03
Organelle large ribosomal subunit	2.60E-03
Plastid large ribosomal subunit	2.60E-03
tRNA metabolic process	3.10E-03
Structural molecule activity	3.10E-03
Adenyl ribonucleotide binding	3.80E-03
Branched chain family amino acid metabolic process	4.10E-03
Drug binding	4.10E-03
Aspartate family amino acid biosynthetic process	5.10E-03
Chloroplast photosystem II	5.10E-03
Peptidyl-prolyl cis-trans isomerase activity	7.30E-03
ATP binding	8.20E-03
Organelle lumen	8.30E-03
Membrane-enclosed lumen	8.30E-03
Cis-trans isomerase activity	9.40E-03
Oxidoreductase activity, acting on sulfur group of donors, NAD or NADP as acceptor	1.00E-02
Reductive pentose-phosphate cycle	1.00E-02

Pathway reconstruction reveals processes that are inversely regulated by *C. fulvum* and *V. dahliae*.

Pathway reconstruction is a valuable tool to identify cellular processes that might otherwise be obscured by the large amount of primary transcription data. Although this tool is widely used for analysis of human and murine transcriptome data, it has hardly been used to analyze plant transcriptome data (van Baarlen et al. 2008; van Esse et al. 2008). Despite the currently available tools that facilitate cellular pathway reconstruction from plant gene expression data, pathway reconstruction in plants remains a challenge, especially in nonmodel plants such as tomato (van Baarlen et al. 2008). Here, we performed pathway reconstruction on the subset of genes that was regulated by both pathogens in the compatible or incompatible interactions but displayed inverse regulation. This set of 164 and 72 inversely regulated genes between the compatible and incompatible interactions, respectively (Fig. 3), may reveal biological processes that are activated by one pathogen and repressed by the other. The BioNetBuilder plugin (Avila-Campillo et al. 2007), an open-source tool that enables generation of

Table 3. Over-representation analysis of differentially regulated genes in roots during the compatible *Verticillium dahliae*–tomato interaction

Gene Ontology subcategory	P value ^a
<i>V. dahliae</i> –tomato induced categories	
Extracellular region	2.50E-04
Manganese ion binding	8.60E-04
Response to ethylene stimulus	1.10E-03
Cation binding	1.10E-03
Transition metal ion binding	1.80E-03
Metal ion binding	2.00E-03
Ion binding	2.00E-03
Zinc ion binding	2.00E-03
Two-component signal transduction system (phosphorelay)	2.00E-03
Defense response, incompatible interaction	2.30E-03
Apoplast	3.30E-03
Response to biotic stimulus	3.70E-03
Response to fungus	4.50E-03
Defense response to fungus	5.70E-03
Protein tyrosine/serine/threonine phosphatase activity	7.90E-03
Response to endogenous stimulus	7.90E-03
<i>V. dahliae</i> –tomato repressed categories	
Intracellular nonmembrane-bounded organelle	5.60E-10
Nonmembrane-bounded organelle	5.60E-10
Ribonucleoprotein complex	2.40E-05
Organelle organization and biogenesis	2.40E-05
Ribosome biogenesis and assembly	2.70E-05
Microtubule cytoskeleton	3.30E-05
Cytoskeletal part	3.40E-05
Macromolecular complex	3.40E-05
Structural constituent of ribosome	4.60E-05
Microtubule-based movement	5.00E-05
Translation	5.70E-05
Cytoskeleton-dependent intracellular transport	6.50E-05
Ribonucleoprotein complex biogenesis and assembly	6.90E-05
Microtubule motor activity	7.90E-05
Ribosome	8.50E-05
Cellular component organization and biogenesis	8.70E-05
Motor activity	9.70E-05
Cytoskeleton	1.70E-04
Structural molecule activity	2.30E-04
Cytoskeleton organization and biogenesis	8.30E-04
Microtubule-based process	1.20E-03
Microtubule-associated complex	1.60E-03
Protein folding	1.80E-03
rRNA processing	2.90E-03
rRNA metabolic process	2.90E-03
Prefoldin complex	4.40E-03
Cytosolic part	5.00E-03
Cajal body	9.10E-03

