The Plant Cell, Vol. 21: 2237–2252, August 2009, www.plantcell.org © 2009 American Society of Plant Biologists

The Mediator Complex Subunit PFT1 Is a Key Regulator of Jasmonate-Dependent Defense in *Arabidopsis* [C][W]

Brendan N. Kidd,[a,b] Cameron I. Edgar,[a,b] Krish K. Kumar,[a,1] Elizabeth A. Aitken,[b] Peer M. Schenk,[b] John M. Manners,[a] and Kemal Kazan[a,2]

[a] Commonwealth Scientific and Industrial Research Organization Plant Industry, Queensland Bioscience Precinct, St. Lucia, Queensland, 4067, Australia
[b] School of Biological Sciences, University of Queensland, St. Lucia, Queensland, 4072, Australia

Jasmonate signaling plays an important role in both plant defense and development. Here, we have identified a subunit of the Mediator complex as a regulator of the jasmonate signaling pathway in *Arabidopsis thaliana*. The Mediator complex is a conserved multiprotein complex that acts as a universal adaptor between transcription factors and the RNA polymerase II transcriptional machinery. We report that the *PHYTOCHROME AND FLOWERING TIME1* (*PFT1*) gene, which encodes the MEDIATOR25 subunit of Mediator, is required for jasmonate-dependent defense gene expression and resistance to leaf-infecting necrotrophic fungal pathogens. Conversely, PFT1 appears to confer susceptibility to *Fusarium oxysporum*, a root-infecting hemibiotrophic fungal pathogen known to hijack jasmonate responses for disease development. Consistent with this, jasmonate gene expression was suppressed in the *pft1* mutant during infection with *F. oxysporum*. In addition, a wheat (*Triticum aestivum*) homolog of PFT1 complemented the defense and the developmental phenotypes of the *pft1* mutant, suggesting that the jasmonate signaling functions of PFT1 may be conserved in higher plants. Overall, our results identify an important control point in the regulation of the jasmonate signaling pathway within the transcriptional machinery.

INTRODUCTION

In response to an attempted infection, plants activate an inbuilt system of defense, which results in the production of a variety of pathogenesis-related (PR) proteins and secondary metabolites, as well as low molecular weight defense-related hormones, such as salicylates (SAs) and jasmonates (JAs). These defense-related hormones act both locally and systemically to orchestrate the plant's defense signaling network through the activation of transcription factors. Thus, transcriptional regulation of defense gene expression plays a major role in determining whether a plant is more or less resistant to pathogen attack (McGrath et al., 2005).

Recently, a new component of the plant's transcriptional machinery has been identified with the purification of the Mediator complex in *Arabidopsis thaliana* (Bäckström et al., 2007). The Mediator complex is a large multiprotein complex that is conserved in all eukaryotes, from yeast to humans, whose characterization had until recently remained elusive in plants. The function of the Mediator complex is to act as a bridge between the RNA polymerase II complex and the myriad of transcription factors present within the cell (Kim et al., 1994; Koleske and Young, 1994). By binding to distal activators/repressors as well as general transcription factors at the promoter site, Mediator fine-tunes diverse regulatory inputs and presents a balanced output to the RNA polymerase II complex to initiate transcription (Malik and Roeder, 2005).

Mediator subunits are organized into three core modules, termed the head, middle, and tail, as well as an additional detachable kinase module. The tail module of Mediator is thought to interact primarily with DNA-bound transcription factors, while the head and middle modules bind to the C-terminal domain of RNA polymerase II (reviewed in Malik and Roeder, 2005). Depending on the organism, the Mediator complex contains ~20 to 30 subunits. For example, the Mediator from the yeast *Saccharomyces cerevisiae* is composed of 25 subunits, of which 22 subunits are at least partially conserved among eukaryotes (Boube et al., 2002; Bourbon et al., 2004). Bäckström et al. (2007) identified 21 conserved and six putative plant-specific Mediator subunits in *Arabidopsis*. It is expected that individual Mediator subunits recognize and respond to a subset of the ~1500 transcription factors present in the *Arabidopsis* genome. Therefore, determining which transcription factors each Mediator subunit recognizes would provide a step forward in the understanding of how the Mediator complex manages to integrate the complex transcriptional information in plants.

Prior to its identification as a Mediator subunit, MEDIATOR25 (MED25) was first described as a positive regulator of shade avoidance in *Arabidopsis* and was termed *PHYTOCHROME AND FLOWERING TIME1* (*PFT1*) (Cerdán and Chory, 2003). Cerdán and Chory (2003) hypothesized that PFT1 might act

[1] Current address: Department of Plant Molecular Biology and Biotechnology, Centre for Plant Molecular Biology, Tamil Nadu Agricultural University, Coimbatore, 641 003, Tamil Nadu, India.
[2] Address correspondence to kemal.kazan@csiro.au.
The author responsible for distribution of materials integral to the findings presented in this article in accordance with the policy described in the Instructions for Authors (www.plantcell.org) is: Kemal Kazan (kemal.kazan@csiro.au).
[C]Some figures in this article are displayed in color online but in black and white in the print edition.
[W]Online version contains Web-only data.
www.plantcell.org/cgi/doi/10.1105/tpc.109.066910

downstream of phytochrome B to promote flowering in response to shade. However, Wollenberg et al. (2008) have since found that the *pft1* mutant did not show an altered flowering response when grown in simulated shade (far-red light–enriched long-day [LD] conditions). As a result, *PFT1* is now considered to be a gene that negatively regulates the phytochrome signaling pathway as opposed to being a component of the phytochrome signaling pathway itself.

Here, we report that PFT1 also acts as a positive regulator of JA signaling that regulates plant defense responses during fungal pathogen infection. In a genome-wide analysis of *Arabidopsis* transcription factor gene expression, we previously reported that *PFT1* expression was reduced in response to methyl jasmonate (MeJA) and during the defense response to an incompatible isolate of the necrotrophic fungal pathogen *Alternaria brassicicola* (McGrath et al., 2005). In this report, we show that PFT1 is required for uncompromised expression of JA-dependent defense genes and resistance to the leaf-infecting necrotrophic pathogens *A. brassicicola* and *Botrytis cinerea*. We have also found that PFT1 is necessary for susceptibility to the root-infecting hemibiotrophic fungal pathogen *Fusarium oxysporum*, which requires intact JA signaling in the host to promote disease symptoms (Thatcher et al., 2009). In addition, through the analysis of several Mediator subunit mutants, we have identified an additional Mediator subunit, MED8, which is also a regulator of both flowering time and disease resistance. These results provide new insights into the regulatory role of plant Mediator subunits in determining fungal disease outcomes as well as the initiation of flowering.

RESULTS

PFT1 Is Required for Basal Resistance to Necrotrophic Fungal Pathogens

Previous research investigating the responses of the *Arabidopsis* transcription factor transcriptome during plant defense had demonstrated that *PFT1* transcript levels were reduced in *Arabidopsis* leaves either challenged with *A. brassicicola* or treated with MeJA (McGrath et al., 2005; see Supplemental Table 1 online). To analyze potential roles of PFT1 in plant defense, we isolated two homozygous lines harboring independent T-DNA insertions in the *PFT1* gene (Alonso et al., 2003). These lines were designated as *pft1-2* and *pft1-3* in sequence after the *pft1-1* mutant previously characterized by Cerdán and Chory (2003). Like the *pft1-1* mutant, the *pft1-2* mutant contains a T-DNA insertion located in the 5th exon of the *PFT1* gene. This insertion lies within a genomic region that encodes a von Willebrand Factor A domain (vWF-A) located at the N terminus of the PFT1 protein (Figure 1A). The vWF-A domain is a widely distributed protein–protein interaction domain (Whittaker and Hynes, 2002) and has been shown to be critical for the binding of MED25 to the Mediator complex in human cell lines (Mittler et al., 2003). The T-DNA in the *pft1-3* mutant is inserted into the 14th exon of *PFT1* (Figure 1A) and disrupts the Gln-rich region predicted to function as a putative transcriptional activation domain (Cerdán and Chory, 2003).

To determine whether PFT1 is required during plant defense, *pft1-2*, *pft1-3*, and wild-type plants were inoculated with the leaf-infecting necrotrophic fungal pathogens *A. brassicicola* and *B. cinerea*. The *A. brassicicola* isolate used here is incompatible on wild-type (Columbia-0 [Col-0]) *Arabidopsis* (Schenk et al., 2000, 2003) but has been shown to be capable of causing lesions on *Arabidopsis* mutants with attenuated plant defenses (Trusov et al., 2006). As shown in Figure 1B, inoculations with *A. brassicicola* resulted in the development of distinct chlorotic regions restricted to older rosette leaves of the *pft1* mutant. In these experiments, 35 and 30% of the inoculated *pft1-2* and *pft1-3* leaves, respectively, showed chlorosis (Figure 1C). Furthermore, the pathogen was often able to sporulate on these chlorotic lesions. By contrast, only 3% of the inoculated leaves from wild-type plants had similar chlorotic regions (Figure 1C). Similarly, *B. cinerea*, although poorly compatible on wild-type plants, produced larger chlorotic regions on the *pft1* mutant leaves than those on wild-type leaves (Figures 1D and 1E). Thus, these pathogen inoculation experiments suggest that PFT1 is an important component of basal resistance to these necrotrophic pathogens in *Arabidopsis*.

PFT1 Is a *F. oxysporum* Susceptibility Gene

To determine whether PFT1 is also required for resistance to a pathogen with a different lifestyle, we conducted inoculation experiments with the root-infecting hemibiotrophic fungal pathogen *F. oxysporum*, which causes vascular wilt disease in a wide range of plants, including *Arabidopsis* (Diener and Ausubel, 2005; Edgar et al., 2006; Berrocal-Lobo and Molina, 2008; Michielse and Rep, 2009). The number of leaves with chlorosis/necrosis and the percentage of plant death after inoculations were substantially lower ($P < 0.01$) in the *pft1* mutants than those in the wild-type plants (Figures 2A to 2C). The increased resistance to *F. oxysporum* and increased susceptibility against *B. cinerea* and *A. brassicicola* in *pft1* mutants was reminiscent of the reaction to these pathogens in the *coi1* mutant, which has impaired JA signaling (Thatcher et al., 2009).

