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Large-scale transcriptome analysis reveals arabidopsis metabolic pathways are frequently influenced by different pathogens

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Abstract

Key message **Through large-scale transcriptional data analyses, we highlighted the importance of plant metabolism in plant immunity and identified 26 metabolic pathways that were frequently influenced by the infection of 14 different pathogens.**

Abstract Reprogramming of plant metabolism is a common phenomenon in plant defense responses. Currently, a large number of transcriptional profiles of infected tissues in Arabidopsis (*Arabidopsis thaliana*) have been deposited in public databases, which provides a great opportunity to understand the expression patterns of metabolic pathways during plant defense responses at the systems level. Here, we performed a large-scale transcriptome analysis based on 135 previously published expression samples, including 14 different pathogens, to explore the expression pattern of Arabidopsis metabolic pathways. Overall, metabolic genes are significantly changed in expression during plant defense responses. Upregulated metabolic genes are enriched on defense responses, and downregulated genes are enriched on photosynthesis, fatty acid and lipid metabolic processes. Gene set enrichment analysis (GSEA) identifies 26 frequently differentially expressed metabolic pathways (FreDE_Paths) that are differentially expressed in more than 60% of infected samples. These pathways are involved in the generation of energy, fatty acid and lipid metabolism as well as secondary metabolite biosynthesis. Clustering analysis based on the expression levels of these 26 metabolic pathways clearly distinguishes infected and control samples, further suggesting the importance of these metabolic pathways in plant defense responses. By comparing with FreDE_Paths from abiotic stresses, we find that the expression patterns of 26 FreDE_Paths from biotic stresses are more consistent across different infected samples. By investigating the expression correlation between transcriptional factors (TFs) and FreDE_Paths, we identify several notable relationships. Collectively, the current study will deepen our understanding of plant metabolism in plant immunity and provide new insights into disease-resistant crop improvement.

Keywords Biotic stresses · Gene set enrichment analysis (GSEA) · Defense response · Large-scale transcriptional data · Metabolic pathways

Introduction

Plants as sessile organisms have to endure a wide variety of attacks from microbes (i.e., biotic stresses) and have evolved a sophisticated immune system to protect them from numerous plant diseases (Jones and Dangl 2006). Plant defense responses are complex, involving large-scale transcriptional and metabolic reprogramming (Moore et al. 2011). The model plant Arabidopsis (*Arabidopsis thaliana*) is highly suitable for systems biology analysis, as diverse omics datasets such as genomic and transcriptional data have been accumulated in the past 15 years. According to

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the current statistics of the plant metabolic network (http://plantcyc.org), the Arabidopsis genome has been annotated to contain more than 600 known metabolic pathways, which cover approximately 3300 metabolic genes. In this context, a systems understanding of the metabolic reprogramming of Arabidopsis in response to biotic stresses is highly required, which will provide guidance to improve disease resistance of crop plants.

Many efforts have been made to study how plant metabolisms are coordinated to meet biotic stresses imposed by different pathogens and significant progress has been achieved (Bolton 2009; Lv et al. 2014; Piasecka et al. 2015; Rojas et al. 2014). Plant metabolisms can be divided into primary metabolisms and secondary metabolisms. Primary metabolisms, which contribute directly to plant growth and development, have been suggested to serve as energy providers for plant defense responses (Bolton 2009; Rojas et al. 2014). Plant defense responses include the reinforcement of cell walls, the generation of reactive oxygen species, the production of plant hormones, such as salicylic acid (SA), jasmonic acid (JA) and ethylene (ET), and the production of pathogenesis-related proteins, as well as the development of a hypersensitive response (Jones and Dangl 2006). Due to their complexity, plant defense responses are often energy consuming. To maintain the homeostasis required for a living organism and to respond dynamically to biotic stresses, a trade-off between plant growth and defense occurs in plants due to limited resources (Huot et al. 2014). Therefore, the upregulation of defense-related genes is often compensated by the downregulation of other metabolic genes (Huot et al. 2014; Rojas et al. 2014). For example, photosynthesis-related genes are suppressed during plant defense responses to different biotic stresses (Bilgin et al. 2010; Jiang et al. 2016b).

The indispensable contribution of secondary metabolites to plant immunity has also been well established (Piasecka et al. 2015), although they are not essential for plants to survive. For example, plant hormones, such as SA, JA and ET, play key roles in regulating plant defense responses (Pieterse et al. 2012). Moreover, other hormones heavily involved in plant development and growth, such as abscisic acid, auxin, gibberellic acid, cytokinin and brassinosteroids, have also been proven to participate in plant defense responses (Pieterse et al. 2012). Experimental evidence suggests that the production of antimicrobial secondary metabolites inhibits the progress of infections (Ahuja et al. 2012). For example, phytoalexin camalexin functions as a defense compound against a broad spectrum of pathogens, including fungi [e.g., *Alternaria brassicicola*, *Botrytis cinerea* (Kliebenstein et al. 2005), *Plectosphaerella cucumerina* (Sanchez-Vallet et al. 2010) and *Golovinomyces orontii* (Pandey et al. 2010)], and oomycetes [e.g., *Phytophthora brassicae* (Schlaeppi et al. 2010)].

Although the role of plant metabolism in plant immunity has been studied for many years, previous metabolic analyses have primarily concentrated on the production of only one metabolite or a single metabolic gene. Importantly, plant defense responses involve massive metabolic genes. Accordingly, to gain a broader perspective of the mechanisms that contribute to plant immunity, it is necessary to investigate genes at the pathway/genome levels rather than evaluating a single gene. Genome-wide transcriptional reprogramming of metabolic genes regarding the plant immune process were not achieved until omics technologies became widely available (Kliebenstein 2012). Indeed, many efforts in this direction have been made to understand how plant metabolisms change as a consequence of pathogen infection (Bilgin et al. 2010; Jiang et al. 2016a; Lv et al. 2014). By focusing on photosynthesis-related metabolic genes, Bilgin et al. (2010) compared transcriptional data from 22 different biotic stresses on eight plant species and showed that photosynthesis-related genes were universally downregulated. Less et al. (2011) proposed a bioinformatics approach termed gene coordination to elucidate the coordinated response of gene networks to external cues and used it to analyze the response of the genes encoding metabolic enzymes and TFs to multiple biotic and abiotic stresses. Their analyses revealed that downregulated genes were particularly associated with photosynthesis, tetrapyrrole biosynthesis, as well as sugar, lipid, and amino acid metabolism, and upregulated genes were enriched on processes associated with energy production. Through integrating co-expression gene pairs and regulatory interactions, Lv et al. (2014) constructed a dynamic regulatory network of secondary metabolic pathways in Arabidopsis to analyze the regulation of secondary metabolites under biotic and abiotic conditions. Studies on plants subjected to various biotic stresses indicate that some induced changes on metabolic genes are common regardless of the applied treatment types (Bilgin et al. 2010).

With the arrival of the 'big data' era, there is an increasing and urgent need to understand plant immunity from a systems perspective through the integration of the huge available omics data deposited in public databases (Bilgin et al. 2010; Dong et al. 2015; Li and Zhang 2016; Lv et al. 2014). Undoubtedly, an integrated pathway-level analysis of large-scale transcriptomics data is also highly desired, which can allow us to obtain a more complete and clear picture of plant defense responses. In this work, we performed a large-scale transcriptome data analysis based on previously published microarray data to explore the expression pattern of Arabidopsis metabolic pathways in response to different pathogens. At the gene level, the global transcriptional changes of metabolic genes across 14 different biotic stresses provide a comprehensive view of the metabolic processes involved in plant defense responses. At the

pathway level, we employed gene set enrichment analysis (GSEA; Subramanian et al. 2005) to identify 26 frequently differentially expressed metabolic pathways (FreDE_Paths) during plant defense responses to 14 different pathogens. Based on the 26 identified FreDE_Paths, we performed the following two analyses. First, in order to verify whether these observations are unique to biotic stresses, we performed a comparative pathway analysis on transcriptome datasets that profiled Arabidopsis gene expression responding to abiotic stresses. Second, we also carried out a correlation analysis to identify potential regulators of the 26 FreDE_Paths.

Results and discussion

Overview of metabolic gene expression during plant defense responses

To understand how metabolic genes expressed during plant defense responses, we collected 135 samples (69 infected samples and 66 control samples) measuring Arabidopsis gene expression following 14 different biotic stress challenges [i.e., *Agrobacterium tumefaciens*, *A. brassicicola, Blumeria graminis*, *B. cinerea*, Cabbage leaf curl virus (CaLCuV), Cucumber mosaic virus (CMV), *G. orontii, Hyaloperonospora arabidopsidis, Phytophthora infestans, P. cucumerina, Pseudomonas syringae, Rhizoctonia solani, Sclerotinia sclerotiorum* and *Verticillium longisporum*] from gene expression omnibus (GEO; Barrett et al. 2013) and ArrayExpress (Brazma et al. 2003). The basic information of these transcriptional data is summarized in Table 1 (See Supplementary Table S1 for more details) and we have also made the raw data of the 135 samples available at http://systbio.cau.edu.cn/SI/index1.php. Differentially expressed genes (DEGs) between the infected samples and their corresponding control samples were inferred using the RankProd package, which was developed from the rank product method (Hong et al. 2006). Rank product is a non-parametric statistical method that uses the ranks of gene expression changes to obtain the combined signatures from multiple studies. In total, we obtained 13168 DEGs that were differentially expressed under at least one of the 14 infection conditions (Supplementary Table S2).