^a False discovery rate.

biological networks based on integrated information from several databases that include the Biomolecular Interaction Network Database (BIND) (Gilbert 2005), Prolinks (Bowers et al. 2004), and the Kyoto Encyclopedia of Genes and Genomes database (KEGG) (Kanehisa et al. 2002), was used by exploiting the previously identified *Arabidopsis* RefSeq identifiers. Subsequently, pathways were visualized in Cytoscape as network graphs by representing molecular components (such as proteins) as nodes and intermolecular interactions as links (edges) between nodes (Shannon et al. 2003). A pathway was identified with 14 interacting gene products implicated in photorespiration, hypoxia, and glyoxylate metabolism (Fig. 5A through C). We subsequently grafted the expression data of the corresponding genes ($P < 0.05$) onto the identified pathway, showing a differential regulation of this pathway by both pathogens in the compatible interaction (Fig. 5A through C) and, similarly, also in the incompatible interaction (data not shown).

Although most components of this pathway are repressed upon *C. fulvum* inoculation (Fig. 5A), they are induced by *V. dahliae* in roots (Fig. 5B) and even more strongly in the foliage (Fig. 5C). In a similar way, a small interaction cascade with two genes that are involved in auxin signaling was identified. Both genes, *AtAux2-11* (Wyatt et al. 1993) and the *IAA16* transcription factor gene (Kim et al. 1997), were repressed by *C. fulvum* and induced by *V. dahliae*. BioNetBuilder allows expansion of gene sets to include neighboring nodes in iterative steps to find new interactions with molecular components identified in the previous step (Avila-Campillo et al. 2007). In this way, after three iterative steps, a predicted network containing 21 proteins was identified based on the two auxin-signaling genes (Fig. 5D through F). For 12 of these 21 proteins, probe sets were present on the tomato GeneChip. Remarkably, nine of those genes were differentially regulated ($P < 0.05$) upon inoculation with least one of the pathogens. Similar to *AtAux-11* and

Fig. 5. Pathway reconstruction reveals protein interaction networks. **A, B,** and **C,** Responses of susceptible tomato upon inoculation of *Cladosporium fulvum* were compared with those of tomato inoculated with *Verticillium dahliae* using the Bionetbuilder plug-in. A protein interaction network implied in both interactions was retrieved and visualized in Cytoscape. Subsequently, expression data monitored with the tomato GeneChip were grafted onto the network. **A,** Network response upon inoculation with *C. fulvum*. **B,** Network response in foliar tissues upon inoculation with *V. dahliae*. **C,** Network response in root tissues upon inoculation with *V. dahliae*. **D, E,** and **F,** Small protein interaction network implied in auxin signaling was retrieved. After three iterative steps in the Bionetbuilder, a protein interaction network was obtained that is visualized in Cytoscape. Subsequently, expression data monitored with the tomato GeneChip were grafted onto the network. Gray nodes indicate proteins for which expression was not considered. **D,** Network response upon inoculation with *C. fulvum*. **E,** Network response in foliar tissues upon inoculation with *V. dahliae*. **F,** Network response in root tissues upon inoculation with *V. dahliae*.

IAA16, the genes encoding the negative auxin-signaling regulator AXR3 (Leyser et al. 1996) and the Skp1-CUL1-F-box protein ASK2 (*Arabidopsis* Skp-1-like 2) (Park et al. 1993) were inversely regulated. Also present in the pathway is the Tir1 (transport inhibitor response) protein, an orthologue of human Skp2, that is able to bind to ASK2 (Ruegger et al. 1998). Interestingly, in addition to Tir1, ASK2 also is known to bind to the negative regulator of jasmonate signaling COI1 (Devoto et al. 2002). The gene encoding AXR3 (Nagpal et al. 2000) was specifically induced in the *V. dahliae*–tomato interaction (Fig. 5D through F). Recently, it has been demonstrated that *Arabidopsis* mutants in AXR3 display 10-fold reduction of *Pseudomonas syringae* pv. *maculicola* colonization (Wang et al. 2007). Finally, the auxin-inducible IAA18 gene (Reed 2001) also was induced by *C. fulvum* and repressed by *V. dahliae* (Fig. 5D through F).