To confirm that the increased *F. oxysporum* resistance was due to a loss-of-function mutation in the *PFT1* gene, the *Fusarium* resistance phenotypes of the *pft1-2* mutant, a *35S-PFT1* overexpressing line (*OX1*) as well as the *pft1* mutant transformed with a genomic copy of *PFT1* (*G1*), were analyzed in replicated inoculation experiments. The *PFT1*-overexpressing line and the *PFT1* genomic complement, designated as *OX1* and *G1*, respectively, have been previously characterized in relation to the function of PFT1 in phytochrome signaling (Cerdán and Chory, 2003). Consistent with the previous inoculation experiments, the *pft1-2* plants were mostly free of visible symptoms, while typical *F. oxysporum* disease symptoms (e.g., vein chlorosis) appeared on a high proportion of the wild-type, *OX1*, and *G1* plants (Figure 2D). The *OX1* plants were the most affected, with ~90% of the *OX1* leaves showing visible symptoms, while the *G1* plants, as expected, showed symptoms comparable to those on wild-type plants (Figures 2D and 2E). Finally, because *G1* was generated in the *pft1-1* background (Cerdán and Chory, 2003), we tested the response of the *pft1-1* mutant to *F. oxysporum*. As expected, *pft1-1* was also resistant to *F. oxysporum*. Together, these

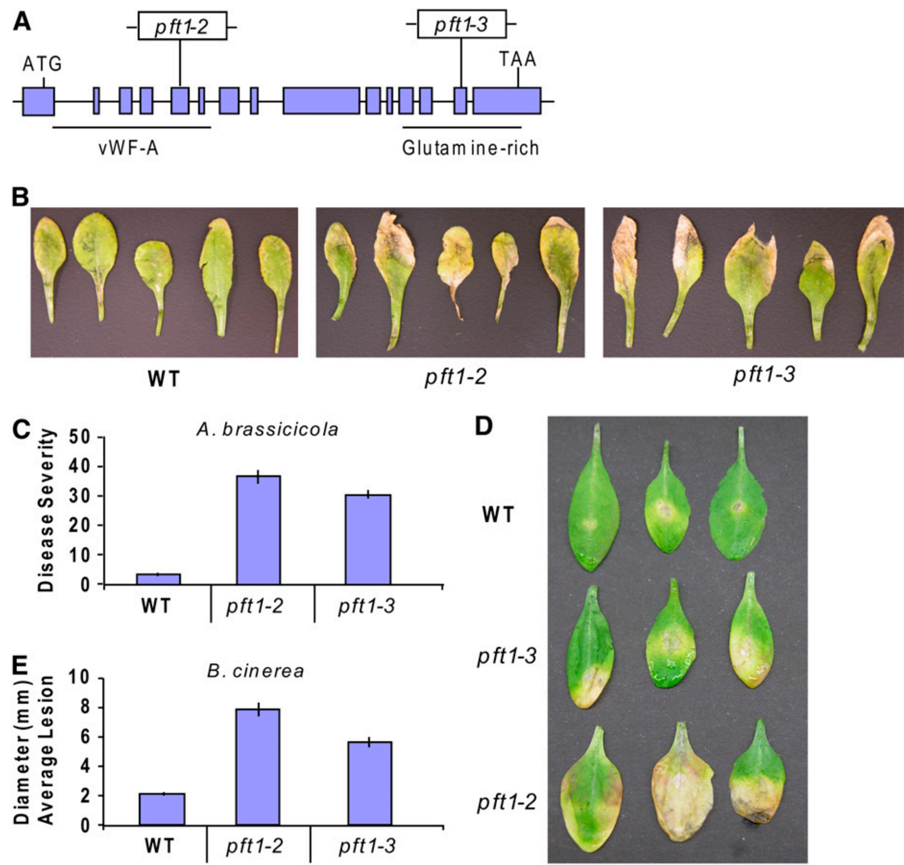

Figure 1. PFT1 Is Required for Basal Resistance to the Leaf-Infecting Necrotrophic Pathogens *A. brassicicola* and *B. cinerea*.

(A) Schematic representation of the *PFT1* gene and the locations of two independent T-DNA insertions designated as *pft1-2* (SALK_129555) and *pft1-3* (SALK_059316). Introns (solid line) and exons (boxes) are indicated.

(B) Symptoms on rosette leaves of 4-week-old soil-grown wild-type, *pft1-2*, and *pft1-3* plants 2 weeks following drop inoculation with freshly harvested spores of *A. brassicicola*.

(C) The percentages of leaves showing chlorotic regions calculated 2 weeks after inoculations with *A. brassicicola*.

(D) Symptoms on rosette leaves of 4-week-old soil-grown wild-type, *pft1-2*, and *pft1-3* plants following drop inoculation with freshly harvested spores of *B. cinerea.*

(E) The average lesion diameter measured 4 d after inoculation with *B. cinerea*.

Error bars in **(C)** and **(E)** represent SE from three replicated experiments that contained 20 to 30 plants each.

experiments clearly demonstrate that PFT1 increases susceptibility to *F. oxysporum*.

To determine when PFT1 may be required for susceptibility, *F. oxysporum*–inoculated roots of *pft1*, wild-type, and *OX1* plants were analyzed by confocal microscopy after staining with a fluorescent wheat germ agglutinin conjugate to differentiate the fungal tissue. These analyses, although qualitative in nature, clearly showed that fungal hyphae were present in the roots of *pft1*, wild-type, and *OX1* plants at the same time periods following inoculations (see Supplemental Figure 1 online). In addition, we could find no evidence of increased callose production or altered structural differences in *pft1* lines upon infection with *F. oxysporum*. This suggests that the *pft1* mutation did not alter the sensitivity of *pft1* roots to *F. oxysporum* infection and that resistance was expressed later in infection as symptoms develop.

PFT1 Is a Positive Regulator of Defense Gene Expression in *Arabidopsis*

Because the contrasting disease phenotypes displayed by the *pft1* mutant against different pathogens resembled those observed in the JA signaling mutant *coi1* (Thomma et al., 1998; Thatcher et al., 2009), we analyzed the expression of a number of JA-responsive defense genes in *pft1* mutants. As shown in Figures 3A to 3D, the JA-responsive genes *PDF1.2*, *HEL*, *CHIB*, and *ESP* showed reduced basal expression levels in *pft1* mutant plants relative to the wild type. In addition, *PDF1.2*, *HEL*, *ESP*, *VSP2*, and *OPR*, although responsive to MeJA, showed reduced expression levels in *pft1* mutants (Figures 3A, 3B, and 3D to 3F). *CHIB* did not show any JA inducibility at this time point; therefore, the expression of *CHIB* in the treated samples was equivalent to that of the mock samples. *MYC2*, encoding a regulator of JA

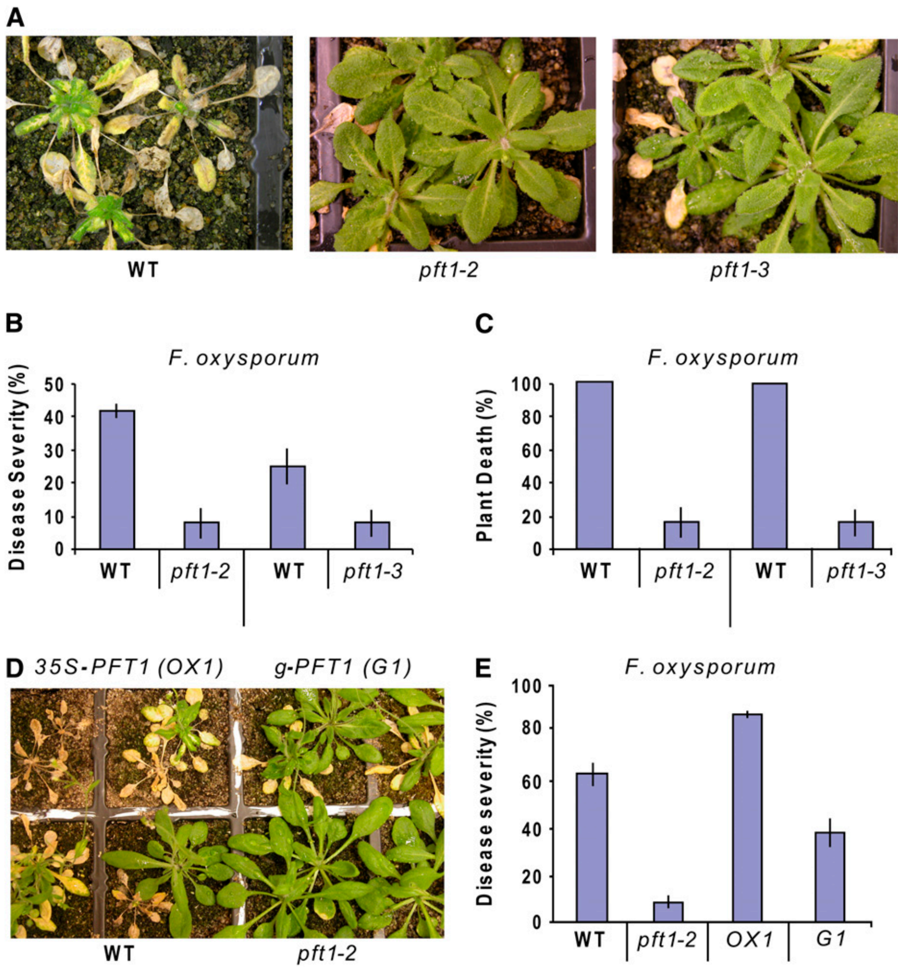

Figure 2. *PFT1* Is an *F. oxysporum* Susceptibility Gene.

(A) Typical disease symptoms and plant death observed on wild-type (Col-0) and *pft1* mutants 2 weeks after inoculations of the roots of 3- to 4-week-old plants.

(B) The number of leaves showing chlorosis and necrosis 10 d after inoculations and expressed as the percentage of total number of leaves.

(C) Percentages of plant death 2 weeks after the inoculation of roots with *F. oxysporum*.

(D) Typical disease phenotypes of wild-type (Col-0), *pft1*, *OX1* (*35S:PFT1*), and *G1* (the *pft1* mutant complemented with a genomic *PFT1*) plants at 12 d after *F. oxysporum* inoculation.

(E) Average percentage of disease severity measured in the same lines shown in **(D)**. Error bars represent SE from three replicated experiments that contained 20 to 30 plants each.

signaling, was inducible by MeJA in the wild type but not inducible in *pft1* mutants (Figure 3G). By contrast, *WRKY70*, encoding a negative regulator of JA signaling, showed higher expression levels in *pft1* mutants in response to MeJA (Figure 3H). In separate sets of experiments, we further examined the expression of these defense genes in wild-type, *G1*, and *OX1* plants and found increased transcript levels of JA-responsive genes in *OX1* plants (see Supplemental Figure 2 online). In addition, *pft1* roots were less sensitive to growth inhibition by MeJA than *G1* and *OX1* roots (Figure 3I). Together, these data suggest a positive regulatory role for PFT1 in JA signaling.

We also noted that *MYC2* and *WRKY70* (differentially regulated in *pft1* mutants) encode a positive and a negative regulator of the anthocyanin pathway, respectively (Li et al., 2006; Dombrecht et al., 2007). In addition, the basal transcript levels of *PAL1*, which encodes a major isoform of the enzyme Phe ammonia lyase involved in phenylpropanoid biosynthesis, was lower in the *pft1* mutant than in wild-type, *G1*, and *OX1* plants (Figure 3J). Consistent with these expression data, the *pft1* mutant showed a lack of anthocyanin production, while wild-type and *OX1* plants produced strong anthocyanin accumulation (Figure 3K).

As the *pft1* mutant showed attenuated JA defense gene expression, we also investigated whether SA-responsive defense genes showed any differential expression in the mutant. Interestingly, the SA-responsive defense genes *PR1*, *PR5*, and *BGL2*, the phytoalexin biosynthesis gene *PAD3*, and the transcriptional activator *WRKY33* all showed attenuated induction in

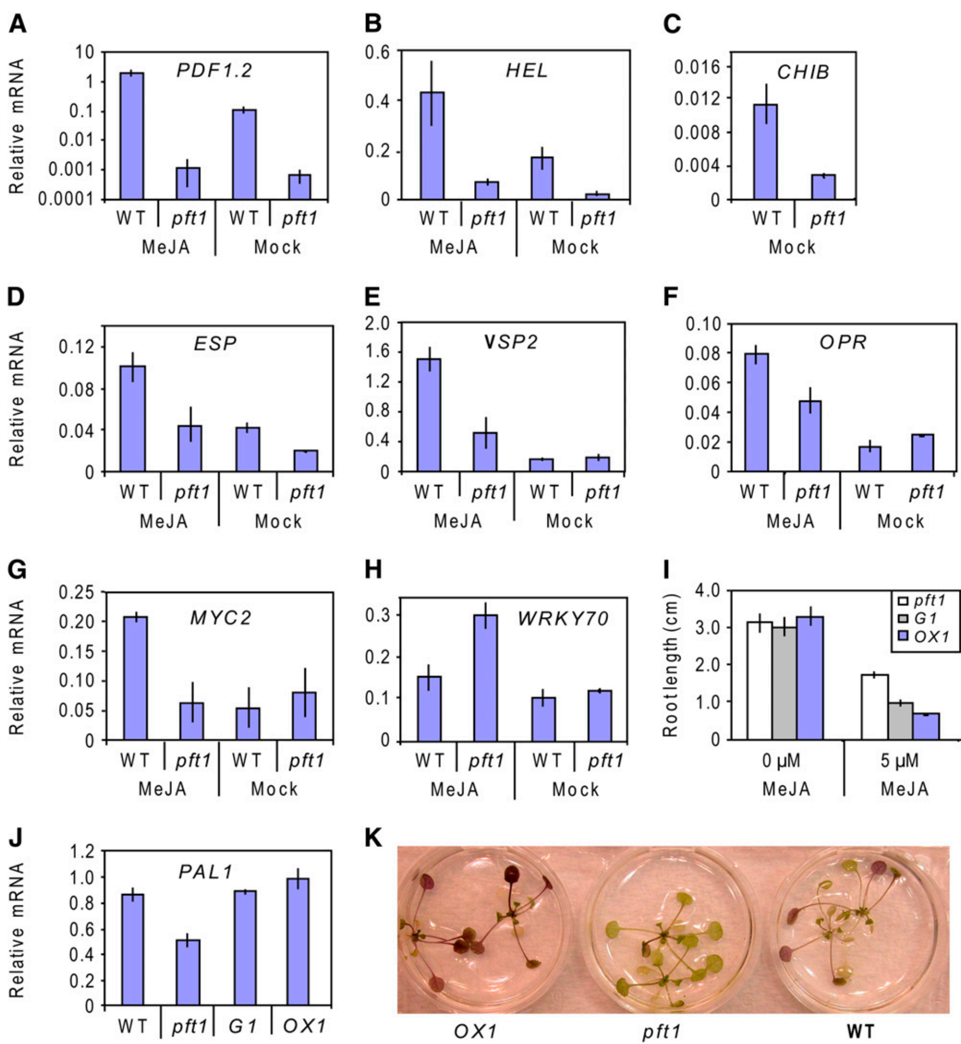

Figure 3. PFT1 Is a Positive Regulator of JA-Dependent Defenses in *Arabidopsis*.