Using the inferred DEGs, we first investigated the global pattern of metabolic gene expression during plant defense responses. For this analysis, we collected 2967 metabolic genes from the Aracyc database (Version 13.0; Mueller

Table 1 Transcriptional data used in this work

Pathogen	Pathogen type	Accession number[a]	Sample number	Treatment number	Mock number	Tissues	Time after inoculation[b]
G. orontii	Fungi	E-MEXP-2371	6	3	3	Leaf	8 h
B. graminis	Fungi	GSE12856	6	3	3	Leaf	12 h
R. solani	Fungi	GSE26206	9	6	3	Seedling	7 d
S. sclerotiorum	Fungi	E-MEXP-3122	12	6	6	Leaf	24, 48 h
A. brassicicola	Fungi	GSE50526	10	5	5	Leaf	9, 24 h
B. cinerea	Fungi	GSE5684	12	6	6	Leaf	18, 48 h
P. cucumerina	Fungi	E-MEXP-3733 E-MTAB-641	12	6	6	Leaf	24 h
V. longisporum	Fungi	GSE62537	6	3	3	Root	48 h
P. infestans	Oomycetes	GSE5616	18	9	9	Leaf	6, 12, 24 h
H. arabidopsidis	Oomycetes	GSE18329	6	3	3	Aerial part	4 d
P. syringae	Bacteria	GSE17500 GSE18978 GSE21920	18	9	9	Leaf	6, 24 h
A. tumefaciens	Bacteria	GSE62749 GSE62750	8	4	4	Leaf Root	8 h
CMV	Viruses	GSE37921	6	3	3	Aerial part	–
CaLCuV	Viruses	E-ATMX-34	6	3	3	Leaf	12 d
Sum			135	69	66		

h hours, *d* days

[a]All the expression profiles were based on the Arabidopsis expression array platform (GPL198), and Arabidopsis ecotype Columbia (Col-0) was used for all experiments

[b]Time for GSE37921 is unclear. Note that the collected transcriptional data are from different time points, which may correspond to different infection stages. Thus, comparative analyses based on these transcriptional data may inevitably yield biased results to some extent

et al. 2003). Compared with the overall Arabidopsis genes, we found that the proportions of DEGs in metabolic genes were significantly higher under all infections by 14 different pathogens (Fig. 1a, Supplementary Table S3). For instance, during the infection of *B. cinerea*, approximately 18.1% of Arabidopsis genes were differentially expressed compared to normal growth, whereas 30.1% (893 of 2967) of metabolic genes were differentially expressed (hypergeometric test, p-value $< 2.2 \times 10^{-16}$). The extensive expression changes of metabolic genes during plant defense responses to all the 14 pathogens indicate that plant metabolisms play critical roles in plant immunity.

Frequently differentially expressed metabolic genes

To explore the metabolic processes that were frequently influenced during plant immune responses, we identified 2087 differentially expressed metabolic genes by selecting metabolic genes that were differentially expressed under at least one infection condition (Supplementary Table S2). Approximately half of these genes (1044 of 2087) were classified as upregulated as these genes are generally more upregulated in all the conditions, whereas the remaining genes (1043) were classified as downregulated. Gene ontology (GO; Consortium GO 2004) enrichment analysis showed that these upregulated and downregulated metabolic genes were enriched on different biological processes (Supplementary Table S4a, b). Photosynthesis, fatty acid and lipid metabolic processes were enriched on downregulated metabolic genes (hypergeometric test, p-value $= 1.71 \times 10^{-25}$, 2.68×10^{-43} and 0, respectively). Photosynthesis genes have been proven to be globally downregulated during the plant immune process in a previous study (Bilgin et al. 2010). Our previous comparative analysis of plant immune responses to biotrophic pathogen *G. orontii* and necrotrophic pathogen *B. cinerea* also showed the repression of plant photosynthesis during plant responses to both pathogens (Jiang et al. 2016b). Upregulated metabolic genes were enriched on plant defense-related GO terms, such as response to biotic stimulus (hypergeometric test, p-value $= 1.62 \times 10^{-15}$), JA metabolic process (hypergeometric test, p-value $= 8.18 \times 10^{-17}$) and defense response (hypergeometric test, p-value $= 3.99 \times 10^{-4}$).

We constructed a Venn diagram to illustrate the overlap of differentially expressed metabolic genes induced by different pathogen types (Fig. 1b). To do so, we first divided the 14 different pathogens into four types (i.e., bacteria, fungi, oomycetes and viruses; Table 1). Then, for each infection type, if a metabolic gene was differentially expressed under more than 80% of infection conditions of that infection type, it was defined as differentially expressed. As shown in Fig. 1b, we observed that some metabolic genes were only differentially expressed under a single infection type. For example, 59 metabolic genes were only differentially expressed during the infection of

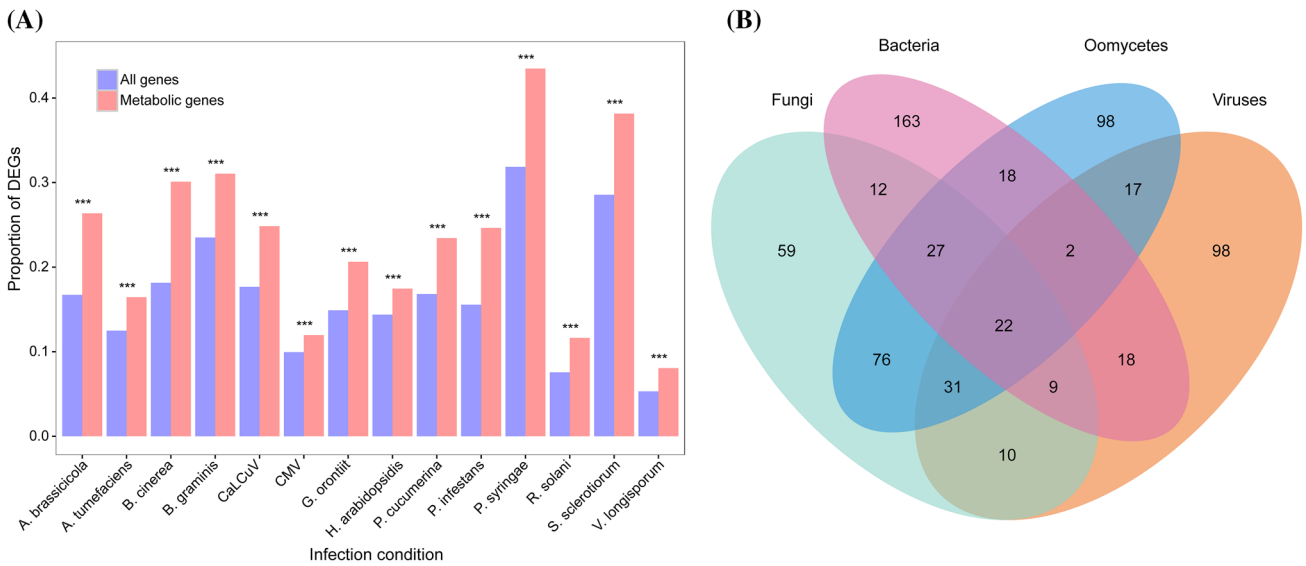

Fig. 1 Differentially expressed metabolic genes during plant defense responses to 14 different pathogens. **a** Metabolic genes were significantly changed in expression under all 14 infection conditions. DEGs were detected using the function RPadvance in the R package RankProd. The *y-axis* shows the proportions of DEGs in overall Arabidopsis genes and metabolic genes induced by the corresponding pathogen. ***Denotes a significant enrichment of metabolic genes on DEGs (p-value < 0.001, hypergeometric test; see Supplementary Table S3 for details). **b** Four-way Venn diagram of the numbers of differentially expressed metabolic genes induced by four different types of pathogens

fungi, whereas 163 metabolic genes were only differentially expressed induced by bacteria. Similarly, 98 metabolic genes were only differentially expressed induced by both oomycetes and viruses. In addition, we also observed large overlaps in metabolic gene expression among plant defense responses to four different pathogen types. For instance, comparing plant defense responses to fungi and oomycetes, there were 156 common differentially expressed metabolic genes. Twenty-two metabolic genes were differentially expressed in response to four different types of pathogens. Annotation analysis showed that these 22 metabolic genes were significantly enriched on "response to biotic stimulus" (hypergeometric test, p-value $= 6.74 \times 10^{-3}$, Supplementary Table S4c).

To identify metabolic genes that were differentially expressed under most infection conditions, we selected metabolic genes that were differentially expressed under more than 12 infection conditions and obtained 23 such metabolic genes (Fig. S1, Table 2). All genes except *FAD5* were upregulated in more than 11 conditions. As

Table 2 23 metabolic genes that are differentially expressed under more than 12 infection conditions