DISCUSSION

In this study, we used microarrays to monitor global transcriptional responses of tomato upon inoculation with the foliar pathogen *C. fulvum* and the vascular pathogen *V. dahliae* in both a compatible and an incompatible interaction. When examining the response to each of the two pathogens separately, a significant overlap was observed in the response of tomato when comparing the compatible and incompatible interactions. These observations are similar to other transcriptomics studies that compared compatible and incompatible plant–pathogen interactions (Eulgem et al. 2004; Navarro et al. 2004; Tao et al. 2003; Thilmony et al. 2006). For both pathogens, approximately two-thirds of the genes that are differentially regulated in the incompatible interaction are also differentially regulated in the compatible interaction (Fig. 2). This complies with the currently held hypothesis that race-specific resistance can largely be seen as an accelerated and amplified basal defense response. Intriguingly, many fewer differentially expressed genes were observed in the interaction with *V. dahliae* than with *C. fulvum*. Possibly, this may be explained by the fact that *C. fulvum* invades living plant tissue, namely the leaf mesophyll, whereas *V. dahliae* resides in the xylem tissues that are actually composed of dead cells. Consequently, direct physical contact of the pathogen with living plant cells that are able to respond is likely to be more extensive in case of *C. fulvum*.

In incompatible interactions, resistance against *C. fulvum* and *V. dahliae* is conveyed by extracellular transmembrane receptors that belong to the class of receptor-like proteins (RLP) (Fradin and Thomma 2006; Thomma et al. 2005; Wang et al. 2008). Resistance against *C. fulvum* is governed by *Cf* genes (Thomma et al. 2005), of which we employed the *Cf-9* gene in this study (Jones et al. 1994) whereas, in all *V. dahliae*-resistant tomato cultivars, resistance is governed by a single locus that contains two resistance genes, *Ve1* and *Ve2* (Kawchuk et al. 2001). Because RLP lack obvious cytoplasmic signaling domains, it has been proposed that they associate with receptor-like kinases (RLK) to relay signals across the plasma membrane (Joosten and de Wit 1999), and different RLP possibly associate with the same RLK to activate a general defense signaling cascade. However, our study suggests that the signaling cascade that is activated by *Cf-9* is different from the cascade that is activated by *Ve1* and *Ve2*, because both pathogens regulate highly divergent gene sets in the incompatible interactions (Fig. 3). Indeed, it was recently shown that the requirement of downstream components for resistance against these two pathogens is largely different (E. F. Fradin and B. P. H. J. Thomma, unpublished results).

C. fulvum and *V. dahliae* display several commonalities in their infection strategies, such as penetration through natural

openings and strictly extracellular growth without the formation of haustoria. Nevertheless, the transcriptional changes induced in tomato by the two pathogens in their compatible interactions hardly overlap. Previously, the transcriptional response of *Arabidopsis* upon challenge with a host (*Erysiphe cichoracearum*) and a nonhost (*B. graminis* f. sp. *hordei*) powdery mildew isolate revealed that *B. graminis* f. sp. *hordei* elicited a more dramatic response than *E. cichoracearum*. It was suggested that plants respond more powerful to *B. graminis* f. sp. *hordei* because it cannot evade or suppress basal defenses as efficiently as the host powdery mildew, *E. cichoracearum* (Stein et al. 2006; Zimmerli et al. 2004). Moreover, attacker-specific transcriptomes have also been observed in *Arabidopsis* upon challenge with different microbial pathogens and feeding insects (de Vos et al. 2005).