(A) to **(H)** Expression from JA-responsive genes *PDF1.2*, *HEL*, *CHIB*, *ESP*, *VSP*, *OPR*, *MYC2*, and *WRKY70* was examined by quantitative RT-PCR in wild-type (Col-0) and *pft1* mutants 6 h following MeJA treatment.

(I) The root lengths of *pft1*, *OX1*, and *G1* seedlings germinated on half-strength Murashige and Skoog (MS) media containing either 0 or 5 μM of MeJA were measured 6 d after germination. Error bars represent the SE from three independent experiments that included ~10 seedlings each.

(J) Gene expression of *PAL1* was examined in 3- to 4-week-old, MeJA-treated wild-type (Col-0), *pft1*, *G1* (genomic complement) , and *OX1* (*35S:PFT1*) plants. The expression data were normalized relative to the expression level of *β-ACTIN* (See Methods) and presented logarithmically for *PDF1.2*. Error bars in **(A)** to **(H)** and **(J)** represent SE from three independent experiments that contained at least 20 plants each.

(K) To visualize anthocyanin accumulation, 2-week-old soil-grown plants of wild-type, *pft1*, and *OX1* were incubated in water until visible anthocyanin developed in the leaves.

[See online article for color version of this figure.]

the *pft1* mutant relative to the wild-type, *G1*, and *OX1* plants after treatment with SA (Figures 4A to 4E). Together, these results suggest that PFT1 is a positive regulator of defense gene expression in *Arabidopsis*.

We noted that in the experiments described above, defense gene expression was only analyzed in the leaf tissue where disease symptoms were scored. Because *F. oxysporum* infects the plants through the roots, we also examined the transcript levels of the same defense genes from the shoot by performing quantitative RT-PCR experiments in the roots of *F. oxysporum*–inoculated *pft1* mutant and wild-type plants. For all defense genes tested, with the exception of *HEL*, no difference in basal expression levels was observed in the *pft1* roots. In addition, no significant upregulation of these genes in the roots of either *pft1* or the wild type by *F. oxysporum* was evident (see Supplemental Figure 3 online). *HEL*, on the other hand, showed increased expression in wild-type roots after *F. oxysporum* infection, but both the basal and *F. oxysporum*–responsive expression level

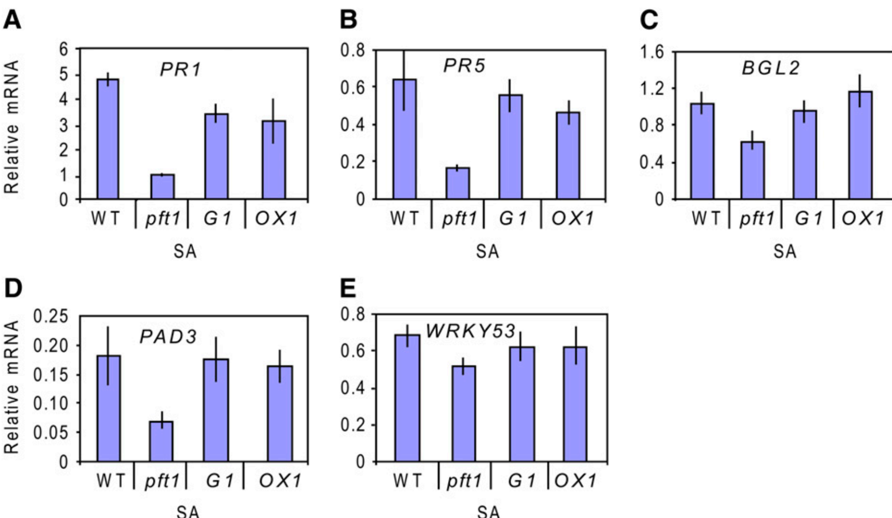

Figure 4. PFT1 Is a Positive Regulator of SA-Responsive Defense Gene Expression.

Expression of *PR1*, *PR5*, *BGL2*, *PAD3*, and *WRKY53* was examined by quantitative RT-PCR in wild-type (Col-0), *pft1*, *G1*, and *OX1* plants 24 h following SA treatment. The expression data were normalized relative to *β-ACTIN* expression (see Methods). Error bars represent SE from three independent experiments that contained ∼20 plants each.
[See online article for color version of this figure.]

was lower in *pft1* roots than the basal wild-type expression level (see Supplemental Figure 3 online). We also conducted quantitative RT-PCR experiments using the shoot tissue of *F. oxysporum*–infected wild-type and *pft1* plants. These analyses also showed a significantly lower expression of defense genes in *pft1* and confirmed the earlier results from the SA and MeJA quantitative RT-PCR experiments (see Supplemental Figure 3 online). We therefore concluded that the *pft1*-mediated *F. oxysporum* resistance occurs independently from elevated defense gene expression in either the roots or the shoots.

F. oxysporum Resistance in *pft1* Is Associated with Attenuated Jasmonate Signaling

As defense against leaf-infecting necrotrophic pathogens requires intact JA signaling, the increased susceptibility of the *pft1* mutant to *A. brassicicola* and *B. cinerea* was most likely due to the attenuated expression of JA-associated defense genes (Thomma et al., 1998, 1999; Zhou et al., 1999). However, the increased *F. oxysporum* resistance observed in the *pft1* mutant despite reduced defense gene expression suggests that an alternative resistance mechanism was operating against this pathogen. To identify the genes that may be contributing to increased *F. oxysporum* resistance in the *pft1* mutant on a genome-wide scale, we performed a microarray experiment using the Affymetrix ATH1 Genome Array, using four independent replicates of *pft1* and wild-type *Arabidopsis* leaves collected 2 d after either a mock treatment or inoculation with *F. oxysporum*. To analyze the microarray data, we performed a two-way analysis of variance (ANOVA; $P < 0.05$) on the entire data set with the inclusion of the Benjamini and Hochberg false discovery rate to reduce the number of potential false positives in our results.

This stringent analysis identified 39 genes that were differentially expressed after *F. oxysporum* treatment, irrespective of the genotype, and 284 genes that were differentially expressed between the genotypes, irrespective of the treatment (see Supplemental Tables 1 and 2 online). Therefore, the genotype had a more substantial effect in this experiment than the *F. oxysporum* treatment. While the number of differentially expressed genes from the *F. oxysporum* treatment was quite low, there were several defense genes found to be induced, signifying that the infection was successful (see Supplemental Table 1 online). Of the 39 *F. oxysporum* differentially expressed genes, 22 genes were also identified as being differentially expressed between *pft1* and the wild type (Table 1; see Supplemental Table 3 online). All except for one of the 22 genes were found to be induced by *F. oxysporum* in both genotypes, but their expression was significantly lower in *pft1* than the wild type under both mock and infected conditions (Table 1). Interestingly, a number of these genes were found to have roles in JA biosynthesis and signaling. Included in the list was the JA carboxyl methyltransferase as well as the JA ZIM-domain gene (*JAZ9*) (Seo et al., 2001; Chini et al., 2007; Farmer, 2007; Thines et al., 2007). Previously mentioned JA-associated genes from the quantitative RT-PCR analysis, such as *PDF1.2* and *HEL*, also appeared in the list as well as other defense-related genes, such as a putative thionin and a lectin family protein.

Analysis of the list using publicly available microarray data revealed a strong enrichment of JA-induced genes, with 14 out of the 22 genes showing a >1.5-fold induction by MeJA and eight out of the 22 genes showing an induction >10-fold (Zimmermann et al., 2004). However, this is probably an underestimate of the proportion of JA-induced genes identified here, as the expression data from the publicly available arrays is only recorded from plants sampled at an early time point after JA treatment.

Table 1. Genes Identified from the Microarray Experiment That Are Differentially Expressed Both between the Wild-Type and *pft1* Genotypes and Also after *F. oxysporum* Treatment (Two-Way ANOVA, P < 0.05 Adjusted by the False Discovery Rate)

AGI Locus	P Value	Infected/Mock	*pft1*/Wild Type	AGI Description	Jasmonate Inducibility
AT1G19640	0.0006	2.5266	0.6016	Jasmonic acid carboxyl methyltransferase (JMT)	**10.46**
AT4G24340	0.0009	2.6703	0.2733	Phosphorylase family protein	**11.45**
AT4G24350	0.0009	2.2642	0.3178	Phosphorylase family protein	**11.45**
AT3G05730	0.0023	2.1376	0.5933	Defensin-like protein	0.92
AT1G70700	0.0031	1.8562	0.5318	Jasmonate Zim-domain protein 9 (JAZ9)	**28.95**
AT1G66100	0.0036	1.8243	0.3275	Thionin, identical to thionin 2.4 precursor	**14.09**
AT3G04720	0.0043	4.4631	0.0841	Hevein-like protein (HEL)	1.15
AT5G24420	0.0068	2.1219	0.6044	Glucosamine/galactosamine-6-phosphate isomerase-related	**33.24**
AT1G06830	0.0134	1.5713	0.7332	Monothiol glutaredoxin-S11 (GRXS11)	0.67
AT1G11580	0.0160	1.8307	0.6279	Pectin methylesterase (ATPMECRA)	**1.87**
AT5G61160	0.0198	6.5067	0.1410	Anthocyanin 5-aromatic acyltransferase 1 (AACT1)	**2.62**
AT5G44420	0.0198	32.7145	0.0125	Plant defensin 1.2a (PDF1.2a)	1.05
AT3G16530	0.0198	3.7444	0.2426	Legume lectin family protein	1.03
AT1G19670	0.0209	2.7618	0.3069	Chlorophyllase 1 (ATCHL1)/coronatine-induced protein 1	**30.83**
AT5G23820	0.0209	1.3360	0.6500	MD-2–related lipid recognition domain-containing protein	**2.09**
AT1G69370	0.0210	1.4756	0.7482	Chorismate mutase3 (CM3)	**3.59**
AT3G62410	0.0256	1.1571	0.8770	CP12 domain-containing protein (CP12-2)	0.61
AT5G40610	0.0326	1.3079	0.7687	Glycerol-3-phosphate dehydrogenase	1.00
AT4G13410	0.0366	1.7372	0.6285	Cellulose synthase like protein (ATCSLA15)	**18.97**
AT2G03980	0.0366	1.2918	0.6883	GDSL-motif lipase/hydrolase family protein	**4.24**
AT5G02940	0.0397	1.4524	0.7374	Phosphotransferase-related protein	**4.60**
AT4G01450	0.0443	0.7104	0.7634	Nodulin MtN21 family protein similar to MtN21	1.31

Values listed under the Infected/Mock and *pft1*/Wild Type columns are the normalized ratios obtained from the entire microarray data set. Analysis of publicly available microarray data revealed an enrichment of JA-induced genes within the list. Values listed under the Jasmonate Inducibility column indicate the expression ratio after MeJA treatment according to Genevestigator (Zimmermann et al., 2004) (values in bold represent genes >1.5-fold induced). AGI, Arabidopsis Genome Initiative.