Locus[a]	Gene name	Up[b]	Down[c]	GO biological process
AT2G26560 (La Camera et al. 2009)	*Patatin-like protein 2, PLP2*	14	0	Plant-type hypersensitive response, defense response to virus
AT1G02930 (Grant et al. 2000)	*Glutathione S-transferase 1, GST1*	14	0	Glutathione metabolic process, defense response to bacterium
AT1G69930 (Wagner et al. 2002)	*Glutathione S-transferase U11, GSTU11*	14	0	Glutathione metabolic process, toxin catabolic process
AT2G02390 (Wagner et al. 2002)	*Glutathione S-transferase 18, GST18*	14	0	Glutathione metabolic process, toxin catabolic process
AT2G02930 (Asano et al. 2012)	*Glutathione S-transferase 16, GST16*	13	0	Glutathione metabolic process, defense response to fungus
AT1G74590 (Wagner et al. 2002)	*Glutathione S-transferase U10, GSTU10*	13	0	Glutathione metabolic process, toxin catabolic process
AT3G13790 (De Coninck et al. 2005)	*Cell wall invertase 1, CWI1*	13	0	Carbohydrate metabolic process
AT4G34230 (Kim et al. 2004b)	*Cinnamyl alcohol dehydrogenase 5, CAD5*	13	1	Oxidation–reduction process
AT5G39050 (Taguchi et al. 2010)	*Phenolic glucoside malonyltransferase 1, PMAT1*	13	0	Response to toxic substance
AT3G22370 (Saisho et al. 1997)	*Alternative oxidase 1a, AOX1A*	13	0	Mitochondria-nucleus signaling pathway, oxidation–reduction process
AT3G13610 (Kai et al. 2008)	*Feruloyl CoA ortho-hydroxylase 1, F6'H1*	13	1	HYDROGEN peroxide-mediated programmed cell death
AT4G25900	–	13	0	Galactose metabolic process
AT4G01700 (Hok et al. 2011)	*AT4G01700*	13	1	Chitin catabolic process, defense response to fungus
AT1G72680 (Kim et al. 2004a)	*Probable cinnamyl alcohol dehydrogenase 1, CAD1*	13	0	Lignin biosynthetic process, oxidation–reduction process
AT3G26830 (Ferrari et al. 2003; van Wees et al. 2003)	*Phytoalexin deficient 3, PAD3*	13	0	Camalexin biosynthetic process, defense response
AT2G43570	*Chitinase, CHI*	13	0	Systemic acquired resistance
AT1G32350 (Clifton et al. 2006)	*Alternative oxidase 1d, AOX1D*	13	0	Oxidation–reduction process
AT4G34135 (Hok et al. 2011)	*Udp-glucosyltransferase 73b2, UGT73B2*	13	0	Flavonol biosynthetic process, response to other organism
AT4G13180	–	13	0	Response to arsenic-containing substance
AT2G43590	–	13	1	Chitinase activity, defense response
AT2G43620	–	12	1	Chitin catabolic process, defense response
AT2G29350 (Lohman et al. 1994)	*Senescence-associated gene 13, SAG13*	12	1	Defense response to insect, oxidation–reduction process
AT3G15850 (Heilmann et al. 2004)	*Fatty acid desaturase 5, FAD5*	1	12	Oxidation–reduction process, oxylipin biosynthetic process, photoinhibition

[a]Genes with known functional roles in plant response to biotic stresses are shown in bold

[b]The number of conditions in which the corresponding gene was significantly upregulated

[c]The number of conditions in which the corresponding gene was significantly downregulated

expected, eight genes (i.e., *CHI, PAD3, PLA2, GST1, GST16, UGT73B2, AT2G43620* and *AT4G01700*) had already been proven to be participating in plant responses to biotic stresses (Table 2). To successfully invade hosts, plant pathogens produce toxins to kill host cells. As a response, plants have evolved detoxification mechanisms to detoxify pathogen toxins, including glutathione-mediated detoxification systems (Ghanta and Chattopadhyay 2011). Five glutathione *S*-transferase (GST) genes were frequently differentially expressed (Table 2). Two GST genes (*GST1* and *GST16*) have already been proven to be participating in plant defense response (Asano et al. 2012; Grant et al. 2000), and the other three genes (*GSTU11, GST18* and *GSTU10*) need further investigation. Chitin molecules are the main structural component in fungal cell walls. Chitin degradation induced by chitinases can inhibit fungal growth and generate elicitors of defense reactions (El Hadrami et al. 2010). Although viruses do not contain or generate chitin, induced expression of chitinases has been reported in response to viruses (Margis-Pinheiro et al. 1993). For instance, two putative chitinases (*AT4G01700* and *CHI*) were found frequently upregulated during plant defense response, although their functional roles in the plant immune response against viruses remain elusive. *Phytoalexin deficient 3* (*PAD3*), a key cytochrome P450 enzyme involved in the biosynthesis of camalexin, was upregulated in 13 infection conditions (Zhou et al. 1999). Arabidopsis *pad3* mutants with decreased camalexin levels are more susceptible to *A. brassicicola* (van Wees et al. 2003) and *B. cinerea* (Ferrari et al. 2003) compared to wild-type Arabidopsis. The other genes with unclear roles in plant immunity were good candidates for further analysis. For example, *AOX1A* and *AOX1D*, two important isoforms of alternative oxidase (AOX) located in the mitochondrial inner membrane, were both upregulated in 13 of 14 conditions. Previous work has shown that the expression of genes encoding AOX under biotic stresses was frequently increased to regulate metabolic and signaling homeostasis (Hanqing et al. 2010; Vanlerberghe 2013). Thus, further analyses are needed to investigate how *AOX1A* and *AOX1D* influence plant defense response.

Expression change of metabolic pathways

We next analyzed the expression changes of Arabidopsis metabolic pathways. To do so, we collected 395 metabolic pathways with at least five genes from the Aracyc database. To identify the pattern of upregulation or downregulation for each metabolic pathway, we employed GSEA to determine the significance of its expression change in each infected sample relative to the corresponding control sample (see the "Materials and Methods" section). Briefly, for each infected sample, we first calculated the value of the fold-change (FC) for each gene relative to the corresponding control sample and ranked genes based on FCs. Then, the pre-ranked GSEA tool (GSEAPreRanked) was used to identify differentially expressed metabolic pathways using FC-based ranked genes as inputs. As there were 69 infected samples in our collected transcriptional datasets (Table 1), 69 GSEAPreRanked analyses were performed. Based on the GSEAPreRanked analyses, we found that 366 of 395 metabolic pathways were differentially expressed under at least one of the 69 infected samples (Supplementary Table S5). For each pathogen, a metabolic pathway was defined as differentially expressed during plant immune responses if it was differentially expressed under at least one of the infected samples infected by the given pathogen. Figure 2a displays the number of differentially expressed pathways detected from infections by 14 different pathogens. Plant immune responses to *P. infestans* resulted in the maximum number of differentially expressed pathways, whereas *B. graminis* caused the minimum number of differentially expressed pathways.

Similar to the analyses performed on metabolic genes, we constructed a Venn diagram to illustrate the overlapping of metabolic pathways induced by different pathogen types (Fig. S2). For each type (i.e., bacteria, fungi, oomycetes and viruses), if a metabolic pathway was differentially expressed under more than 60% infection conditions belonging to the given type, it was defined as differentially expressed. In total, we obtained 98 metabolic pathways that were differentially expressed under infections by at least one pathogen type. We observed that some pathways overlapped between different types of pathogens and some were unique to an individual pathogen type. Fungi and oomycetes not only contain many similar morphological and physiological features but also share common infection processes in pathogenesis (Meng et al. 2009). Approximately 76% (26 of 34) of the differentially expressed metabolic pathways induced by fungi overlapped with those pathways induced by oomycetes, which was larger than the overlap between fungi and bacteria or viruses. This indicated that the plant metabolism responses to fungi and oomycetes were more similar to some extent. Unlike cellular pathogens (bacteria, fungi and oomycetes), plant viruses are obligate intracellular parasites that can only replicate in the cytoplasm of a host cell (Hull 2002). The overlap between metabolic pathways induced by viruses and the other three types of pathogens were generally small. Approximately 36% (15 of 41) metabolic pathways induced by viruses were specific to viruses. This showed that plant defense response to viruses was different from other pathogens from the perspective of plant metabolisms.

We were interested in metabolic pathways that were frequently differentially expressed by different pathogens. To help determine this, we defined a metabolic pathway