Apart from the commonalities in the colonization strategies of *C. fulvum* and *V. dahliae*, there are also obvious differences. The most significant difference is the tissue that is colonized; the leaf apoplast by *C. fulvum* and the xylem by *V. dahliae*. Whereas *C. fulvum* is in close contact with mesophyll cells, *V. dahliae* resides in dead tracheids and may not have direct physical contact with living plant cells. Furthermore, during colonization, *V. dahliae* has been reported to secrete potent phytotoxins into the xylem that are dispersed throughout the plant with the sap stream, whereas *C. fulvum* is not known to utilize toxin activity (Fradin and Thomma 2006; Thomma et al. 2005). Several studies show that the fungus enters the xylem vessels of the root and starts sporulation after 2 to 5 days (Chen et al. 2004; Gold and Robb 1995; Heinz et al. 1998). After 1 week, this sporulation results in colonization of stem vessels, at which time fungal elimination as a consequence of plant defense also occurs. In a compatible interaction, the pathogen is able to overcome this elimination (Chen et al. 2004; Gold and Robb 1995; Heinz et al. 1998). Because *V. dahliae* is a wilt pathogen, the more prominent expression of genes that are associated with water stress and dehydration, such as several δ -TIP aquaporin-like genes, and transporters may be expected. The prominent occurrence of the manganese ion-binding category in the ORA of the compatible *V. dahliae*–tomato interaction in roots is interesting because it concerns four germin-like proteins that may play a role in reinforcement plant cell walls upon pathogen attack (Schweizer et al. 1999; Wei et al. 1998). Therefore, cell wall fortifications may be more important in defense against *V. dahliae* (Fradin and Thomma 2006) when compared with *C. fulvum*. The over-representation of gene categories involved in cytoskeletal and microtubule organization, movement, and transport in the set of *V. dahliae*-repressed genes further supports this observation.

A remarkable difference between the transcriptional changes induced in tomato by both pathogens is the prominent occurrence of WRKY transcription factors in the *C. fulvum* interaction whereas, in the interaction with *V. dahliae*, these transcription factors are not induced. Upon *C. fulvum* infection, the tomato homologs of the *Arabidopsis* WRKY transcription factors 2, 3, 4, 6, 7, 23, 51, 53, and 71 are differentially regulated. Of these, WRKY 4, 6, 7, 51, and 53 have previously been implicated in plant defense and senescence responses (Dong et al. 2003; Eulgem and Somssich 2007; Eulgem et al. 2000; Kalde et al. 2003; Robatzek and Somssich 2001). Furthermore, induction of glutathione S-transferases and serine-type protease inhibitors is specifically observed in the *C. fulvum*–tomato interaction. Both types of proteins may attenuate the oxidative burst (Lamb and Dixon 1997) that plays a role in tomato defense against *C. fulvum* (Hammond-Kosack et al. 1996). Other genes that are specifically induced by *C. fulvum* in tomato are those that code for NAM-like proteins (Souer et al. 1996) that, together with ATAF and CUP-SHAPED COTYLEDON (CUC),

are part of a larger NAC (for NAM, ATAF, and CUC) protein family of transcription factors (Aida et al. 1997). Some NAC genes, such as the *ATAF1* and *ATAF2* genes from *Arabidopsis* and the *StNAC* gene from potato, are induced by pathogen attack and wounding (Aida et al. 1997; Collinge and Boller 2001). Furthermore, many NAC genes were differentially regulated in the interaction between the nematode *Heterodera glycines* and soybean (Klink et al. 2007). Recently, the barley NAC gene *HvNAC6* was implicated in basal defense against the barley powdery mildew pathogen *Botrytis graminis* f. sp. *hordei* (Jensen et al. 2007). Furthermore, *Arabidopsis* ATAF2 is known to repress *PR* genes, and *ATAF2*-overexpressing plants showed a higher susceptibility to the soilborne vascular fungal pathogen *Fusarium oxysporum* (Delessert et al. 2005). Other *Arabidopsis* NAC members are involved in secondary cell wall thickening (Mitsuda et al. 2005; Zhong and Ye 2007) and in auxin and ethylene signaling (Guo et al. 2005; He et al. 2005). Also, in a cDNA amplified fragment length polymorphism analysis on tomato transgenes that undergo a controlled hypersensitive response due to heterologous expression of *C. fulvum* Avr4 in *Cf-4* plants, transcripts for NAM-like proteins and a WRKY transcription factor were identified (Gabiñels et al. 2006). The activation of different defense cascades is also visible in the ORA of the compatible interactions with *C. fulvum* and *V. dahliae* with over-represented gene categories such as jasmonic acid and salicylic acid responses, as well as expression of tomato homologues *Jar1* (Staswick et al. 1992), *Pad4* (Glazebrook et al. 1997), *Npr1* (Cao et al. 1994), and *Rin4* (Mackey et al. 2002) by *C. fulvum* but not by *V. dahliae*.