Therefore, well-known JA-responsive genes, such as *PDF1.2* and *HEL*, which respond more slowly to JA, are not indicated in this comparison as being JA induced. However, one particularly interesting gene that was identified was the At *CLH1* gene (also known as *CORONATINE INDUCED PROTEIN1*), that encodes a pathogen- and JA-inducible chlorophyllase (Kariola et al., 2005). Kariola et al. (2005) found that a reduction in At *CLH1* expression, as can be seen in the *pft1* mutant, resulted in a decrease in JA defenses and an increased susceptibility to *A. brassicicola*. The microarray results suggest that infection with *F. oxysporum* induces a number of JA-associated genes and that these genes have reduced expression in the *pft1* mutant. This suggests that an attenuated JA signaling observed in the *pft1* mutant might be responsible not only for the reduced *F. oxysporum* symptom development but also the loss of basal resistance to the leaf-infecting necrotrophic pathogens.

PFT1 Function Is Conserved in Plants

PFT1 is a single-copy gene in *Arabidopsis* with homologs in diverse plant species (Hecht et al., 2005; Bäckström et al., 2007). The conserved nature of the PFT1 protein indicates the possibility that the role of PFT1 homologs in disease and development might also be conserved in other plants. To test this possibility, we cloned a *PFT1* cDNA homolog named here as Ta *PFT1* from wheat (*Triticum aestivum*) and introduced this cDNA under the control of the cauliflower mosaic virus 35S promoter into the *Arabidopsis pft1* mutant. As shown in Figure 5A for the *PDF1.2* gene, a high level of Ta *PFT1* expression in the *pft1-2* mutant background has led to a significant increase in JA-dependent defense gene expression. The *35S:TaPFT1*-expressing *pft1* mutant lines also displayed a lesion-mimic phenotype (Figure 5B), possibly due to constitutive activation of defenses. This lesion-mimic phenotype was particularly visible in the rosette leaves after bolting. In the plants grown under LD conditions (16 h), this lesion mimic phenotype was aggravated, with older leaves dying prematurely in parallel to the development of multiple shoots (Figure 5B). The *35S:TaPFT1*/*pft1* lines also showed increased *F. oxysporum* susceptibility (Figures 5C and 5D) and early flowering phenotypes (Figures 5E and 5F), suggesting that Ta *PFT1* complements the defense and developmental defects of the *pft1* mutant. Although further experiments are required to fully assess the potential roles of PFT1 in wheat, these complementation experiments in *Arabidopsis* suggest that the role of PFT1 in both plant defense and the control of flowering may be conserved across diverse plant species.

The Role of Mediator Subunits in Plant Defense and Flowering Time Control

Given that Mediator is a multiprotein complex, the loss of PFT1 function could affect the overall function of Mediator. If this was

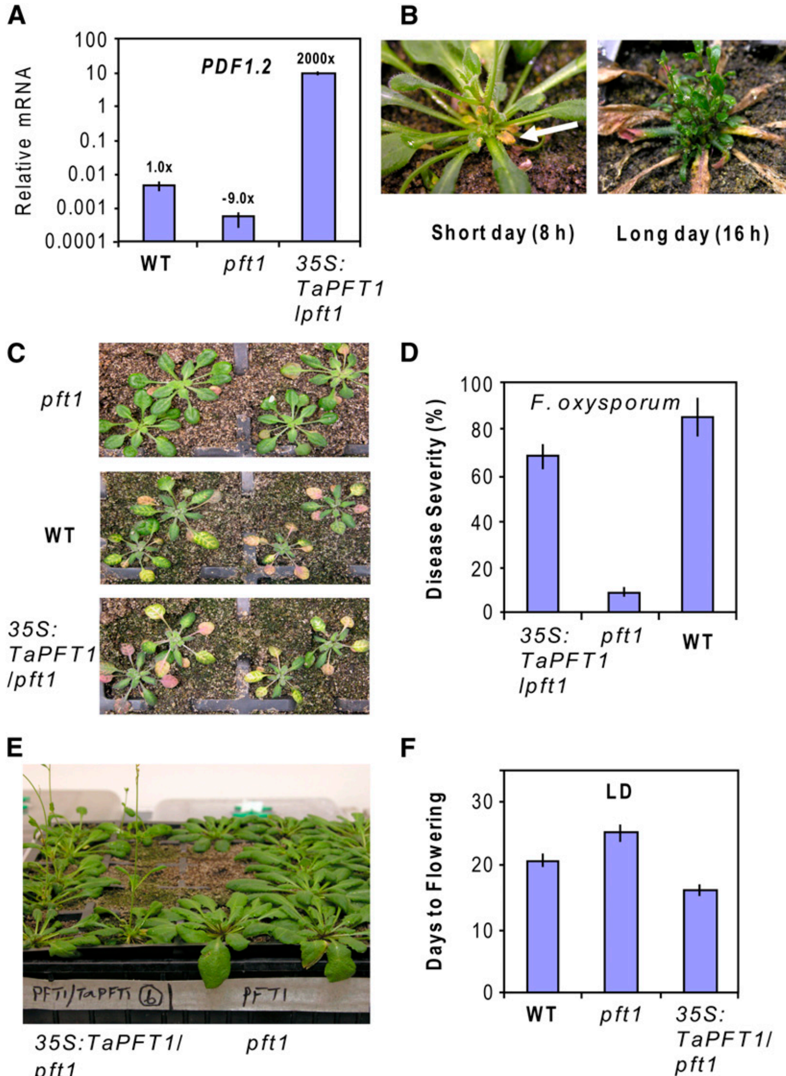

Figure 5. PFT1 Function Is Conserved in Plants.

(A) Transgenic expression of Ta *PFT1*, a PFT1 homolog from wheat, in the *pft1* mutant background complements the compromised defense gene expression.

(B) The *35S:TaPFT1/pft1* line shows spontaneous lesion development (arrow).

(C) and **(D)** Expression of Ta *PFT1* complements the increased *F. oxysporum* resistance of the *pft1* mutant.

(E) and **(F)** Expression of Ta *PFT1* complements the delayed flowering phenotype of *pft1*. *PDF1.2* transcript levels were examined by quantitative RT-PCR, and the expression data were normalized relative to *β-ACTIN* expression and presented logarithmically.

Numbers on each bar in **(A)** represent fold difference in expression of *PDF1.2* in untreated plants of *pft1* and *35S:TaPFT1/pft1* relative to untreated wild-type plants. Error bars represent SE from three independent replicates that contained ~20 plants each. *F. oxysporum* inoculation experiments were conducted as described above, and symptom development was scored 10 d after inoculation.

the case, then inactivation of other Mediator subunits could lead to similar defense and developmental phenotypes observed in the *pft1* plants. To test this hypothesis, we isolated homozygous T-DNA insertion lines for 11 individual *Arabidopsis* Mediator subunit mutants publicly available in the SALK *Arabidopsis* T-DNA insertion collection (see Methods). Of the Mediator mutants tested, only an insertion in the MED8 subunit (Figure 6A; see Supplemental Figure 4 online) produced an *F. oxysporum* resistance phenotype that was comparable to that of the *pft1* mutant in terms of delayed symptom development (Figures 6B and 6C) and increased survival rates (see Supplemental Figure 5 online). In addition, the *med8* mutant had increased susceptibility to *A. brassicicola* with 42% of the inoculated leaves showing relatively large chlorotic lesions (Figure 6D) as compared with 21% of wild-type leaves that showed some degree of chlorosis in these experiments.

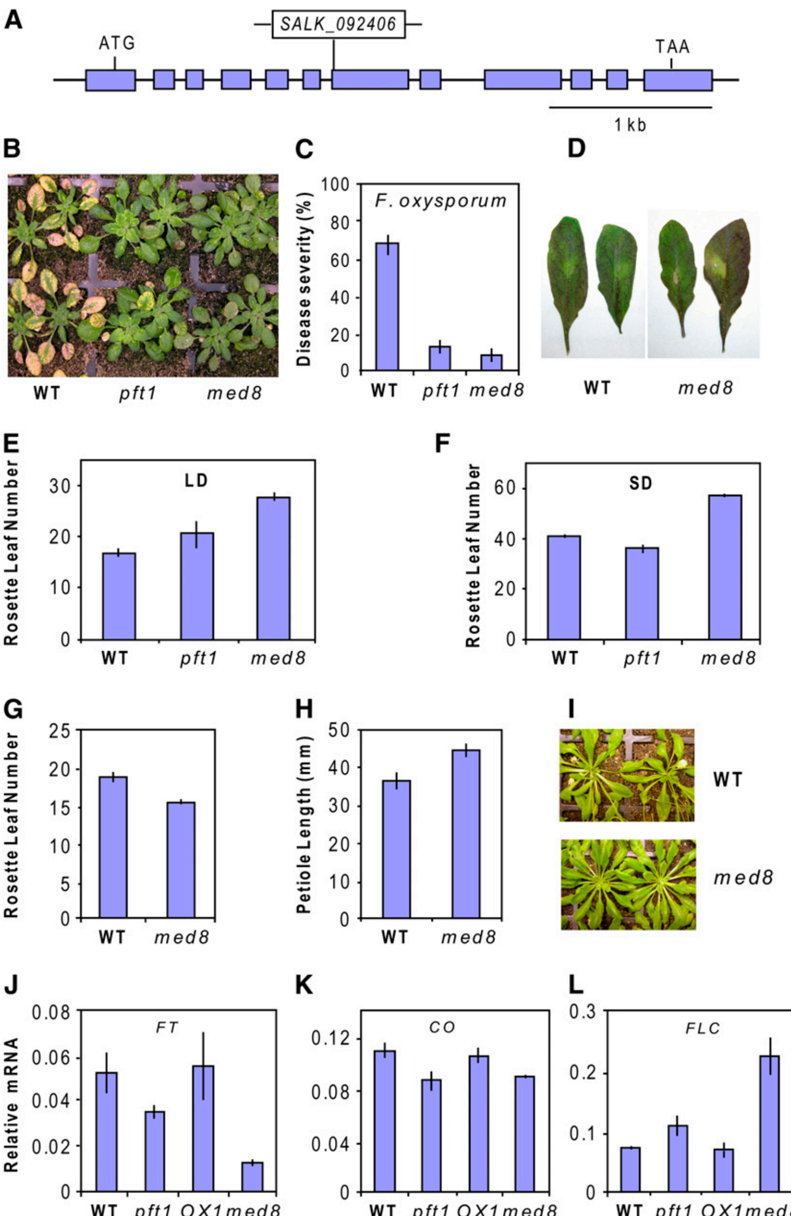

Figure 6. MED8 Is a Regulator of Plant Defense and Flowering.

(A) Schematic representation of the *MED8* gene and the location of the T-DNA insertion (SALK_092406). Introns (solid line) and exons (boxes) are indicated.

(B) and **(C)** The *med8* mutant shows increased resistance to *F. oxysporum*.

(D) The *med8* mutant shows increased susceptibility to *A. brassicicola*.

(E) and **(F)** The *med8* mutant shows delayed flowering under both LDs and SDs (expressed as the number of rosette leaves at the time of bolting).

(G) to **(I)** The *med8* mutant has reduced petiole lengths and an increased vegetative leaf number compared with the wild type at equivalent growth stages, shown in **(I)**. Measurements for **(G)** and **(H)** were taken by counting the total leaves from 10 wild-type and *med8* plants that were 8 weeks old. Flowering measurements were taken from two independent experiments with 18 plants per line in each experiment. The rosette leaf number was recorded when the shoot bud had extended 5 mm.