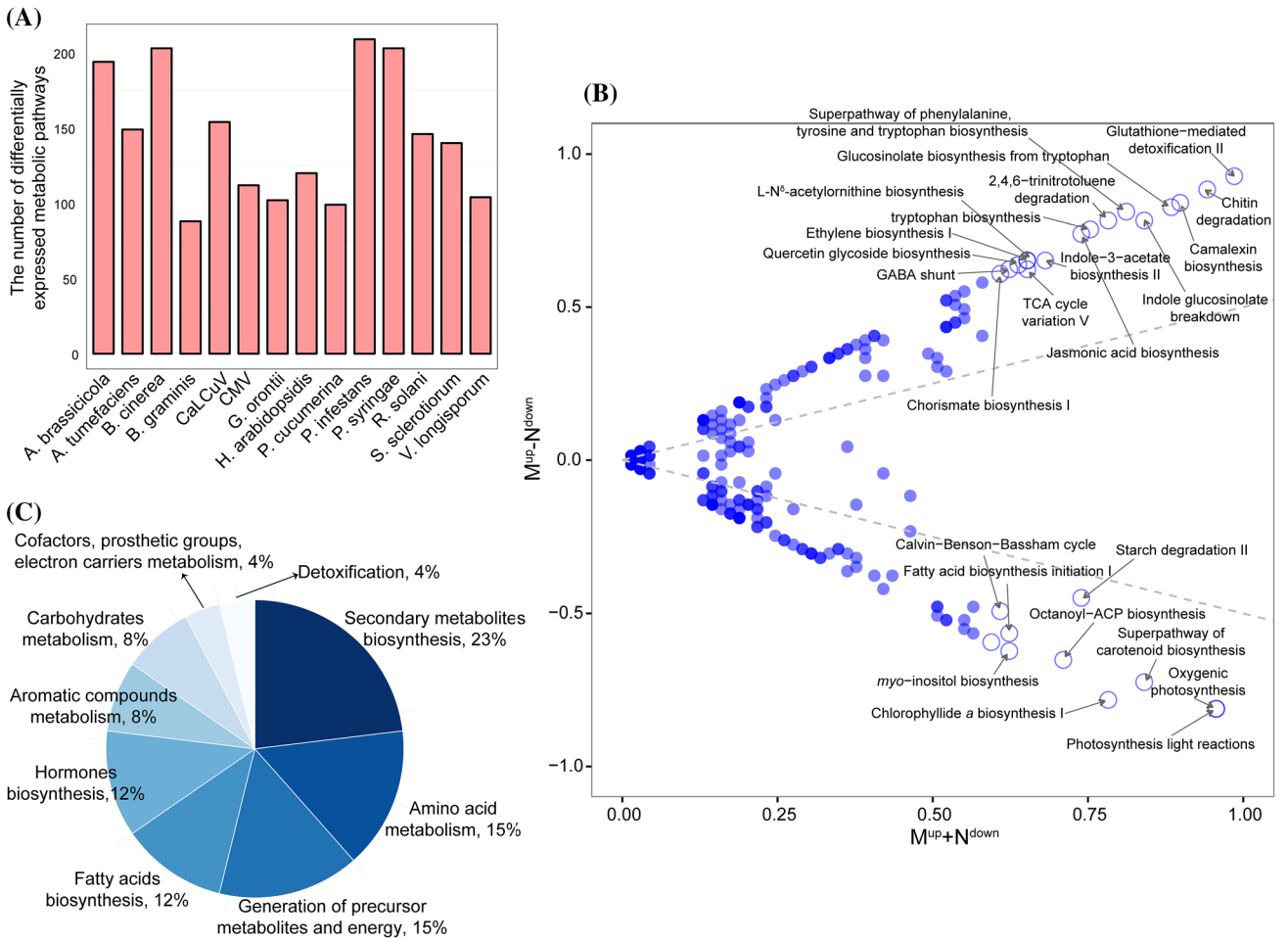

Fig. 2 Differentially expressed pathways detected using GSEA. A pathway is defined as differentially expressed if its normal *p*-value is lower than 0.05. **a** The number of differentially expressed pathways during plant immune response to 14 differential pathogens. **b** Expression patterns of individual metabolic pathways during plant immune response. The metabolic pathways defined in the Aracyc database are shown in the coordination of $N^{up}+N^{down}$ and $N^{up}-N^{down}$. N^{up} and N^{down} represent the fraction of infected samples, in which a pathway is significantly upregulated and downregulated, respectively. The dashed lines demarcate the region where the absolute value of $N^{up}-N^{down}$ is <50% of $N^{up}+N^{down}$ and are generated for visualization purposes only. FreDE_Paths are marked with the corresponding pathway names and the overlapping nodes are displayed in node transparency. Frequently upregulated pathways occupy positions in the upper right corner, whereas frequently downregulated pathways appear in the lower right corner. **c** The distribution of the categories for 26 FreDE_Paths. The category for each pathway is obtained according to its belonging to a super pathway in the Aracyc database

differentially expressed in at least 60% of the 69 infected samples as a FreDE_Path. Based on this criterion, we obtained 26 FreDE_Paths (Fig. 2b; Table 3). Note that COMPLETE-ARO-ARA-PWY is the super pathway of TRPSYN-PWY, and PHOTOALL-PWY is the super pathway of PWY-101 and CALVIN-PWY. Among the 26 FreDE_Paths, 16 pathways were upregulated in a majority of infection conditions, and the remaining ten metabolic pathways were downregulated. According to their super pathways in the Aracyc database, these pathways were classified into nine different categories (Fig. 2c). According to the number of pathways in each category, the top three categories were 'secondary metabolites biosynthesis,' 'amino acid metabolism' and 'generation of precursor metabolites and energy.' Secondary metabolisms have long been thought to contribute to plant–pathogen interactions (Bennett and Wallsgrove 1994). Previous studies have revealed that many secondary metabolites play important roles in plant defense responses, such as glucosinolates (Clay et al. 2009) and camalexin (Ahuja et al. 2012). Both camalexin biosynthesis and glucosinolate biosynthesis metabolic pathways were upregulated in the majority of infected samples (Table 3). Amino acids are precursors for the biosynthesis of protective plant natural products (such as glucosinolates and phytoalexin) that directly exert defenses to pathogens (Zeier 2013). In addition to their roles as precursors for defense components, amino acid metabolisms have also been proven to directly impact plant-pathogen interactions.

Table 3 26 FreDE_Paths during plant immune responses to 14 different pathogens

Unique-ID	Name	Up[a]	Down[b]
PWY-6842	Glutathione-mediated detoxification II	66	2
PWY-101	Photosynthesis light reactions	5	61
PHOTOALL-PWY	Oxygenic photosynthesis	5	61
PWY-6902	Chitin degradation II	63	2
CAMALEXIN-SYN	Camalexin biosynthesis	60	2
PWY-601	Glucosinolate biosynthesis from tryptophan	59	2
CAROTENOID-PWY	Superpathway of carotenoid biosynthesis	4	54
PWYQT-4477	Indole glucosinolate breakdown	56	2
COMPLETE-ARO-ARA-PWY	Superpathway of phenylalanine, tyrosine and tryptophan biosynthesis	56	0
CHLOROPHYLL-SYN	Chlorophyllide *a* biosynthesis I	0	54
PWY-6051	2,4,6-Trinitrotoluene degradation	54	0
TRPSYN-PWY	Tryptophan biosynthesis	52	0
PWY-6724	Starch degradation II	10	41
PWY-735	JA biosynthesis	51	0
PWY-7388	Octanoyl–ACP biosynthesis	2	47
PWY-581	Auxin biosynthesis	46	1
PWYQT-4481	TCA cycle variation V (plant)	44	1
PWY-6922	L-N^{δ}-acetylornithine biosynthesis	45	0
ETHYL-PWY	Ethylene biosynthesis I (plants)	45	0
PWY-5321	Quercetin glycoside biosynthesis	44	0
PWY-4381	Fatty acid biosynthesis initiation I	2	41
GLUDEG-I-PWY	GABA shunt	43	0
PWY-2301	*myo*-inositol biosynthesis	0	43
ARO-PWY	Chorismate biosynthesis I	42	0
CALVIN-PWY	Calvin–Benson–Bassham cycle	4	38
PWY-5080	Very long chain fatty acid biosynthesis I	0	41

[a]The number of infected samples with significantly upregulated expression

[b]The number of infected samples with significantly downregulated expression

Four amino acid metabolism pathways were upregulated in many infected samples (Table 3). Plant defense responses require a significant amount of energy, as they involve massive transcriptional reprogramming (Eulgem 2005). As expected, pathways for the generation of precursor metabolites and energy, such as 'TCA cycle variation V,' were significantly upregulated in many infected samples. Moreover, ET, JA and auxin biosynthesis pathways were all upregulated in more than 65% infected samples, which was consistent with the importance of plant hormones in plant immune responses.

In contrast to the upregulation of a pathway related to generation of precursor metabolites and energy, three other pathways (i.e., photosynthesis light reactions, oxygenic photosynthesis and Calvin–Benson–Bassham cycle; Table 3) from the same category were downregulated in the majority of infected samples. Oxygenic photosynthesis, photosynthesis light reactions and Calvin–Benson–Bassham cycle are all subpathways of photosynthesis. Previous analyses have already shown that photosynthesis genes are globally downregulated during biotic stresses (Bilgin et al. 2010). Two mechanisms of the downregulation of photosynthesis have been proposed: (1) suppression triggered by pathogen effectors and (2) feedback regulation mediated by sugar signals (Rojas et al. 2014). Other downregulated pathways include fatty acids biosynthesis, two secondary metabolite biosynthesis pathways and a starch degradation II metabolic pathway.

Signatures of plant defense responses

To test the ability of the 26 FreDE_Paths in classifying infected and control samples, we performed a clustering analysis for the 135 samples used in this work based on the pathway scores. To do so, we defined the pathway score of a given metabolic pathway as the average expression value of metabolic genes included in the given metabolic pathway. As shown in Fig. 3, infected samples and control samples are clustered into four different clusters. Cluster 2 and Cluster 4 are largely comprised of control samples. We

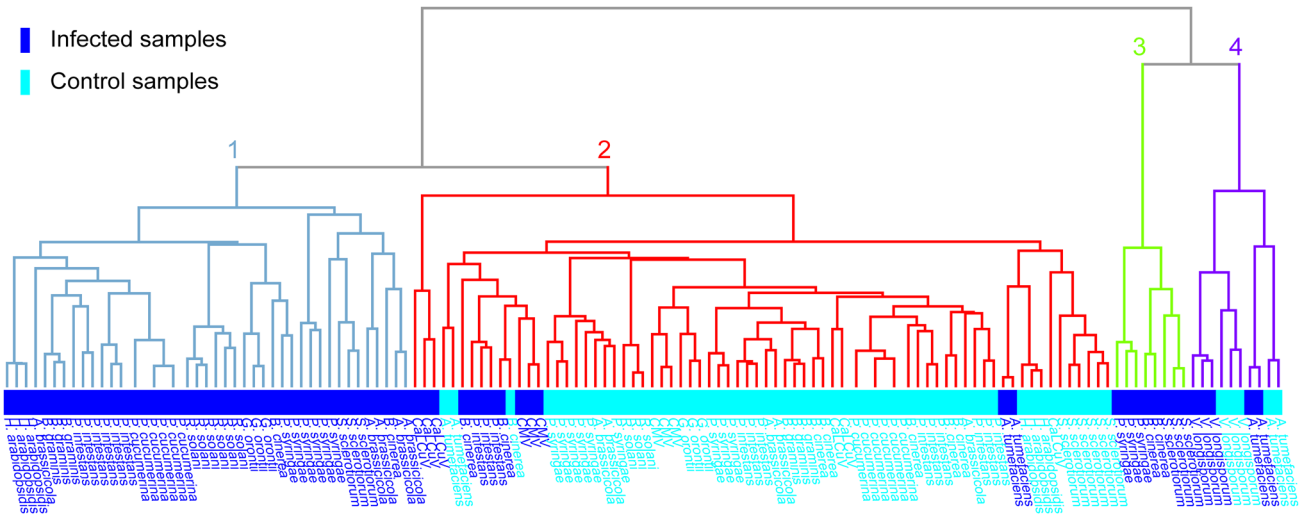

Fig. 3 Hierarchal clustering of 135 samples based on the 26 FreDE_Paths. Infected and control samples were shown in *different colors*

noticed that all ten samples of Cluster 4 were from roots and Cluster 2 did not contain samples from root. It is possible that root-specific expression patterns lead to the separation of Cluster 2 and Cluster 4, since it has been reported that roots have distinct gene expression patterns from aerial tissues (He et al. 2016). Cluster 1 and Cluster 3 mainly consist of infected samples, but why these infected samples are separated into two clusters needs further investigation. Taken together, clustering analysis showed that these 26 FreDE_Paths can successfully classify 135 samples into infection and control groups, meaning that the 26 FreDE_Paths had different expression patterns in infected and control samples. These results further showed the importance of these pathways in plant defense responses, and we concluded that these 26 FreDE_Paths can act as signatures of plant defense responses.