A surprising observation is the inverse regulation of photosynthesis in tomato upon challenge by *C. fulvum* and *V. dahliae*. Strong repression of photosynthesis, as in *C. fulvum*-challenged tomato, is a typical plant response to pathogen attack (Berger et al. 2007; Dowd et al. 2004; Scheideler et al. 2002; Zimmerli et al. 2004). However, local stimulation of photosynthesis has been noted, too, because *Albugo candida* infection of *Arabidopsis* and *Botrytis cinerea* infection of tomato results in decreased photosynthesis at the infection site, surrounded by a zone of enhanced photosynthesis (Berger et al. 2004; Chou et al. 2000). In a recent study, transcription profiles were determined for stem tissue of Craigella tomato plants infected with two different *V. dahliae* isolates using a cDNA array to interrogate expression of approximately 8,600 tomato genes (Robb et al. 2007). Infection with isolate Vd1 resulted in a compatible interaction while infection with isolate E6 resulted in a tolerant interaction. Also in this study, as with our findings, stimulation of photosynthesis by *V. dahliae* was observed in both the tolerant and compatible interaction (Robb et al. 2007). Moreover, Robb and associates (2007) noted induction of a gene encoding a 14-3-3 protein in the compatible interaction with *V. dahliae*, whereas this gene was repressed in a tolerant tomato genotype that develops few symptoms despite substantial fungal colonization, suggesting that this gene may be elementary for the tolerance phenotype. However, in a tolerant cotton genotype, a gene encoding a 14-3-3 protein was found to be activated upon *V. dahliae* challenge (Hill et al. 1999). In our study, genes encoding 14-3-3 proteins were found to be induced in both the compatible and incompatible interactions with *V. dahliae* in leaves and roots but also in the interactions with *C. fulvum*.

Few microarray studies have been performed to monitor the transcriptional changes of different plant tissues upon pathogen infection. One example is the profiling in cotton roots and hypocotyls in response to infection with *F. oxysporum* (Dowd et al. 2004). Also, in this study, it appeared that tissues responded quite differently to infection. Substantially more induced plant genes were identified in infected cotton hypocotyl tissues than

in root tissues, suggesting that the fungus may be suppressing plant defense responses in the root tissue (Dowd et al. 2004). Also, our transcriptional profiling of roots and foliage of *V. dahliae*-infected tomato in the compatible interaction revealed significant differences in expression profiles between the two tissues. In contrast to the *F. oxysporum*-infected cotton, in our study, more genes were induced in the roots than in the foliage.