(J) to **(L)** The transcript levels of *FT*, *CO*, and *FLC* were examined by quantitative RT-PCR and the expression data normalized relative to *β-ACTIN*. Error bars represent SE from three replicates of 30 plants each.

Interestingly, the *med8* mutant also demonstrated an altered flowering time with a strong delay in flowering under both short-day (SD) and LD conditions (Figures 6E and 6F). The *med8* mutant also possessed an increased number of leaves and shorter petioles under vegetative conditions, giving it a distinctive phenotype (Figures 6G to 6I). In addition to *med8*, we also measured the flowering time of *pft1* as a comparison. Our results confirmed the results of Cerdán and Chory (2003), with a small but statistically significant ($P < 0.05$) decrease and increase in flowering time under SD and LD, respectively, when determined by rosette leaf number (Figures 6E and 6F), and an increased flowering time under both SD and LD when determined by the number of days to bolting (see Supplemental Figure 6 online).

To explore the molecular mechanism behind the late flowering phenotype of *med8*, we also quantified the expression of the key flowering genes, *FLOWERING LOCUS T* (*FT*) and *CONSTANS* (*CO*), which positively regulate flowering, and *FLOWERING LOCUS C* (*FLC*), which negatively regulates flowering (reviewed in Farrona et al., 2008) in LD-grown *pft1* and *med8* plants (Figures 6J to 6L). Cerdán and Chory (2003) previously reported reduced *FT* and *CO* expression in *pft1*, and our results confirmed this finding. We also found reduced expression of the floral promoting genes *FT* and *CO* in *med8* compared with the wild type. In addition, we found increased expression of the floral repressor *FLC* in both *pft1* and *med8.* Furthermore, the levels of *FT* and *FLC* expression in *pft1* and *med8* correlated well with the severity of the flowering delay seen in these two mutants, with the later flowering *med8* having a noticeably lower and higher level of *FT* and *FLC* expression, respectively, than *pft1* and the wild type (Figures 6J and 6L). Together, these experiments suggest that MED8, similar to PFT1, is a regulator of both plant defense and flowering time.

Genetic Evidence for the Independent and Additive Functions of PFT1 and MED8

As the *pft1* and *med8* mutants appear to similarly affect both flowering time and pathogen resistance, we sought to determine whether these two mutations act independently to produce similar phenotypes. To test this possibility genetically, we constructed a *pft1 med8* double mutant and analyzed its defense and flowering time phenotypes together with those of the *pft1* and *med8* single mutants. First, we looked at the expression of *PDF1.2* in the double and single mutants after treatment with MeJA. We found that the expression of *PDF1.2* was slightly lower in both untreated and 6-h MeJA-treated *med8* plants than in similarly treated wild-type plants (Figure 7A). However, in the *pft1 med8* double mutant, *PDF1.2* expression was greatly reduced, with the expression level in the double mutant being ~300- and 370-fold less in the MeJA-treated and untreated plants, respectively, than similarly treated *pft1* plants (Figure 7A). Inoculation of the *pft1 med8* double mutant with *F. oxysporum* also revealed a relatively small but additive increase in resistance relative to the individual single mutants (Figures 7B and 7C). At 24 d after inoculation, the increased resistance of the *pft1 med8* double mutant was more evident, with a noticeable difference in the survival rate and overall vigor of the double mutant compared with the *pft1* and *med8* lines (Figure 7C).

In addition, we compared the flowering time of the double mutant to the wild-type and the single *pft1* and *med8* mutants. We found that the *pft1 med8* double mutant had a similar number of leaves at flowering under LD to the *pft1* mutant (see Supplemental Figure 7 online). However, the double mutant flowered later than *pft1* and *med8* when flowering time was measured by days to flowering under LD, suggesting an additive effect on flowering time (Figure 7D). The stronger effects of the *pft1 med8* double mutant on flowering time and defense than either of the single mutants suggest that the *pft1* and *med8* mutations might be affecting these two phenotypes by independent and additive mechanisms.

DISCUSSION

The Mediator complex was first purified from yeast in the early 1990s (Kim et al., 1994). Subsequent studies have discovered that the Mediator complex is an essential part of the transcriptional machinery in all eukaryotes (Bourbon, 2008). Surprisingly, the existence of the Mediator complex in plants has only recently been shown in *Arabidopsis* (Bäckström et al., 2007). Using a biochemical purification strategy, Bäckström et al. (2007) identified 21 conserved subunits and six plant-specific Mediator subunits. Two of the Mediator subunits identified by Bäckström et al. (2007), MED14 and MED25, were previously studied plant proteins, SWP1 and PFT1, which are involved in regulating leaf cell number in aerial organs and phytochrome signaling, respectively (Autran et al., 2002; Cerdán and Chory, 2003). More recently, another Mediator subunit, MED21, has been shown to be required for resistance to necrotrophic pathogens in *Arabidopsis* (Dhawan et al., 2009), although the mechanism of this resistance is currently unknown.

In this article, we show that in addition to its known role in phytochrome signaling, PFT1 is also an important component of the plant's basal defense and is required for uncompromised expression of JA-dependent defenses as well as resistance to necrotrophic fungal pathogens, such as *A. brassicicola* and *B. cinerea* (Figures 1 to 4). Interestingly, PFT1 is also essential for susceptibility to the root-infecting fungus *F. oxysporum*, which is thought to use the host JA pathway to promote host senescence and necrosis (Thatcher et al., 2009).

JA-dependent defense genes and the phytoalexin camalexin have previously been shown to be active against necrotrophic pathogens, such as *A. brassicicola* and *B. cinerea* (Thomma et al., 1998, 1999; Zhou et al., 1999). Therefore, it is likely that the attenuated expression of JA-responsive defense genes as well as *PAD3* is the cause of increased *A. brassicicola* and *B. cinerea* susceptibility in *pft1*.

The mechanism(s) behind the increased *F. oxysporum* resistance was initially less apparent. However, as recently reported, the *coi1* mutant with compromised JA signaling and JA-dependent gene expression shows a remarkable increase in resistance to this pathogen (Thatcher et al., 2009). Through stringent analysis of the microarray data, we identified that a large proportion of the genes that were differentially expressed after infection by *F. oxysporum* treatment, as well as between the wild-type and *pft1* genotypes, were genes that are also regulated by

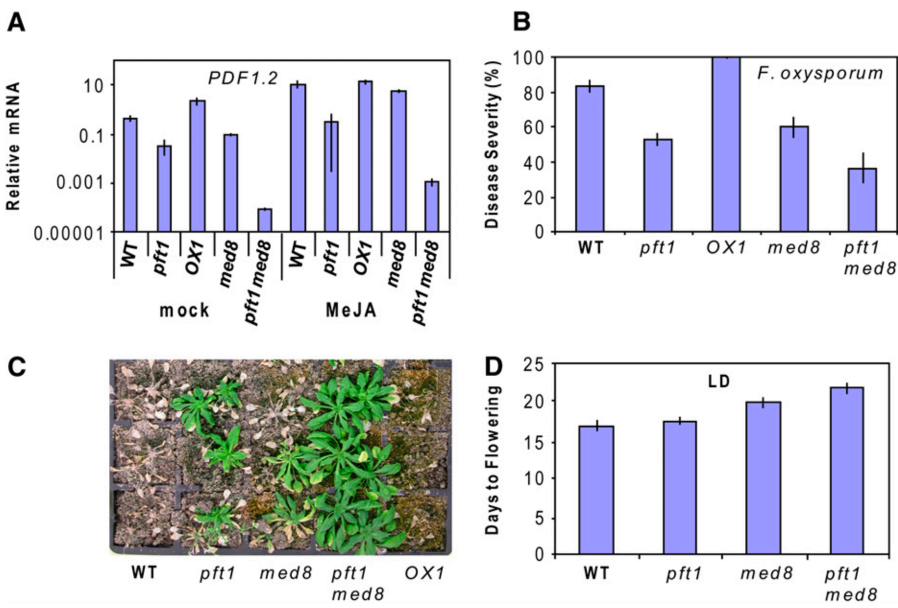

Figure 7. The *pft1 med8* Double Mutant Shows an Additive Increase in Both the Flowering Time and the Pathogen Defense Phenotypes Compared with the *pft1* and *med8* Single Mutants.

(A) The expression of *PDF1.2* is greatly reduced in the double mutant relative to the wild type and *pft1* and *med8* single mutants in both untreated and MeJA-treated plants.

(B) and **(C)** The double mutant also shows reduced symptoms and greater survival after inoculation with *F. oxysporum*.

(D) The flowering time of the double mutant is more delayed than the individual single mutants. *PDF1.2* transcript levels were examined by quantitative RT-PCR, and the expression data were normalized relative to *β-ACTIN* expression and presented logarithmically. The disease symptoms and survival were assessed 14 and 24 d after inoculation, respectively. The error bars from the *F. oxysporum* inoculation and defense gene experiments are the SE from three replicates of ~30 plants each, whereas the error bars for the flowering time experiment are the SE from 18 plants and are representative of two independent replicated experiments.

[See online article for color version of this figure.]

JA. Importantly, all of the JA-regulated genes that were found to be induced by *F. oxysporum* in the wild type had significantly reduced expression in the *pft1* mutant, under both mock and inoculated conditions (Table 1). Therefore, it is likely that a reduction in JA-responses in *pft1* may be providing an increased tolerance to *F. oxysporum*, as is seen with the *coi1* mutant (Thatcher et al., 2009).

Interestingly, we also found a reduction in SA defense gene expression in *pft1* from the quantitative RT-PCR experiments. The SA and the JA signaling pathways have been reported to act in a mutually antagonistic manner (Spoel et al., 2003; reviewed in Kazan and Manners, 2008; Koornneef and Pieterse, 2008); therefore, reduced expression from both the JA- and SA-associated defense genes in *pft1* was not expected. However, given the fact that *Arabidopsis* contains >1500 transcription factors and only a limited number of Mediator subunits (Bäckström et al., 2007), it is plausible that PFT1 interacts with several activators/repressors involved in multiple aspects of the disease response pathways. If this was the case, then removal of PFT1 could potentially result in the suppression of both SA and JA defenses. The possibility that PFT1 is a convergence point for the activation of both SA and JA defense signaling within the Mediator complex is an intriguing hypothesis that would justify further investigation. However, we noted that the differential expression of the SA-associated defense genes could not be detected under basal conditions, with a difference seen only after treatment with SA. Therefore, the regulation of JA defense genes in *pft1*, which occurs under both basal and JA-treated conditions in the *pft1* mutant, may be considered to be the primary role of PFT1.

The PFT1 protein is highly conserved across diverse eukaryotes. For example, PFT1 shows sequence similarity to the *Drosophila melanogaster* and human MED25 proteins, particularly in the N-terminal vWF-A domain but less so in the C-terminal regions of the protein. Interestingly, like the *Arabidopsis* PFT1, both the *Drosophila* and human MED25 proteins appear to have a function in host defense. RNA interference–mediated suppression of *Drosophila MED25* results in attenuated induction of the *AttA* gene encoding the antibacterial peptide attacin in response to lipopolysaccharide treatment (Kim et al., 2004). In addition, the human MED25 has been shown to be the cellular target of both VP16, the well-studied activator of herpes simplex virus (Mittler et al., 2003; Yang et al., 2004), as well as the activator IE62, from the closely related Varicella Zoster virus, the virus responsible for chicken pox and shingles (Yang et al., 2008). By functionally complementing the defensive and developmental phenotypes of the *Arabidopsis pft1* mutant with a wheat ortholog, we obtained initial evidence that the function of PFT1 is also conserved in plants. In addition to heightened defense gene expression and

accelerated flowering relative to the *pft1* mutants, the *35S:TaPFT1/pft1* plants displayed a spontaneous lesion phenotype. Transgenic expression of the vWF-A domain of the BONZAI/COPINE1 protein of *Arabidopsis* in transgenic tobacco (*Nicotiana tabacum*) activates defense responses and also produces a lesion mimic phenotype (Liu et al., 2005), suggesting that the lesion mimic phenotype we observed in Ta *PFT1*-expressing *pft1* mutant plants could be mediated by the vWF-A domain of Ta PFT1.