Different expression patterns resulting from biotic stresses and abiotic stresses

The above analysis identified 26 metabolic pathways that were frequently influenced (including upregulation and downregulation) during the infection of various pathogens. In addition, we also showed that microarray samples of different conditions can be separated using information within the expression profiles of those 26 pathways. We questioned whether the expression patterns of those 26 pathways are unique to biotic stresses. Hence, we investigated the data from abiotic stresses using the same approach. We applied GSEA to Arabidopsis expression data under eight different abiotic stresses (i.e., cold, drought, genotoxic, heat, osmotic, salt, UV and wound), which are composed of 106 abiotic stress-processing samples and 16 control samples in shoot tissues (Kilian et al. 2007; Supplementary Table S6). A total of 392 pathways were identified as differentially expressed under at least one of 106 abiotic stress-processing samples (Supplementary Table S7). Similar to the definition of FreDE_Paths under biotic stresses (i.e., differentially expressed under at least 60% of abiotic stress-processing samples), only three pathways were detected from abiotic stresses (Fig. S3). Interestingly, all three pathways also belong to the list of 26 FreDE_Paths from biotic stresses (Table 3). As shown in Fig. S3, these three FreDE_Paths are distributed inside the region demarcated by two dashed lines, which are different from the expression patterns of the corresponding pathways in biotic stresses. For instance, the pathway PWY-6842 (Glutathione-mediated detoxification II) was upregulated in 50 out of 106 abiotic stress-processing samples, while it was downregulated in 26 samples. Comparatively, PWY-6842 was upregulated in 66 out of 69 infected samples during biotic stress.

We employed a heat map to systematically compare the expression patterns of these 26 FreDE_Paths detected from different biotic stresses with the corresponding expression changes in abiotic stresses (Fig. 4). In this heat map, we used the normalized enrichment scores (NESs) resulting from GSEA to quantify the differences of expression patterns. The larger the absolute value of the NES, the more likely a pathway tended to be differentially expressed. A pathway with a positive/negative NES indicates that the pathway is upregulated/downregulated. As shown in Fig. 4, the expression patterns of the 26 FreDE_Paths were generally more consistent in response to biotic stresses in comparison to abiotic stresses, although some abiotic stress-processing samples also shared similar expression patterns with the biotic stresses. These results indicate that plant defense responses to different pathogens result in the consistent upregulation or downregulation of several metabolic

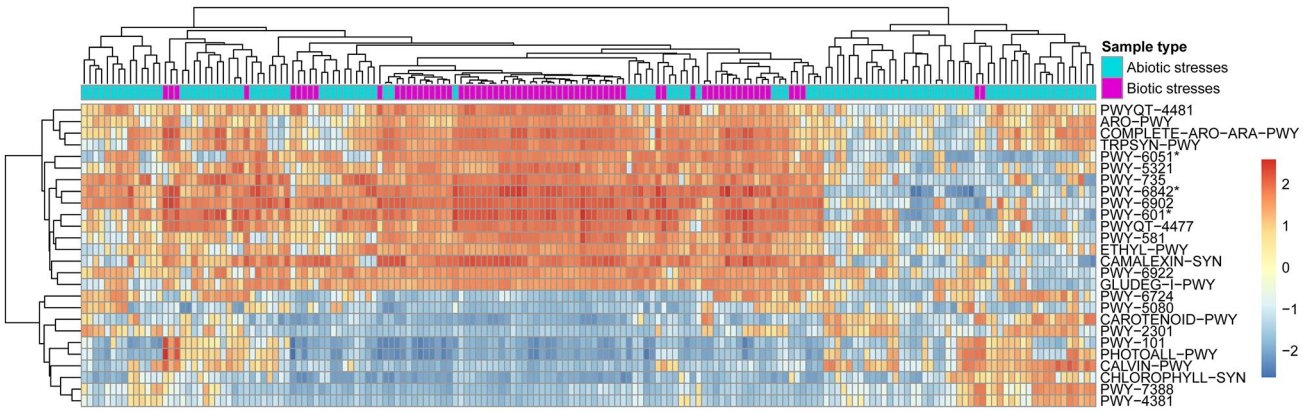

Fig. 4 Heat map of 26 common pathways under biotic and abiotic stresses. NES values for the 26 FreDE_Paths in 69 biotic stress processing samples and 106 abiotic stress processing samples were used to draw the heat map. Three pathways that were identified as frequently *differentially* expressed in both biotic and abiotic stresses were marked with an *asterisk*. *Both rows and columns* were clustered using hierarchical clustering

pathways, which is different from plant response to abiotic stresses.

Detection of significant correlation between metabolic pathways and TFs

To gain an in-depth understanding of how plants regulate these 26 FreDE_Paths during plant defense responses, we used a list of 1707 TFs collected from PlantTFDB (Jin et al. 2014) and AtTFDB (Yilmaz et al. 2011). Based on the assumption that significant correlation implies a potential regulatory relationship, the context likelihood of relatedness (CLR) algorithm (Faith et al. 2007) was used to identify significant correlations between TFs and metabolic pathways (see the "Materials and Methods" section for details). The CLR algorithm has been used to infer regulatory links based on expression profiles and proven superior to other methods (Faith et al. 2007). Hu et al. (2013) used the CLR algorithm to infer significant expression correlations between metabolic pathways and signaling genes and identified several notable relationships. In this work, given a Z-score cutoff of 2.0 and a Pearson correlation coefficient (PCC) cutoff of 0.6/−0.6, the resulting network contained 353 relationships between 171 TFs and 26 FreDE_Paths (Supplementary Table S8). Figure 5 shows the network representation of the predicted TF-metabolic pathway relationships of Arabidopsis. On average, each pathway was predicted to be regulated by approximately 13 TFs, and each TF regulated two pathways. The metabolic pathway 'very long chain fatty acid biosynthesis I' (Pathway ID: PWY-5080) was the pathway regulated by the largest number of TFs and was predicted to be regulated by 32 TFs. As a predicted regulator shared by the largest number of metabolic pathways, the TF *WRKY45* potentially regulated ten different metabolic pathways, including ARO-PWY, CAMALEXIN-SYN, COMPLETE-ARO-ARA-PWY, ETHYL-PWY, PWY-5080, PWY-581, PWY-601, PWY-6051, PWY-6842 and TRPSYN-PWY. Even though the role of *WRKY45* in plant immunity has not been reported, extensive studies have firmly established the importance of numerous distinct WRKY members in plant immunity (Tsuda and Somssich 2015). Moreover, *WRKY45* has significantly enhanced expression in plant defense response to an avirulent strain of *P. syringae* (Dong et al. 2003), chitosan (Povero et al. 2011) or CaLCuV (Ascencio-Ibanez et al. 2008). In this work, *WRKY45* was also identified as upregulated in 13 of 14 infection conditions. These evidences show the potential role of *WRKY45* in plant immunity.

The CLR algorithm identified several regulatory relationships that have been reported in previous studies. For example, the camalexin biosynthesis pathway had high mutual information with TFs *MYB51* and *NAC042*. Notably, the expression of the two TFs was positively correlated with the expression of the camalexin biosynthesis pathway (Fig. 5). Previous work has shown that *MYB51* is a key regulator of camalexin biosynthesis (Frerigmann et al. 2015), and *NAC042* has also been proven to be involved in the regulation of camalexin biosynthesis (Saga et al. 2012). Moreover, regulatory relationships between *MYB51* and two metabolic genes (i.e., *CYP79B2* and *CYP79B3*) in camalexin biosynthesis metabolic pathways have already been identified (Gigolashvili et al. 2007). We also collected 149 TFs assigned as defense-related genes from the review article of Tsuda and Somssich (Tsuda and Somssich 2015). Among 171 TFs, 25 were overlapped with these 149 plant defense-related TFs, and this overlap was statistically significant compared with the random control (hypergeometric