With pathway reconstruction, it was attempted to uncover additional differentially regulated biological processes in the global transcription profiles. Although it has become accepted presently that, with careful experiment design and rigorous statistical analysis, confirmation of expression data by using an alternative technique is not necessary for high-quality, commercially available array platforms (Wise et al. 2007), the identification of differentially regulated biological processes further substantiates the results obtained by the transcriptional profiling. Among the gene sets inversely regulated by the two pathogens is a pathway that can be implicated in photorespiration, hypoxia, and glyoxylate metabolism. It has previously been shown that these processes are repressed upon pathogen attack (Berger et al. 2007; Scheideler et al. 2002; Zimmerli et al. 2004). Glutamate:glyoxylate aminotransferase (GGT1) is a peroxisomal enzyme that plays a central role in the photorespiratory pathway (Liepman and Olsen 2003). Peroxisomal alanine:glyoxylate aminotransferases AGT1 and AGT2 and alanine:2-oxoglutarate aminotransferase AOAT2 have also been implicated in photorespiration, although the exact metabolic function of AGT2 and AOAT2 remain unclear (Igarashi et al. 2003; Liepman and Olsen 2003). ALAT1 and ALAT2 have recently been shown to be induced by oxygen deprivation (Miyashita et al. 2007). Interestingly, malate synthase is induced during the *V. dahliae*-tomato interaction and repressed during the *C. fulvum*-tomato interaction. Malate synthase is part of the glyoxylate cycle and expression of this gene is repressed when sucrose is being synthesized (e.g., in photosynthetic tissues), although induction has also been observed in senescent organs (Graham et al. 1992). Interestingly, it has been noted previously that *V. dahliae* induces early senescence in several host plants (du Toit et al. 2005; Joaquim and Rowe 1991; Sadras et al. 2000; Veronese et al. 2003). Peroxisomal glycolate oxidases have a clear role in plant defense and programmed cell death (Grant and Loake 2000; Hammond-Kosack and Jones 1996) but also play a role in photorespiration (Fukao et al. 2002). Another gene in the pathway, PSBO-2, is part of the photosystem II and clearly links this pathway to the differential regulation of photosynthetic genes. Altogether, this pathway analysis shows that, apart from photosynthesis, the photorespiratory system of tomato also is differentially regulated upon infection by the two pathogens.

A similar situation as for the photorespiratory system occurs for an auxin-signaling pathway. Previously, auxin signaling has been implicated in the response of cotton to *F. oxysporum* infection (Dowd et al. 2004). With respect to the role of auxin in plant defenses, it has been shown that an *Arabidopsis* miRNA contributes to resistance against *P. syringae* by repressing auxin signaling (Navarro et al. 2006), and salicylic acid was found to inhibit the growth of pathogens via repression of the auxin-signaling pathway (Wang et al. 2007). Recently, the *P. syringae* type III effector AvrRpt2 was found to alter auxin physiology in *Arabidopsis* (Chen et al. 2007), suggesting that modulation of auxin signaling contributes to host susceptibility. Furthermore, some strains of the bacterial pathogen *Ralstonia solanacearum* are known to produce auxin, the production of which is controlled by an *hrp* master regulatory gene whose activity is induced in the presence of plant cells (Valls et al. 2006). Finally, auxin produced by *P. syringae* pv. *savastanoi* appears to be required for the inhibition of plant defenses (Robinette and Matthysse 1990).

Overall, it can be concluded that global transcriptional profiling of tomato during compatible and incompatible interactions with the foliar pathogenic fungus *C. fulvum* and the vascular pathogenic fungus *V. dahliae* revealed only limited overlap. Furthermore, with novel strategies that allowed the use of state-of-the-art tools, differentially regulated pathways were identified that provide new insight into pathogen biology even though tomato is not a genetic model organism.

MATERIALS AND METHODS

Cultivation of microorganisms and plant inoculations.

C. fulvum and *V. dahliae* were cultured at room temperature on half-strength potato dextrose broth (Oxoid, Basingstoke, England) supplemented with technical agar at 7 g/liter (Oxoid). All tomato plants were grown in soil under standard greenhouse conditions: 21 and 19°C during day and night periods of 16 and 8 h, respectively; 70% relative humidity (RH); and supplemental light of 100 W/m² when the intensity dropped below 150 W/m². For inoculations of tomato with *C. fulvum*, 5 ml of conidial suspension (1×10^6 conidia/ml) was sprayed on the lower surface of leaves of 5-week-old tomato plants (van Esse et al. 2007). Plants were kept at high RH under a plastic cover for 48 h after inoculation. For inoculation with *V. dahliae*, 10-day-old soil-grown tomato plants were uprooted and inoculated by dipping the roots for 2 min in a conidial suspension (10^6 conidia/ml) in water. After replanting in soil, plants were incubated at standard greenhouse conditions of 16- and 8-h light and dark regime, respectively, and 70% RH.