In addition to PFT1, our study implicated another Mediator subunit, MED8, in both plant defense and flowering time regulation. The MED8 subunit has been found to be located within the head module of the Mediator complex in other eukaryotes (Kang et al., 2001), while the location of PFT1 within the Mediator complex is currently unknown. However, we noted that the *med8* mutant also possesses additional phenotypes that are not present in the *pft1* mutant. For instance, the *med8* mutant shows a stronger delay in flowering time than *pft1* under both LD and SD conditions. Our results also indicated only a slight reduction in *PDF1.2* expression in the *med8* mutant (Figure 7A). Therefore, it is possible that the effect of the *med8* mutation on resistance might also be due to other effects, such as delayed senescence (Schenk et al., 2005; Thatcher et al., 2009), a natural consequence of delayed flowering. However, the effect of the *med8* mutation on *PDF1.2* expression was more visible in the *pft1* mutant background, as the *pft1 med8* double mutant showed an additional decrease in *PDF1.2* expression over that of the *pft1* mutant. The *med8* mutation also had an additive effect on both *F. oxysporum* resistance and flowering time phenotypes of the *pft1* mutant. These results suggest that these two Mediator subunits may act independently within the Mediator complex to affect similar developmental and defensive processes as proposed in the model given in Figure 8.

Currently, which activator(s) might be involved in relaying JA and phytochrome signals to the Mediator complex through PFT1 is not known. However, recent studies revealed that COI1-dependent ubiquitination of JAZ repressors, which interact with the transcriptional regulator MYC2, modulates downstream transcriptional responses in the JA signaling pathway (Chini et al., 2007). Therefore, one can speculate that MYC2 and/or some of the MYC2-regulated transcriptional activators (Dombrecht et al., 2007) might be involved in relaying JA signals to the transcriptional machinery through PFT1. Because JA signaling is known to act in a positive feedback loop (Kazan and Manners, 2008), inactivation of PFT1 attenuates the JA-dependent expression of a number of JA biosynthesis and signaling genes, including *MYC2* and a number of MYC2-regulated genes, such as *JAZ9*. Therefore, we tentatively place PFT1 downstream from the COI1-JAZ-MYC2 complex in our model given in Figure 8.

Similar to PFT1 and MED8, it has been recently shown that MED21, which is located in the middle module of the Mediator complex (Boube et al., 2002), is also required for resistance to necrotrophic pathogens in *Arabidopsis* (Dhawan et al., 2009). While *med21* shares similar pathogen phenotypes to *pft1* and *med8*, it is unknown whether MED21 possesses altered defense gene expression or flowering time. T-DNA insertions of *MED21* show embryo lethality, and an altered flowering time phenotype has not been reported for the *MED21* RNA interference lines

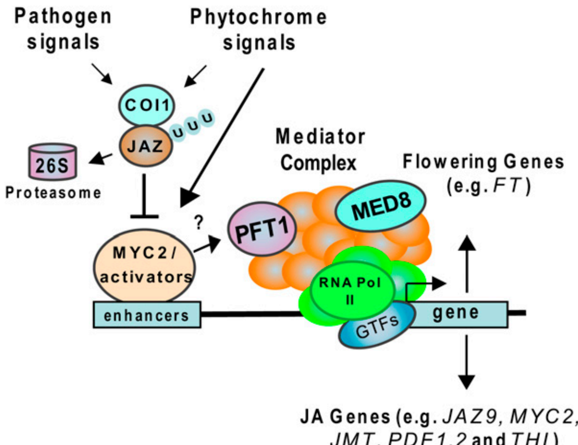

Figure 8. The Plant Mediator Complex Is a Key Regulator of JA-Dependent Plant Defense and Flowering Time.

The *Arabidopsis* Mediator subunit PFT1, possibly by acting downstream from a COI1-JAZ-MYC2 complex, integrates JA-dependent defense and developmental (e.g., phytochrome) cues by coordinating information from DNA-bound activators. This enables the RNA polymerase II complex to initiate the transcription of genes involved in flowering time and JA-dependent pathogen defense. GTFs, general transcription factors. [See online article for color version of this figure.]

(Dhawan et al., 2009). Nevertheless, our results together with those of Dhawan et al. (2009) suggest that at least three Mediator subunits are required for necrotrophic pathogen resistance in *Arabidopsis*.

Our discovery of PFT1 and MED8 as integrators of both plant defense and flowering time raises an intriguing question as to why these two seemingly different but important pathways may be linked. Similarly to what we found in *pft1*, it has been previously reported that many flowering time mutants also show altered defense gene expression (Wilson et al., 2005). Furthermore, the alteration of flowering times in pathogen-infected plants has been previously reported (Korves and Bergelson, 2003; Veronese et al., 2003). In addition, an intriguing link between phytochrome control and JA responses has recently been discovered (Moreno et al., 2009). The authors found impaired JA responses in the *phyB* mutant as well as in wild-type plants treated with far-red light. Therefore, the reason why the *pft1* mutant possesses reduced JA responses might be due to its involvement in phytochrome regulation. The involvement of MED8 in phytochrome regulation is currently unknown. However, it is plausible that the plant Mediator complex acts as a control panel in integrating both phytochrome and defense-related signals from multiple transcriptional regulators (Figure 8).

At the molecular level, the effect of these two Mediator subunits on defense and flowering time may be due to the involvement of Mediator in chromatin remodeling. In several cases, the Mediator complex has been shown to interact with chromatin modification complexes, such as SWI/SNF and histone deacetylases and acetylases (Sharma and Fondell, 2002; He et al., 2008; Malik and Roeder, 2008). The human MED25

protein has been shown to interact with histone acetylases (Black et al., 2006; Lee et al., 2007). Chromatin remodeling complexes are known to be functionally conserved evolutionarily (Farrona et al., 2008). Thus, if this function of MED25 is conserved between plants and animals, then one would expect that *Arabidopsis* PFT1 would also play a role in chromatin modification. SWP1/MED14 is known to interact with the transcriptional corepressor LEUNIG, which interacts with the histone deacetylase HDA19 (Gonzalez et al., 2007). Interestingly, a number of genes involved in chromatin modification and remodeling affect both flowering and plant defense (Wagner and Meyerowitz, 2002; He and Amasino, 2005; Zhou et al., 2005; Walley et al., 2008; March-Díaz et al., 2008; Wu et al., 2008).

Similar to our results with the *pft1* and *med8* mutants, a recent study found that the loss of activity in SPLAYED, one of the SWI/SNF classes of chromatin remodeling ATPases in *Arabidopsis*, leads to reduced JA-responsive defense gene expression and increased susceptibility to *B. cinerea* (Walley et al., 2008). The *syd* mutants also show defects in reproductive development and flowering time (Wagner and Meyerowitz, 2002). Similarly, chromatin modification by the histone deacetylases HDA19 and HDA6 is required for JA-responsive defense gene expression and resistance to necrotrophic pathogens (Zhou et al., 2005; Wu et al., 2008). Again, similarly to *pft1* and *med8*, loss-of-function mutations in the *HDA6* gene delays flowering (Wu et al., 2008). HDA6 is also known to interact with COI1 (Devoto et al., 2002), further linking JA responses to chromatin modification.

Finally, the Mediator subunit MED21, required for necrotrophic pathogen resistance, has been shown to interact with HUB1, an E3 ligase responsible for the ubiquination of H2B histones. The *hub1* mutant also shows alterations in flowering time (Dhawan et al., 2009). Although further research is required to determine whether perturbation of the Mediator complex in *pft1* and *med8* would compromise chromatin remodeling, overall, these studies support the view that multiple components of the plant transcriptional machinery are required for the regulation of both plant development, such as flowering time and pathogen defense.

In conclusion, our results reported here link two plant Mediator subunits as integrators of flowering time and JA-dependent defense-related signals to the transcriptional machinery. Future research should reveal new insights into the specific roles of the remaining Mediator subunits and help to advance our understanding of the transcriptional regulation of gene expression in plants.

METHODS

Plant Growth Conditions, Chemical Treatments, and Pathogen Inoculations

Plant growth conditions and MeJA and SA treatments were described previously (Schenk et al., 2000; Campbell et al., 2003; Anderson et al., 2004). Briefly, plants were grown in a controlled environment room with a temperature of 24°C and a light intensity of 150 μmol·m^{-2}·s^{-1}. Photosynthetically active radiation was supplied by high pressure metal halide lamps (Sylvania) and tungsten halogen lamps (Phillips). The red:far-red ratio was ~1.13, which is within 10% of the observed daylight. All plants used were in the *Arabidopsis thaliana* Col-0 background. Mutant lines used are listed in the Accession Numbers section at the end of Methods. Homozygous plants of *pft1*, *med8*, and the other Mediator subunit mutants were identified using the primer sequences given at http://signal.salk.edu/tdnaprimers.html and used in the experiments described here. The *pft1 med8* double mutant was created by pollinating an emasculated *med8* floral bud with the pollen from the *pft1.2* mutant. All treatments were performed on soil-grown 4- to 5-week-old plants, unless otherwise stated. The *Fusarium oxysporum* isolate used in this study was strain Fo5176 obtained from Roger Shivas, Queensland Plant Pathology Herbarium, Queensland Department of Primary Industries and Fisheries (QDPIandF), Brisbane, Australia. Inoculations were performed as described by Anderson et al. (2004) and Edgar et al. (2006). The *Alternaria brassicicola* (UQ4273) infection assays were performed as described previously (Campbell et al., 2003). *Botrytis cinerea* (BRIP25539) infection assays were performed in a similar manner to the *A. brassicicola* assays by harvesting spores from half-strength potato dextrose agar plates and pipetting 5-μL droplets (1 $\times$ 10^6 spores/mL) on mature *Arabidopsis* leaves.

Quantitative RT-PCR Expression Analyses

Quantitative RT-PCR experiments were done as described previously using the Applied Biosystems 7900HT Fast real-time PCR system in conjunction with SYBR Green fluorescence to detect transcript levels (McGrath et al., 2005). Briefly, for all data analysis, the PCR primer efficiency (E-value) of each primer pair in each reaction was calculated from the ΔRn values of each amplification plot using LinReg PCR software (Ramakers et al., 2003). Amplification plots were analyzed using a cycle threshold value of 0.2 across all experiments. Absolute gene expression levels relative to the previously validated reference genes *β-ACTIN2*, *β-ACTIN7*, and *β-ACTIN8* were used for each cDNA sample using the equation: relative abundance = (Egene^(–Ct gene))/(E*ACTIN*^(–Ct *ACTIN*)). The mean expression range of the reference gene was found to be within ±1 Ct across all experiments. Three biological replicates of mock and treated samples were used, and the average ratio of these values was used to determine the fold change in transcript level in treatment samples compared with control. The sequences of the primer pairs have been previously published (Anderson et al., 2004; McGrath et al., 2005; Dombrecht et al., 2007).

Flowering Time Measurements

Flowering time measurements were recorded from at least 18 plants per genotype that were grown in soil under either LD or SD conditions. Plants grown in LD conditions had 16 h of light at 28°C and a night period of 21°C. The plants grown in SD conditions had 8 h of light at 24°C and a night period of 21°C. Plants were grown in trays containing 30 (5 $\times$ 5 cm) cells and were separated into individual cells once the first true leaves had expanded. The rosette leaf number as well as days to flowering were recorded when the shoot bud had extended 5 mm. For quantitative RT-PCR analysis of flowering control genes, 30 plants of each genotype were grown in LD conditions for 4 weeks before being harvested at the end of the 16-h photoperiod. RNA was extracted, converted to cDNA, and used for quantitative RT-PCR analysis as described above.