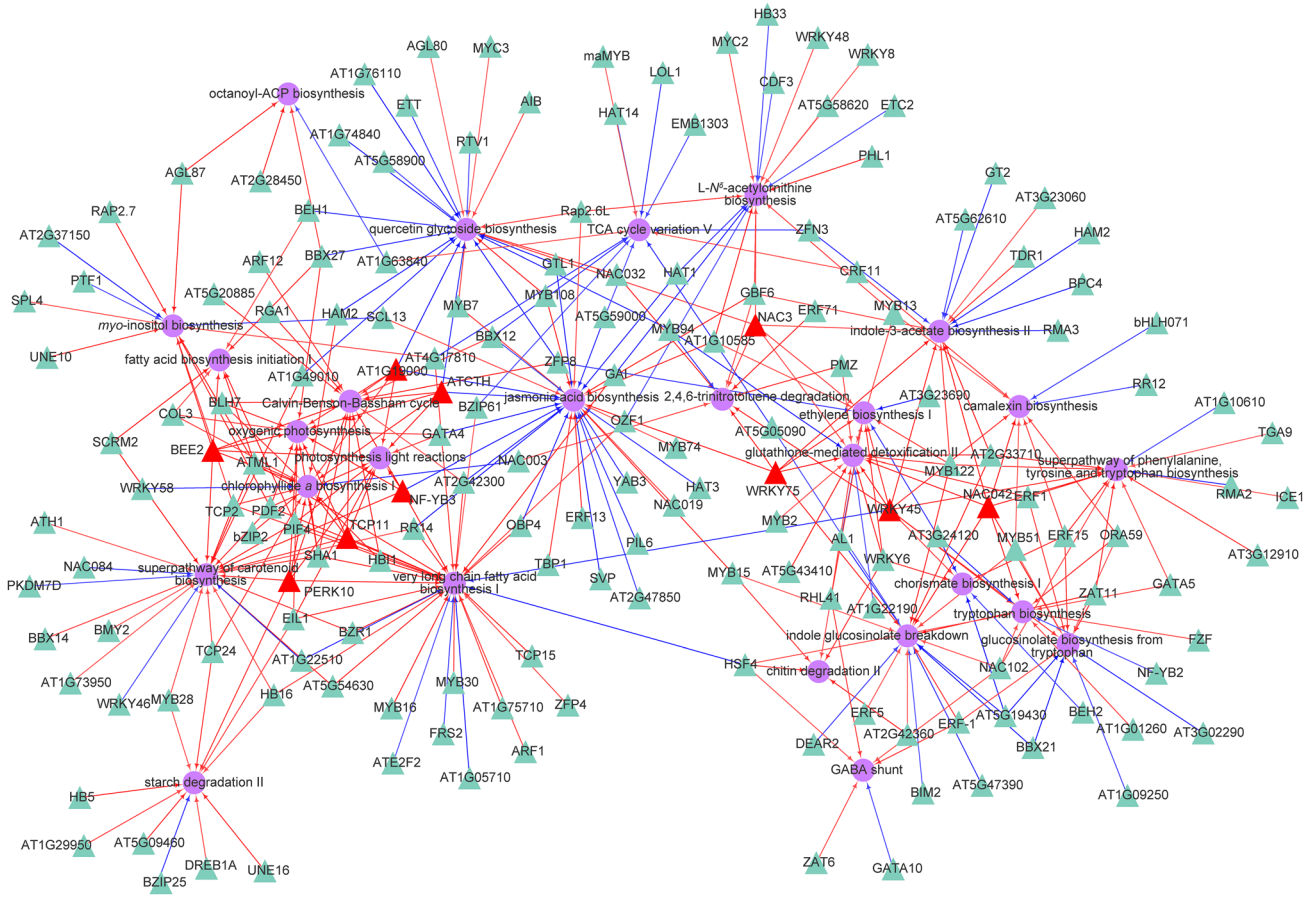

Fig. 5 Network representation of the predicted regulatory relationship between 171 TFs and 26 FreDE_Paths. *Triangle nodes* denote TFs, while *circular nodes* stand for pathways. *Arrows* show potential regulatory relationship between TFs and FreDE_Paths. The *color scheme of edges* represents PCCs ranging from −1 (*blue*) to 1 (*red*). This figure was prepared using Cytoscape. The top 10 TFs that regulated the largest number of pathway were colored in *red*

test, p-value = 9.24×10^{-7}). The significant overlap further showed the importance of 171 TFs in plant immunity.

The high mutual information between these TFs and metabolic pathways may provide new hints on how these TFs regulate plant immunity. For example, overexpression of *WRKY75* in Arabidopsis enhances resistance to *S. sclerotiorum* (Choi et al. 2014) and reduces bacterial growth of *Pectobacterium carotovorum* by positively regulating JA-mediated defense signaling (Choi et al. 2014). In our CLR analysis, we observed high mutual information between *WRKY75* and all three frequently upregulated hormone biosynthesis pathways (i.e., JA biosynthesis, auxin biosynthesis and ET biosynthesis; Supplementary Table S8). It is possible that *WRKY75* regulates plant defense by regulating the biosynthesis of plant hormones. Another example of the predicted significant regulatory relationships was *BEE2* (*brassinosteroid enhanced expression2*) and three frequently downregulated photosynthesis-related pathways, namely, oxygenic photosynthesis, photosynthesis light reactions and the Calvin–Benson–Bassham cycle. Overexpression of *BEE2* partially inhibits immunity, and it is speculated that *BEE2* acts redundantly with *HBI1* (*Homolog of BEE2 interacting with IBH1*; Malinovsky et al. 2014). It was established that *HBI1* might inhibit immunity through regulating photosynthesis (Fan et al. 2014). Here, we also observed high mutual information between *HBI1* and the Calvin–Benson–Bassham cycle. These results further enhanced the speculation that both *BEE2* and *HBI1* inhibit plant immunity by regulating photosynthesis.

Conclusions

We performed a large-scale transcriptome analysis based on previously published microarray data to explore the reprogramming of metabolic pathways during plant defense responses to 14 different pathogens. This analysis represents the largest transcriptome analysis of plant metabolism reprogramming during plant defense responses, and

we would like to emphasize the following major findings. First, we highlighted the extensive changes of metabolic genes during plant defense responses by differential expression analysis and identified 23 frequently differentially expressed metabolic genes. Second, we identified 26 metabolic pathways that were frequently differentially expressed in more than 60% of infected samples, such as camalexin biosynthesis, JA biosynthesis, chitin degradation and photosynthesis, demonstrating the existence of common pathways responding to different pathogens. Clustering analysis showed that infected and control samples were divided into distinct groups based on the expression levels of 26 FreDE_Paths. This finding further supports that these pathways represent common responsive pathways in plant immunity to different pathogens. Third, by comparing with abiotic stresses, we showed that expression pattern of FreDE_Paths identified from biotic stresses were more consistent than these from abiotic stresses. Finally, we identified a number of associations between 171 TFs and the 26 FreDE_Paths, such as the regulation between *MYB51* and camalexin biosynthesis. These predicted associations provide information about how plants regulate these pathways when responding to pathogens. Taken together, it is hoped that our current analyses can provide new direction for the development of broad-spectrum disease-resistant crops. For instance, it is possible that fine-tuning of these FreDE_Paths might result in the enhancement of plant resistance without compromising crop yield.

Materials and methods

Data collection and preprocessing

Raw transcriptional data of Arabidopsis in response to the infections of 14 different pathogens were downloaded from GEO and ArrayExpress. Raw transcriptional data in response to eight different abiotic stresses were downloaded from AtGenExpress (http://jsp.weigelworld.org/AtGenExpress/resources/). We only used transcriptional datasets obtained from the most comprehensive Arabidopsis expression array platform GPL198. The above raw data were normalized by RMA using the Bioconductor R package affy (Gautier et al. 2004). Probe sets were mapped to their corresponding AGI (Arabidopsis Genome Initiative) gene identifiers according to the annotation file from GEO and replicated probes of the same gene were averaged.

Arabidopsis metabolic pathway information was retrieved from the Aracyc database (Version 13.0) at the plant metabolic network (http://www.plantcyc.org/). Genes in each pathway were filtered using expression data, and only pathways with genes detected on the microarray were kept for further analysis. Genes from metabolic pathways were defined as metabolic genes.

Differential expression analysis

For the infection of each pathogen, the function RPadvance in the BioConductor package RankProd (Hong et al. 2006), which is specifically designed for meta-analysis by taking into consideration the different origins of samples, was used to identify DEGs between infected samples and the corresponding control samples. Briefly, the FCs of genes between infected and control samples from individual studies were first translated to the ranks of genes. Then, the combined rank of each gene from multiple studies was defined as the rank product. Independent permutated expression data were used to calculate the null density of the rank product and to determine the *p*-value associated with each gene. Finally, in this work, a gene was defined as differentially expressed with a *p*-value less than 0.05 and an average FC larger than 1.5.

Gene set enrichment analysis

GSEA was used to assess metabolic pathways significantly differentially expressed during plant defense response. GSEA is a statistical method that determines if an a priori defined set of genes shows statistically significant concordant upregulated or downregulated expression between two conditions (i.e., infection and control in this work; Subramanian et al. 2005). By excluding gene sets with fewer than five or more than 1000 genes, 395 metabolic pathways were used for GSEA. To identify metabolic pathways with significant expression changes for each infected sample, we use the pre-ranked GSEA tool (GSEAPreRanked; http://www.broadinstitute.org/gsea/, version 2.2.1) directly. GSEAPreranked runs GSEA against a user-supplied ranked list of genes. For each gene, we first calculated its expression change ($\log_2$ FC) in each infected sample relative to the corresponding normal samples. Then, for each infected sample, genes were ranked from the highest to the lowest by $\log_2$ FC. Ranked genes were then used as inputs to GSEAPreRanked with the default options except that the permutation type was set to Gene_set with 1000 permutations. Metabolic pathways with *p*-values less than 0.05 were defined as significantly differentially expressed metabolic pathways.

GO enrichment analysis

GO enrichment analysis was performed using the plugin BiNGO 3.02 (Maere et al. 2005) in Cytoscape (Shannon et al. 2003) with the "GO biological process" category. Using the whole annotations of Arabidopsis genes as the

reference set and the hypergeometric test as the statistical test, over-represented terms were selected with a significance level of 0.05 after the Benjamini–Hochberg correction.

Clustering analysis

Expression samples were clustered using a hierarchical clustering method (Eisen et al. 1998) with an average linkage algorithm (Sokal 1958). Briefly, the pathway score for a given metabolic pathway was first calculated as the average expression value of genes included in the pathway. Then, the Euclidean distance function was used to measure the distance between two samples based on the pathway scores of 26 FreDE_Paths. Finally, the hierarchical clustering algorithm was employed to cluster different samples based on the Euclidean distances.