Microarray sample preparation and data analyses.

All samples were collected in three independently repeated experiments. A race 5 strain of *C. fulvum* (5 Kim) was used for inoculation. MoneyMaker *Cf-0* was used as a *C. fulvum*-susceptible genotype because it is devoid of functional *Cf* resistance genes, while the isogenic MoneyMaker *Cf-9* line was used as a resistant genotype because it recognizes the Avr9 elicitor that is produced by the race 5 *C. fulvum* strain. At 0, 3, 7, and 10 DPI, leaf material was harvested from the secondary and tertiary leaves of three plants, pooled, and flash frozen in liquid nitrogen. Material harvested at 0 DPI was used as the reference sample to which all other samples were compared.

For *V. dahliae*, a race 1 strain (St14.01) was used for inoculation on MoneyMaker and Motelle tomato plants. MoneyMaker was used as a susceptible genotype because it lacks *Verticillium* resistance, while Motelle contains the *Ve* locus that provides resistance against race 1 *Verticillium* strains (Schaible et al. 1951). At 3 and 7 DPI, foliar and root material was harvested from nine plants, pooled, and flash frozen in liquid nitrogen. As the reference sample to which all other samples were compared, foliar and root material of mock-inoculated plants at 3 DPI was used.

For RNA extraction, the frozen plant material was ground using a mortar and pestle, and approximately 100 mg of the material was homogenized in Trizol reagent (Invitrogen, Carlsbad, CA, U.S.A.). After phase separation, isopropanol was added to the aqueous phase, which was subsequently further purified with the NucleoSpin RNA plant kit (Macherey-Nagel GmbH, Düren, Germany). In this way, total RNA was obtained that was hybridized onto custom-designed Affymetrix tomato GeneChip array (Syngenta Biotechnology, Inc., Research Triangle Park, NC, U.S.A.) that contains probe sets to interrogate 22,721 tomato transcripts (van Esse et al. 2007). Probe preparations and GeneChip hybridizations were carried out at ServiceXS (Leiden, The Netherlands).

Microarray data analyses.

Bioconductor packages (Gentleman et al. 2004) were used to analyze the scanned Affymetrix arrays. The Bioconductor packages were integrated in the automated online MADMAX pipeline. The arrays were normalized using quantile normalization and expression estimates were compiled using RMA applying the empirical Bayes approach (Wu et al. 2004). They were considered of sufficiently high quality if they showed less than 10% of specks in fitPLM model images, were not deviating in RNA degradation and density plots, were not significantly deviating in NUSE and RLE plots, and were within each other's range in boxplots. Differentially expressed probe-sets were identified using linear models, applying moderated *t* statistics that implement empirical Bayes regularization of standard errors (Smyth 2004). Venn diagrams and basic comparisons were performed in Microsoft Excel. HCL and K-Means clustering were performed using the TM4 microarray software suite and data were imported as Tab Delimited, Multiple Sample Files (TDMS) (*.*) files. The TM4 software suite runs in a Java environment (Sun Microsystems, Inc. Santa Clara, CA, U.S.A.).

Microarray data.

Microarray data have been deposited in ArrayExpress as accession E-MEXP-1844.

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AUTHOR-RECOMMENDED INTERNET RESOURCES

ArrayExpress: www.ebi.ac.uk/microarray-as/ae
 BioConductor, an open source software for bioinformatics:
www.bioconductor.org
 BioNetBuilder plugin: err.bio.nyu.edu/cytoscape/bionetbuilder
 Java: www.java.com/en
 RefSeq database: www.ncbi.nlm.nih.gov/RefSeq
 TM4 microarray software suite: www.tm4.org/mev.html