Microarray Analysis

Wild-type and *pft1* plants were grown and inoculated as described above. Four independent biological replicates consisting of 20 plants each were root dipped in either water or a *F. oxysporum* spore suspension of 10^6 spores/mL in water and replanted in soil. The shoot material was harvested 48 h later and total RNA extracted using the RNeasy plant mini kit (Qiagen). The RNA was labeled, hybridized, washed, and scanned by the Australian Genome Research Facility onto 16 ATH1 GeneChip arrays and the resulting data analyzed using GenespringGX 7.3.1 (Agilent) as previously described (Dombrecht et al., 2007). Briefly, the raw CEL files

were normalized using the RMA algorithm, and then the resulting expression values were normalized per chip to the median across all chips. A two-way ANOVA was used to investigate differentially expressed genes between both the treatment and genotype. A P value cutoff of 0.05 as well as multiple testing correction using the Benjamini and Hochberg false discovery rate was applied to the data, and the significant genes in both treatment and genotype parameters were obtained. The microarray data have been submitted to the National Center for Biotechnology Information (NCBI) Gene Expression Omnibus (http://www.ncbi.nlm.nih.gov/geo) under accession number GSE15236.

Root Growth Inhibition and Anthocyanin Assays

Surface-sterilized *Arabidopsis* seeds were plated on half-strength MS medium (supplied with 5% sucrose and 0.7% Bacto Agar, pH 6.0) supplemented with either 0.01% ethanol (mock treatment) or 5 μM MeJA (Aldrich; solubilized in ethanol). Plates were incubated under continuous light at 22°C, and seedlings were monitored 6 d for root growth. For anthocyanin assays, 2-week-old *pft1*, wild-type, and *OX1* plants were detached from their roots and incubated in 6-well microtitre plates (Iwaki) containing distilled water for 3 weeks under SD conditions. Two independent experiments were performed with each containing four plants per genotype.

Generation of *35S:TaPFT1/pft1 Arabidopsis* Lines

The PFT1 protein of *Arabidopsis* (NP_173925.3) was used to search the homologous gene of wheat in The Institute of Genomic Research database using tBLASTn program. The search resulted in identification of the UniGene (Ta.39294) that is 61% identical to the *Arabidopsis* PFT1 protein. Ta.39294 is named here as Ta PFT1. To clone Ta *PFT1*, total RNA was isolated from young seedling of the wheat (*Triticum aestivum*) variety Kennedy using Promega SV total RNA isolation system. cDNA synthesis was done using the cDNA synthesis kit Superscript III (Invitrogen). The Ta *PFT1* cDNA was amplified using the following primers: 5′-CCCGGATCCCGGATTCGCGAGGGCGAG-3′ and 5′-CCCGGATCCACTCGCAATGCTCTGTAC-3′. The amplification product was cloned into the pBlunt vector (Invitrogen) and confirmed by sequencing. Ta *PFT1* was released by digesting the plasmid with *Bam*HI and cloned into the *Bam*H1-digested binary vector *pPCV91* (Strizhov et al., 1996), which was then mobilized into the *Agrobacterium tumefaciens* strain GV3101. The *pft1* mutant plants were transformed using the floral dip method, and the seeds collected from infiltrated plants were grown on half-strength MS medium containing 15 mg/L hygromycin (Sigma-Aldrich) to select the transformants. The presence of Ta *PFT1* was confirmed by PCR. Homozygous lines were used in gene expression and phenotypic analyses.

Accession Numbers

Sequence data from this article can be found in the Arabidopsis Genome Initiative or GenBank/EMBL databases under the following accession numbers: *PFT1* (At1g25540, NP_173925.3), *MED8* (At2g03070), *PDF1.2* (At5g44420), *CHIB* (At3g12500), *PR1* (At2g14610), *PR5* (At1G75040), *PAL1* (At2g37040), *MYC2* (At1g32640), *HEL* (At3g04720), *ESP* (At1g54040), *OPR* (At1g17990 and At1g18020), *BGL2* (At3g57260), *PAD3* (At3g26830), *WRKY70* (At3g56400), *WRKY53* (At4g23810), *iASK* (At5g26751), *FT* (At1G65480), *CO* (At5G15840), *FLC* (At5G10140), *β-ACTIN2* (At3g18780), *β-ACTIN7* (At5g09810), *β-ACTIN8* (At1g49240), and Ta *PFT1* (Unigene Ta.39294). The microarray data have been submitted to the NCBI Gene Expression Omnibus (http://www.ncbi.nlm.nih.gov/geo) under the accession number GSE15236. The following mutant lines were used: *pft1-2* (At1g25540, SALK_129555), *pft1-3* (SALK_059316), *med6* (At3g21350, SALK_055723C), *med8* (At2g03070, SALK_092406), *med9* (At1g55080, SALK_115775), *med10a* (At5g41910, SALK_115673), *med22b* (At1g07950, SALK_001024C), *med31* (At5g19910, SALK_051025), *med19a* (At5g12230, SALK_020936), *med32* (At1g11760, SALK_028490), *med33a* (At3g23590, SALK_119561), *med33b* (At2g48110, SALK_015532), and *med34* (At1g31360, CS87663).

Supplemental Data

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

Supplemental Figure 1. Confocal Microscopy and Fungal Quantification of *F. oxysporum*–Infected Wild-Type, *pft1*, and *OX1* Lines.

Supplemental Figure 2. Quantitative RT-PCR Expression of JA-Associated Genes in the Wild-Type, *G1*, and *OX1* Lines.

Supplemental Figure 3. The Expression of Defense Genes after *F. oxysporum* Infection in the Roots and Shoots.

Supplemental Figure 4. Disease Severity following the Inoculation of 11 Mediator Subunit Mutant Lines with *F. oxysporum.*

Supplemental Figure 5. The Survival of Wild-Type, *pft1*, *OX1*, *med8*, and the *pft1 med8* Double Mutant after Infection with *F. oxysporum*.

Supplemental Figure 6. The Flowering Phenotype of *pft1* and *med8*.

Supplemental Figure 7. The Flowering Phenotype of the *pft1 med8* Double Mutant.

Supplemental Table 1. The List of Genes Differentially Regulated by the *F. oxysporum* Treatment from the Microarray.

Supplemental Table 2. The List of Genes Differentially Regulated by the Genotype from the Microarray.

Supplemental Table 3. The List of Genes That Are Differentially Regulated by Both the Genotype and the *F. oxysporum* Treatment.

ACKNOWLEDGMENTS

B.N.K. and C.I.E. were supported by postgraduate scholarships from the Cooperative Research Centre for Tropical Plant Protection as well as the Grains Research and Development Corporation. K.K.K. was the recipient of a fellowship from the Department of Biotechnology of the Indian Government. We thank the ABRC for the seeds of *Arabidopsis* T-DNA insertion lines used in the study; Pablo Cerdán and Joan Chory for kindly providing the *pft1-1*, *OX1*, and *G1* seeds; Roger Shivas for the *F. oxysporum* and *B. cinerea* isolates used in the study; Christina Bakker and Carol Kistler for the isolation of homozygous lines of the *PFT1* T-DNA insertion lines; Christina Bakker for optimization of the *B. cinerea* inoculation assay; Rosemary White for assistance in confocal microscopy; Bruno Dombrecht for useful discussions at the early stages of this work; and Louise Thatcher, Donald Gardiner, and Timothy Fitzgerald for critical manuscript reading and useful discussions.

Received March 16, 2009; revised June 26, 2009; accepted July 25, 2009; published August 11, 2009.

REFERENCES

Alonso, J.M., et al. (2003). Genome-wide insertional mutagenesis of *Arabidopsis thaliana.* Science **301:** 653–657.

Anderson, J.P., Badruzsaufari, E., Schenk, P.M., Manners, J.M., Desmond, O.J., Ehlert, C., Maclean, D.J., Ebert, P.R., and Kazan, K. (2004). Antagonistic interaction between abscisic acid and jasmonate-ethylene signaling pathways modulates defense gene

expression and disease resistance in *Arabidopsis*. Plant Cell **16:** 3460–3479.

Autran, D., Jonak, C., Belcram, K., Beemster, G.T., Kronenberger, J., Grandjean, O., Inze, D., and Traas, J. (2002). Cell numbers and leaf development in Arabidopsis: A functional analysis of the STRUWWELPETER gene. EMBO J. **21:** 6036–6049.

Bäckström, S., Elfving, N., Nilsson, R., Wingsle, G., and Björklund, S. (2007). Purification of a plant Mediator from *Arabidopsis thaliana* identifies PFT1 as the Med25 subunit. Mol. Cell **26:** 717–729.

Berrocal-Lobo, M., and Molina, A. (2008). Arabidopsis defense response against *Fusarium oxysporum.* Trends Plant Sci. **13:** 145–150.

Black, J.C., Choi, J.E., Lombardo, S.R., and Carey, M. (2006). A Mechanism for coordinating chromatin modification and preinitiation complex assembly. Mol. Cell **23:** 809–818.

Boube, M., Joulia, L., Cribbs, D.L., and Bourbon, H.M. (2002). Evidence for a Mediator of RNA polymerase II transcriptional regulation conserved from yeast to man. Cell **110:** 143–151.

Bourbon, H.M. (2008). Comparative genomics supports a deep evolutionary origin for the large, four-module transcriptional Mediator complex. Nucleic Acids Res. **36:** 3993–4008.

Bourbon, H.M., et al. (2004). A unified nomenclature for protein subunits of Mediator complexes linking transcriptional regulators to RNA polymerase II. Mol. Cell **14:** 553–557.

Campbell, E.J., Schenk, P.M., Kazan, K., Penninckx, I.A., Anderson, J.P., Maclean, D.J., Cammue, B.P., Ebert, P.R., and Manners, J.M. (2003). Pathogen-responsive expression of a putative ATP-binding cassette transporter gene conferring resistance to the diterpenoid sclareol is regulated by multiple defense signaling pathways in Arabidopsis. Plant Physiol. **133:** 1272–1284.

Cerdán, P.D., and Chory, J. (2003). Regulation of flowering time by light quality. Nature **423:** 881–885.

Chini, A., Fonseca, S., Fernández, G., Adie, B., Chico, J.M., Lorenzo, O., García-Casado, G., López-Vidriero, I., Lozano, F.M., Ponce, M.R., Micol, J.L., and Solano, R. (2007). The JAZ family of repressors is the missing link in jasmonate signalling. Nature **448:** 666–671.

Devoto, A., Nieto-Rostro, M., Xie, D., Ellis, C., Harmston, R., Patrick, E., Davis, J., Sherratt, L., Coleman, M., and Turner, J.G. (2002). COI1 links jasmonate signalling and fertility to the SCF ubiquitin-ligase complex in Arabidopsis. Plant J. **32:** 457–466.

Dhawan, R., Luo, H., Foerster, A.M., AbuQamar, S., Du, H.-N., Briggs, S.D., Scheid, O.M., and Mengiste, T. (2009). HISTONE MONOUBIQUITINATION1 interacts with a subunit of the Mediator complex and regulates defense against necrotrophic fungal pathogens in *Arabidopsis*. Plant Cell **21:** 1000–1019.

Diener, A.C., and Ausubel, F.M. (2005). RESISTANCE TO FUSARIUM OXYSPORUM 1, a dominant Arabidopsis disease-resistance gene, is not race specific. Genetics **171:** 305–321.

Dombrecht, B., Xue, G.P., Sprague, S.J., Kirkegaard, J.A., Ross, J.J., Reid, J.B., Fitt, G.P., Sewelam, N., Schenk, P.M., Manners, J.M., and Kazan, K. (2007). MYC2 differentially modulates diverse jasmonate-dependent functions in *Arabidopsis*. Plant Cell **19:** 2225–2245.

Edgar, C.I., McGrath, K.C., Dombrecht, B., Manners, J.M., Schenk, P.M., Maclean, D.J., and Kazan, K. (2006). Salicylic acid mediates resistance to the vascular wilt pathogen *Fusarium oxysporum* in the model host *Arabidopsis thaliana.* Australas. Plant Pathol. **35:** 581–591.

Farmer, E.E. (2007). Jasmonate perception machines. Nature **448:** 659–660.