TF-metabolic pathway regulation analysis

The CLR method (Faith et al. 2007) was used to identify significant relationships between 1707 Arabidopsis TFs and 26 FreDE_Paths. The set of 1707 TFs were collected from PlantTFDB (Jin et al. 2014) and AtTFDB, a sub database of the Arabidopsis gene regulatory information server (AGRIS) (Yilmaz et al. 2011) and filtered using expression data. For gene i in the infected sample m, we first calculated its expression change ΔE_i^m as the FC between the infected sample m and the average of the corresponding control samples. For pathway j in the infected sample m, the expression change was calculated as $\Delta E\_path_j^m = \sum_{n=1}^{n_j} \Delta E_n^m / n_j$, where n_j is the number of genes within the metabolic pathway j. Then, the mutual information (MI) between TF i and metabolic pathway j was calculated across all 69 infected samples:

$$MI_{ij} = MI(\Delta E_i, \Delta E\_path_j) \quad (1)$$

Similar to Hu et al. (2013), all MI values were computed using ten bins of ΔE and ΔE_path. Next, the background distribution was constructed from two sets of MI values: the set of MI values between TF i and 26 FreDE_Paths $(MI_{i,1}, MI_{i,2}, MI_{i,3}, ..., MI_{i,26})$, and the set of MI values between metabolic pathway j and 1707 TFs $(MI_{1,j}, MI_{2,j}, MI_{3,j}, ..., MI_{1707,j})$. After that, the z-scores z_i and z_j of M_{ij} relative to the background distribution were calculated. Finally, the CLR interaction Z-score for the pair between gene i and pathway j was calculated as

$$Z_{ij} = \sqrt{\left(z_i^2 + z_j^2\right)/2} \quad (2)$$

In the meantime, we calculated the PCC for the gene i and the pathway j based on their expression changes across 69 infected samples. In our work, a TF-pathway relationship with a Z-score no less than 2.0 and an absolute value of PCC no less than 0.6 was regarded as significant.

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Author contributions ZJ designed the study, performed the analyses and drafted the manuscript. ZZ and FH revised the manuscript. ZZ supervised the study.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

References

Ahuja I, Kissen R, Bones AM (2012) Phytoalexins in defense against pathogens. Trends Plant Sci 17:73–90. doi:10.1016/j.tplants.2011.11.002

Asano T, Kimura M, Nishiuchi T (2012) The defense response in *Arabidopsis thaliana* against *Fusarium sporotrichioides*. Proteome Sci 10:61. doi:10.1186/1477-5956-10-61

Ascencio-Ibanez JT, Sozzani R, Lee TJ, Chu TM, Wolfinger RD, Cella R, Hanley-Bowdoin L (2008) Global analysis of Arabidopsis gene expression uncovers a complex array of changes impacting pathogen response and cell cycle during geminivirus infection. Plant Physiol 148:436–454. doi:10.1104/pp.108.121038

Barrett T et al (2013) NCBI GEO: archive for functional genomics data sets—update. Nucleic Acids Res 41:D991–D995. doi:10.1093/nar/gks1193

Bennett RN, Wallsgrove RM (1994) Secondary metabolites in plant defence mechanisms. New Phytol 127:617–633. doi:10.1111/j.1469-8137.1994.tb02968.x

Bilgin DD, Zavala JA, Zhu JIN, Clough SJ, Ort DR, DeLucia EH (2010) Biotic stress globally downregulates photosynthesis genes. Plant Cell Environ 33:1597–1613. doi:10.1111/j.1365-3040.2010.02167.x

Bolton MD (2009) Primary metabolism and plant defense—fuel for the fire. Mol Plant Microbe Interact 22:487–497. doi:10.1094/mpmi-22-5-0487

Brazma A et al (2003) ArrayExpress–a public repository for microarray gene expression data at the EBI. Nucleic Acids Res 31:68–71. doi:10.1093/nar/gkg091

Choi C, Park YH, Kwon SI, Yun C, Ahn I, Park SR, Hwang D-J (2014) Identification of AtWRKY75 as a transcriptional regulator in the defense response to Pcc through the screening of Arabidopsis activation-tagged lines. Plant Biotechnol Rep 8:183–192. doi:10.1007/s11816-013-0308-x

Clay NK, Adio AM, Denoux C, Jander G, Ausubel FM (2009) Glucosinolate metabolites required for an Arabidopsis innate immune response. Science 323:95–101. doi:10.1126/science.1164627

Clifton R, Millar AH, Whelan J (2006) Alternative oxidases in Arabidopsis: A comparative analysis of differential expression in the gene family provides new insights into

function of non-phosphorylating bypasses. Biochim Biophys Acta 1757:730–741. doi:10.1016/j.bbabio.2006.03.009
Consortium GO (2004) The Gene Ontology (GO) database and informatics resource. Nucleic Acids Res 32:D258–D261. doi:10.1093/nar/gkh036
De Coninck B et al (2005) Arabidopsis AtcwINV3 and 6 are not invertases but are fructan exohydrolases (FEHs) with different substrate specificities. Plant Cell Environ 28:432–443. doi:10.1111/j.1365-3040.2004.01281.x
Dong J, Chen C, Chen Z (2003) Expression profiles of the Arabidopsis WRKY gene superfamily during plant defense response. Plant Mol Biol 51:21–37. doi:10.1023/A:1020780022549
Dong X, Jiang Z, Peng Y-L, Zhang Z (2015) Revealing shared and distinct gene network organization in Arabidopsis immune responses by integrative analysis. Plant Physiol 167:1158–1185. doi:10.1104/pp.114.254292
Eisen MB, Spellman PT, Brown PO, Botstein D (1998) Cluster analysis and display of genome-wide expression patterns. Proc Natl Acad Sci U S A 95:14863–14868
El Hadrami A, Adam LR, El Hadrami I, Daayf F (2010) Chitosan in plant protection. Mar Drugs 8:968–987. doi:10.3390/md8040968
Eulgem T (2005) Regulation of the Arabidopsis defense transcriptome. Trends Plant Sci 10:71–78. doi:10.1016/j.tplants.2004.12.006
Faith JJ et al (2007) Large-scale mapping and validation of *Escherichia coli* transcriptional regulation from a compendium of expression profiles. PLoS Biol 5:e8. doi:10.1371/journal.pbio.0050008
Fan M et al. (2014) The bHLH transcription factor HBI1 mediates the trade-off between growth and pathogen-associated molecular pattern–triggered immunity in Arabidopsis. Plant Cell. doi:10.1105/tpc.113.121111
Ferrari S, Plotnikova JM, De Lorenzo G, Ausubel FM (2003) Arabidopsis local resistance to *Botrytis cinerea* involves salicylic acid and camalexin and requires EDS4 and PAD2, but not SID2, EDS5 or PAD4. Plant J 35:193–205. doi:10.1046/j.1365-313X.2003.01794.x
Frerigmann H, Glawischnig E, Gigolashvili T (2015) The role of MYB34, MYB51 and MYB122 in the regulation of camalexin biosynthesis in *Arabidopsis thaliana*. Front Plant Sci 6:654. doi:10.3389/fpls.2015.00654
Gautier L, Cope L, Bolstad BM, Irizarry RA (2004) Affy—analysis of Affymetrix GeneChip data at the probe level. Bioinformatics 20:307–315. doi:10.1093/bioinformatics/btg405
Ghanta S, Chattopadhyay S (2011) Glutathione as a signaling molecule: another challenge to pathogens. Plant Signal Behav 6:783–788. doi:10.4161/psb.6.6.15147
Gigolashvili T, Berger B, Mock HP, Muller C, Weisshaar B, Flugge UI (2007) The transcription factor HIG1/MYB51 regulates indolic glucosinolate biosynthesis in *Arabidopsis thaliana*. Plant J 50:886–901. doi:10.1111/j.1365-313X.2007.03099.x
Grant JJ, Yun BW, Loake GJ (2000) Oxidative burst and cognate redox signalling reported by luciferase imaging: identification of a signal network that functions independently of ethylene, SA and Me-JA but is dependent on MAPKK activity. Plant J 24:569–582. doi:10.1046/j.1365-313x.2000.00902.x
Hanqing F, Kun S, Mingquan L, Hongyu L, Xin L, Yan L, Yifeng W (2010) The expression, function and regulation of mitochondrial alternative oxidase under biotic stresses. Mol Plant Pathol 11:429–440. doi:10.1111/j.1364-3703.2010.00615.x
He F, Yoo S, Wang D, Kumari S, Gerstein M, Ware D, Maslov S (2016) Large-scale atlas of microarray data reveals the distinct expression landscape of different tissues in Arabidopsis. Plant J 86:472–480. doi:10.1111/tpj.13175
Heilmann I, Mekhedov S, King B, Browse J, Shanklin J (2004) Identification of the Arabidopsis palmitoyl-monogalactosyldiacylglycerol Δ7-desaturase gene FAD5, and effects of plastidial retargeting of Arabidopsis desaturases on the fad5 mutant phenotype. Plant Physiol 136:4237–4245. doi:10.1104/pp.104.052951
Hok S, Danchin EG, Allasia V, Panabieres F, Attard A, Keller H (2011) An Arabidopsis (malectin-like) leucine-rich repeat receptor-like kinase contributes to downy mildew disease. Plant Cell Environ 34:1944–1957. doi:10.1111/j.1365-3040.2011.02390.x
Hong F, Breitling R, McEntee CW, Wittner BS, Nemhauser JL, Chory J (2006) RankProd: a bioconductor package for detecting differentially expressed genes in meta-analysis. Bioinformatics 22:2825–2827. doi:10.1093/bioinformatics/btl476
Hu J et al (2013) Heterogeneity of tumor-induced gene expression changes in the human metabolic network. Nat Biotechnol 31:522–529. doi:10.1038/nbt.2530
Hull R (2002) Virus Infection, Plant. In: Encyclopedia of Molecular Biology. Wiley, Hoboken. doi:10.1002/047120918X.emb1662
Huot B, Yao J, Montgomery BL, He SY (2014) Growth-defense tradeoffs in plants: a balancing act to optimize fitness. Mol Plant 7:1267–1287. doi:10.1093/mp/ssu049
Jiang Z, Dong X, Li Z-G, He F, Zhang Z (2016a) Differential coexpression analysis reveals extensive rewiring of Arabidopsis gene coexpression in response to *Pseudomonas syringae* infection. Sci Rep 6:35064. doi:10.1038/srep35064
Jiang Z, Dong X, Zhang Z (2016b) Network-based comparative analysis of *Arabidopsis* immune responses to *Golovinomyces orontii* and *Botrytis cinerea* infections. Sci Rep 6:19149. doi:10.1038/srep19149
Jin J, Zhang H, Kong L, Gao G, Luo J (2014) PlantTFDB 3.0: a portal for the functional and evolutionary study of plant transcription factors. Nucleic Acids Res 42:D1182–D1187. doi:10.1093/nar/gkt1016
Jones JDG, Dangl JL (2006) The plant immune system. Nature 444:323–329. doi:10.1038/nature05286
Kai K et al (2008) Scopoletin is biosynthesized via ortho-hydroxylation of feruloyl CoA by a 2-oxoglutarate-dependent dioxygenase in *Arabidopsis thaliana*. Plant J 55:989–999. doi:10.1111/j.1365-313X.2008.03568.x
Kilian J et al (2007) The AtGenExpress global stress expression data set: protocols, evaluation and model data analysis of UV-B light, drought and cold stress responses. Plant J 50:347–363. doi:10.1111/j.1365-313X.2007.03052.x
Kim S-J et al (2004a) Functional reclassification of the putative cinnamyl alcohol dehydrogenase multigene family in Arabidopsis. Proc Natl Acad Sci U S A 101:1455–1460. doi:10.1073/pnas.0307987100
Kim SJ et al (2004b) Functional reclassification of the putative cinnamyl alcohol dehydrogenase multigene family in Arabidopsis. Proc Natl Acad Sci U S A 101:1455–1460. doi:10.1073/pnas.0307987100
Kliebenstein DJ (2012) Plant defense compounds: systems approaches to metabolic analysis. Annu Rev Phytopathol 50:155–173. doi:10.1146/annurev-phyto-081211-172950
Kliebenstein DJ, Rowe HC, Denby KJ (2005) Secondary metabolites influence Arabidopsis/Botrytis interactions: variation in host production and pathogen sensitivity. Plant J 44:25–36. doi:10.1111/j.1365-313X.2005.02508.x
La Camera S et al (2009) The Arabidopsis patatin-like protein 2 (PLP2) plays an essential role in cell death execution and differentially affects biosynthesis of oxylipins and resistance to pathogens. Mol Plant Microbe Interact 22:469–481. doi:10.1094/MPMI-22-4-0469
Less H, Angelovici R, Tzin V, Galili G (2011) Coordinated gene networks regulating Arabidopsis plant metabolism in response to various stresses and nutritional cues. Plant Cell 23:1264–1271. doi:10.1105/tpc.110.082867

Li H, Zhang Z (2016) Systems understanding of plant-pathogen interactions through genome-wide protein-protein interaction networks. Front Agr Sci Eng 3:102–112. doi:10.15302/j-fase-2016100

Lohman KN, Gan S, John MC, Amasino RM (1994) Molecular analysis of natural leaf senescence in *Arabidopsis thaliana*. Physiol Plant 92:322–328. doi:10.1111/j.1399-3054.1994.tb05343.x

Lv Q, Cheng R, Shi T (2014) Regulatory network rewiring for secondary metabolism in *Arabidopsis thaliana* under various conditions. BMC Plant Biol 14:180. doi:10.1186/1471-2229-14-180

Maere S, Heymans K, Kuiper M (2005) BiNGO: a Cytoscape plugin to assess overrepresentation of Gene Ontology categories in biological networks. Bioinformatics 21:3448–3449. doi:10.1093/bioinformatics/bti551

Malinovsky FG et al (2014) Antagonistic regulation of growth and immunity by the Arabidopsis basic helix-loop-helix transcription factor homolog of brassinosteroid enhanced expression interacting with increased leaf inclination binding bHLH1. Plant Physiol 164:1443–1455. doi:10.1104/pp.113.234625

Margis-Pinheiro M, Martin C, Didierjean L, Burkard G (1993) Differential expression of bean chitinase genes by virus infection, chemical treatment and UV irradiation. Plant Mol Biol 22:659–668

Meng S, Torto-Alalibo T, Chibucos MC, Tyler BM, Dean RA (2009) Common processes in pathogenesis by fungal and oomycete plant pathogens, described with Gene Ontology terms. BMC Microbiol 9:S7. doi:10.1186/1471-2180-9-s1-s7

Moore JW, Loake GJ, Spoel SH (2011) Transcription dynamics in plant immunity. The Plant Cell 23:2809–2820. doi:10.1105/tpc.111.087346

Mueller LA, Zhang P, Rhee SY (2003) AraCyc: A biochemical pathway database for Arabidopsis. Plant Physiol 132:453–460. doi:10.1104/pp.102.017236

Pandey SP, Roccaro M, Schon M, Logemann E, Somssich IE (2010) Transcriptional reprogramming regulated by WRKY18 and WRKY40 facilitates powdery mildew infection of Arabidopsis. Plant J 64:912–923. doi:10.1111/j.1365-313X.2010.04387.x

Piasecka A, Jedrzejczak-Rey N, Bednarek P (2015) Secondary metabolites in plant innate immunity: conserved function of divergent chemicals. New Phytol 206:948–964. doi:10.1111/nph.13325

Pieterse CMJ, Does DVd, Zamioudis C, Leon-Reyes A, Wees SCMV (2012) Hormonal modulation of plant immunity. Annu Rev Cell Dev Biol 28:489–521. doi:10.1146/annurev-cellbio-092910-154055

Povero G et al (2011) Transcript profiling of chitosan-treated Arabidopsis seedlings. J Plant Res 124:619–629. doi:10.1007/s10265-010-0399-1

Rojas CM, Senthil-Kumar M, Tzin V, Mysore K (2014) Regulation of primary plant metabolism during plant-pathogen interactions and its contribution to plant defense. Front Plant Sci 5:17. doi:10.3389/fpls.2014.00017

Saga H et al (2012) Identification and characterization of ANAC042, a transcription factor family gene involved in the regulation of camalexin biosynthesis in Arabidopsis. Mol Plant Microbe Interact 25:684–696. doi:10.1094/MPMI-09-11-0244

Saisho D, Nambara E, Naito S, Tsutsumi N, Hirai A, Nakazono M (1997) Characterization of the gene family for alternative oxidase from *Arabidopsis thaliana*. Plant Mol Biol 35:585–596. doi:10.1023/a:1005818507743

Sanchez-Vallet A, Ramos B, Bednarek P, Lopez G, Pislewska-Bednarek M, Schulze-Lefert P, Molina A (2010) Tryptophan-derived secondary metabolites in *Arabidopsis thaliana* confer non-host resistance to necrotrophic *Plectosphaerella cucumerina* fungi. Plant J 63:115–127. doi:10.1111/j.1365-313X.2010.04224.x

Schlaeppi K, Abou-Mansour E, Buchala A, Mauch F (2010) Disease resistance of Arabidopsis to Phytophthora brassicae is established by the sequential action of indole glucosinolates and camalexin. Plant J 62:840–851. doi:10.1111/j.1365-313X.2010.04197.x

Shannon P et al (2003) Cytoscape: a software environment for integrated models of biomolecular interaction networks. Genome Res 13:2498–2504. doi:10.1101/gr.1239303

Sokal RR (1958) A statistical method for evaluating systematic relationships. Univ Kans Sci Bull 38:1409–1438.

Subramanian A et al (2005) Gene set enrichment analysis: A knowledge-based approach for interpreting genome-wide expression profiles. Proc Natl Acad Sci U S A 102:15545–15550. doi:10.1073/pnas.0506580102

Taguchi G, Ubukata T, Nozue H, Kobayashi Y, Takahi M, Yamamoto H, Hayashida N (2010) Malonylation is a key reaction in the metabolism of xenobiotic phenolic glucosides in Arabidopsis and tobacco. Plant J 63:1031–1041. doi:10.1111/j.1365-313X.2010.04298.x

Tsuda K, Somssich IE (2015) Transcriptional networks in plant immunity. New Phytol 206:932–947. doi:10.1111/nph.13286

van Wees SC, Chang HS, Zhu T, Glazebrook J (2003) Characterization of the early response of Arabidopsis to *Alternaria brassicicola* infection using expression profiling. Plant Physiol 132:606–617. doi:10.1104/pp.103.022186

Vanlerberghe GC (2013) Alternative oxidase: a mitochondrial respiratory pathway to maintain metabolic and signaling homeostasis during abiotic and biotic stress in plants. Int J Mol Sci 14:6805–6847. doi:10.3390/ijms14046805

Wagner U, Edwards R, Dixon DP, Mauch F (2002) Probing the Diversity of the Arabidopsis glutathione S-Transferase Gene Family. Plant Mol Biol 49:515–532. doi:10.1023/a:1015557300450

Yilmaz A, Mejia-Guerra MK, Kurz K, Liang X, Welch L, Grotewold E (2011) AGRIS: the Arabidopsis gene regulatory information server, an update. Nucleic Acids Res 39:D1118–D1122. doi:10.1093/nar/gkq1120

Zeier J (2013) New insights into the regulation of plant immunity by amino acid metabolic pathways. Plant Cell Environ 36:2085–2103. doi:10.1111/pce.12122

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