Farrona, S., Coupland, G., and Turck, F. (2008). The impact of chromatin regulation on the floral transition. Semin. Cell Dev. Biol. **19:** 560–573.

Gonzalez, D., Bowen, A.J., Carroll, T.S., and Conlan, R.S. (2007). The transcription corepressor LEUNIG interacts with the histone deacetylase HDA19 and Mediator components MED14 (SWP) and CDK8 (HEN3) to repress transcription. Mol. Cell. Biol. **15:** 5306–5315.

He, Q., Battistella, L., and Morse, R.H. (2008). Mediator requirement downstream of chromatin remodeling during transcriptional activation of CHA1 in yeast. J. Biol. Chem. **283:** 5276–5286.

He, Y., and Amasino, R.M. (2005). Role of chromatin modification in flowering-time control. Trends Plant Sci. **10:** 30–35.

Hecht, V., Foucher, F., Ferrándiz, C., Macknight, R., Navarro, C., Morin, J., Vardy, M.E., Ellis, N., Beltrán, J.P., Rameau, C., and Weller, J.L. (2005). Conservation of Arabidopsis flowering genes in model legumes. Plant Physiol. **137:** 1420–1434.

Kang, J.S., Kim, S.H., Hwang, M.S., Han, S.J., Lee, Y.C., and Kim, Y.J. (2001). The structural and functional organization of the yeast mediator complex. J. Biol. Chem. **276:** 42003–42010.

Kariola, T., Brader, G., Li, J., and Palva, E.T. (2005). Chlorophyllase 1, a damage control enzyme, affects the balance between defense pathways in plants. Plant Cell **17:** 282–294.

Kazan, K., and Manners, J.M. (2008). Jasmonate signaling: toward an integrated view. Plant Physiol. **146:** 1459–1468.

Kim, T.W., Kwon, Y.J., Kim, J.M., Song, Y.H., Kim, S.N., and Kim, Y.J. (2004). MED16 and MED23 of Mediator are coactivators of lipopolysaccharide- and heat-shock-induced transcriptional activators. Proc. Natl. Acad. Sci. USA **101:** 12153–12158.

Kim, Y.J., Bjorklund, S., Li, Y., Sayre, M.H., and Kornberg, R.D. (1994). A multiprotein Mediator of transcriptional activation and its interaction with the C-terminal repeat domain of RNA polymerase II. Cell **4:** 599–608.

Koleske, A.J., and Young, R.A. (1994). An RNA polymerase II holoenzyme responsive to activators. Nature **368:** 466–469.

Koornneef, A., and Pieterse, C.M. (2008). Cross talk in defense signaling. Plant Physiol. **146:** 839–844.

Korves, T.M., and Bergelson, J. (2003). A developmental response to pathogen infection in Arabidopsis. Plant Physiol. **133:** 339–347.

Lee, H.K., Park, U.H., Kim, E.J., and Um, S.J. (2007). MED25 is distinct from TRAP220/MED1 in cooperating with CBP for retinoid receptor activation. EMBO J. **26:** 3545–3557.

Li, J., Brader, G., Kariola, T., and Palva, E.T. (2006). WRKY70 modulates the selection of signaling pathways in plant defense. Plant J. **46:** 477–491.

Liu, J., Jambunathan, N., and McNellis, T.W. (2005). Transgenic expression of the von Willebrand A domain of the BONZAI 1/COPINE 1 protein triggers a lesion-mimic phenotype in Arabidopsis. Planta **221:** 85–94.

Malik, S., and Roeder, R.G. (2005). Dynamic regulation of pol II transcription by the mammalian Mediator complex. Trends Biochem. Sci. **30:** 256–263.

Malik, S., and Roeder, R.G. (2008). Epigenetics? Mediator does that too! Mol. Cell **31:** 305–306.

March-Díaz, R., García-Domínguez, M., Lozano-Juste, J., León, J., Florencio, F.J., and Reyes, J.C. (2008). Histone H2A.Z and homologues of components of the SWR1 complex are required to control immunity in Arabidopsis. Plant J. **53:** 475–487.

McGrath, K.C., Dombrecht, B., Manners, J.M., Schenk, P.M., Edgar, C.I., Maclean, D.J., Scheible, W.R., Udvardi, M.K., and Kazan, K. (2005). Repressor- and activator-type ethylene response factors functioning in jasmonate signaling and disease resistance identified via a genome-wide screen of Arabidopsis transcription factor gene expression. Plant Physiol. **139:** 949–959.

Michielse, C.B., and Rep, M. (2009). Pathogen profile update: *Fusarium oxysporum.* Mol. Plant Pathol. **10:** 311–324.

Mittler, G., Stuhler, T., Santolin, L., Uhlmann, T., Kremmer, E., Lottspeich, F., Berti, L., and Meisterernst, M. (2003). A novel

docking site on Mediator is critical for activation by VP16 in mammalian cells. EMBO J. **22:** 6494–6504.

Moreno, J.E., Tao, Y., Chory, J., and Ballaré, C.L. (2009). Ecological modulation of plant defense via phytochrome control of jasmonate sensitivity. Proc. Natl. Acad. Sci. USA **106:** 4935–4940.

Ramakers, C., Ruijter, J.M., Deprez, R.H.L., and Moorman, A.F.M. (2003). Assumption-free analysis of quantitative real-time polymerase chain reaction (PCR) data. Neurosci. Lett. **339:** 62–66.

Schenk, P.M., Kazan, K., Manners, J.M., Anderson, J.P., Simpson, R.S., Wilson, I.W., Somerville, S.C., and Maclean, D.J. (2003). Systemic gene expression in Arabidopsis during an incompatible interaction with *Alternaria brassicicola.* Plant Physiol. **132:** 999–1010.

Schenk, P.M., Kazan, K., Rusu, A.G., Manners, J.M., and Maclean, D.J. (2005). The *SEN1* gene of Arabidopsis is regulated by signals that link plant defense responses and senescence. Plant Physiol. Biochem. **43:** 997–1005.

Schenk, P.M., Kazan, K., Wilson, I., Anderson, J.P., Richmond, T., Somerville, S.C., and Manners, J.M. (2000). Coordinated plant defense responses in Arabidopsis revealed by microarray analysis. Proc. Natl. Acad. Sci. USA **97:** 11655–11660.

Seo, H.S., Song, J.T., Cheong, J.J., Lee, Y.H., Lee, Y.W., Hwang, I., Lee, J.S., and Choi, Y.D. (2001). Jasmonic acid carboxyl methyltransferase: A key enzyme for jasmonate-regulated plant responses. Proc. Natl. Acad. Sci. USA **98:** 4788–4793.

Sharma, D., and Fondell, J.D. (2002). Ordered recruitment of histone acetyltransferases and the TRAP/Mediator complex to thyroid hormone-responsive promoters in vivo. Proc. Natl. Acad. Sci. USA **99:** 7934–7939.

Spoel, S.H., et al. (2003). NPR1 modulates cross-talk between salicylate- and jasmonate-dependent defense pathways through a novel function in the cytosol. Plant Cell **15:** 760–770.

Strizhov, N., Keller, M., Mathur, J., Koncz-Kalman, Z., Bosch, D., Prudovsky, E., Schell, J., Sneh, B., Koncz, C., and Zilberstein, A. (1996). A synthetic cryIC gene, encoding a *Bacillus thuringiensis* delta-endotoxin, confers Spodoptera resistance in alfalfa and tobacco. Proc. Natl. Acad. Sci. USA **93:** 15012–15017.

Thatcher, L.F., Manners, J.M., and Kazan, K. (2009). *Fusarium oxysporum* hijacks COI1-regulated jasmonate signaling to promote disease in Arabidopsis. Plant J. **58:** 927–939.

Thines, B., Katsir, L., Melotto, M., Niu, Y., Mandaokar, A., Liu, G.H., Nomura, K., He, S.Y., Howe, G.A., and Browse, J. (2007). JAZ repressor proteins are targets of the SCFCO11 complex during jasmonate signalling. Nature **448:** 661–665.

Thomma, B., Eggermont, K., Penninckx, I., Mauch-Mani, B., Vogelsang, R., Cammue, B.P.A., and Broekaert, W.F. (1998). Separate jasmonate-dependent and salicylate-dependent defense response pathways in Arabidopsis are essential for resistance to distinct microbial pathogens. Proc. Natl. Acad. Sci. USA **95:** 15107–15111.

Thomma, B.P., Nelissen, I., Eggermont, K., and Broekaert, W.F. (1999). Deficiency in phytoalexin production causes enhanced susceptibility of *Arabidopsis thaliana* to the fungus *Alternaria brassicicola.* Plant J. **19:** 163–171.

Trusov, Y., Rookes, J.E., Chakravorty, D., Armour, D., Schenk, P.M., and Botella, J.R. (2006). Heterotrimeric G proteins facilitate Arabidopsis resistance to necrotrophic pathogens and are involved in jasmonate signaling. Plant Physiol. **140:** 210–220.

Veronese, P., Narasimhan, M.L., Stevenson, R.A., Zhu, J.K., Weller, S.C., Subbarao, K.V., and Bressan, R.A. (2003). Identification of a locus controlling Verticillium disease symptom response in *Arabidopsis thaliana.* Plant J. **35:** 574–587.

Wagner, D., and Meyerowitz, E.M. (2002). SPLAYED, a novel SWI/SNF ATPase homolog, controls reproductive development in Arabidopsis. Curr. Biol. **12:** 85–94.

Walley, J.W., Rowe, H.C., Xiao, Y., Chehab, E.W., Kliebenstein, D.J., Wagner, D., and Dehesh, K. (2008). The chromatin remodeler SPLAYED regulates specific stress signaling pathways. PLoS Pathog. **4:** e1000237.

Whittaker, C.A., and Hynes, R.O. (2002). Distribution and evolution of von Willebrand/integrin A domains: Widely dispersed domains with roles in cell adhesion and elsewhere. Mol. Biol. Cell **13:** 3369–3387.

Wilson, I.W., Kennedy, G.C., Peacock, J.W., and Dennis, E.S. (2005). Microarray analysis reveals vegetative molecular phenotypes of Arabidopsis flowering-time mutants. Plant Cell Physiol. **46:** 1190–1201.

Wollenberg, A.C., Strasser, B., Cerdán, P.D., and Amasino, R.M. (2008). Acceleration of flowering during shade-avoidance in *Arabidopsis thaliana* alters the balance between *FLOWERING LOCUS C*-mediated repression and photoperiodic induction of flowering. Plant Physiol. **148:** 1681–1694.

Wu, K., Zhang, L., Zhou, C., Yu, C.W., and Chaikam, V. (2008). HDA6 is required for jasmonate response, senescence and flowering in Arabidopsis. J. Exp. Bot. **59:** 225–234.

Yang, F., DeBeaumont, R., Zhou, S., and Naar, A.M. (2004). The activator-recruited cofactor/Mediator coactivator subunit ARC92 is a functionally important target of the VP16 transcriptional activator. Proc. Natl. Acad. Sci. USA **101:** 2339–2344.

Yang, M., Hay, J., and Ruyechan, W.T. (2008). The Varicella Zoster Virus IE62 protein utilizes the human Mediator complex in promoter activation. J. Virol. **82:** 12154–12163.

Zhou, C., Zhang, L., Duan, J., Miki, B., and Wu, K. (2005). HISTONE DEACETYLASE19 is involved in jasmonic acid and ethylene signaling of pathogen response in Arabidopsis. Plant Cell **17:** 1196–1204.

Zhou, N., Tootle, T.L., and Glazebrook, J. (1999). *Arabidopsis PAD3*, a gene required for camalexin biosynthesis, encodes a putative cytochrome P450 monooxygenase. Plant Cell **11:** 2419–2428.

Zimmermann, P., Hirsch-Hoffmann, M., Hennig, L., and Gruissem, W. (2004). GENEVESTIGATOR. Arabidopsis microarray database and analysis toolbox. Plant Physiol. **136:** 2621–2632.