Title: Transcriptional reprogramming during effector/flg22-triggered immune is independent of defense phytohormone signaling networks

Short Title: ETI and PTI independent of SA/JA/ET/PAD4 signaling

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One-sentence summary: JA/ethylene/PAD4/SA signaling network is required for resistance to *Pseudomonas syringae* but not for the activation of PTI and ETI.

Authors' contributions

NZ conceived the original research plan; NZ analyzed the data, performed statistical analyses, data visualization, and wrote the manuscript; ZF authored and reviewed drafts of the paper. All authors read and approved the final manuscript.

Abstract

Plants rely on the innate immune system to sense and respond to a wide range of lifestyle pathogens and to facilitate their survival in natural ecosystems. Pathogen-associated molecular patterns (PAMP)-triggered immunity (PTI) and effector-triggered immunity (ETI) are designated as a two-branched system of innate immunity. Although PTI/ETI share a series of downstream molecular events, systematic analysis of convergent and divergent signaling in PTI/ETI is currently lacking. The phytohormones salicylic acid (SA) and jasmonic acid (JA) are considered to constitute the hormonal backbone of plant immunity, are functionally antagonistic, and play essential roles in defending against biotrophic and necrotrophic pathogens, respectively. However, the distinct performance of two phytohormones in PTI/ETI remains unclear. Here, we systemically investigate

and validate the reprogramming of molecular networks during PTI and ETI. Using publicly available *Arabidopsis* RNA sequence data from 560 samples, we construct a co-expression network under Mock conditions and then explore the differential expression/co-expression changes during PTI, ETI, and *Pto* DC3000 infection. During PTI and ETI, one-third of genes in the *Arabidopsis* genome exhibit the same directional differential expression in a manner independent of JA/ethylene/PAD4/SA signaling but show differential co-expression patterns. However, the defense phytohormone network is required for defense against *Pto* DC3000 infection. We also exhibit the use of this network in prioritizing genes that functioned closely with the proteins directly targeted by elicitors. Overall, this study will deepen our understanding of plant transcriptome in plant immunity and provide new insights into the mode of action of elicitors.

Keyword: PAMP-triggered immunity, Effector-triggered immunity, Salicylic acid, Jasmonic acid, Differential co-expression network analysis

Introduction

Through evolution, plants have developed pre-formed (chemical or physical) barriers and a two-tiered innate immune system to resist numerous microbial infections (Jones and Dangl, 2006). Their first line of defense is activated by conserved microbial molecules (e.g. flg22, elf18, and chitin) that are recognized by cell surface-localized pattern recognition receptors (e.g. receptor-like kinases or receptor-like proteins; RLKs and RLPs), which trigger pattern-triggered immunity (PTI) (Macho and Zipfel, 2014). While these measures are effective in defending against non-pathogenic microbes, adapted pathogens (e.g. *Pto* DC3000) can deliver effector proteins into plant cells where they thwart PTI and intercept the immune response (Jones and Dangl, 2006; Macho and Zipfel, 2015). Fortunately, receptors in plant cells (e.g. nucleotide-binding/leucine-rich-repeat (NLR) receptors, also known as resistant genes (R genes)) can either recognize these effectors directly or sense their interactions with other accessory proteins to trigger effector-triggered immunity (ETI) (Cui et al., 2015). The PTI and ETI systems evolve sequentially and have long been considered to act independently (Chisholm et al., 2006; Jones and Dangl, 2006). In some cases, they largely share downstream signaling machinery (e.g. hormone signaling, hypersensitivity response, and reactive oxygen species (ROS) burst)(Tsuda and Katagiri, 2010; Peng et al., 2017). However, the immune response elicited during ETI is stronger and more persistent than PTI (Tsuda and Katagiri, 2010).

60 Recently, PTI is found to be an indispensable component of ETI during bacterial infection, and ETI
 61 act through PTI-specific receptors (Yuan et al., 2020); Yuan et al. and Ngou et al. also showed that
 62 PAMPs- and effector-activated plant immunity synergistically activate stronger defense, and that the
 63 activation of ETI can promote unique PTI responses (e.g. activations of multiple protein kinases and
 64 NADPH oxidases, ROS production, and callose deposition) (Ngou et al., 2020; Yuan et al., 2020).
 65 These suggest that PTI and ETI may act on the same gene sets to elicit similar defense responses.
 66 Although previous studies have shown some overlap between flg22-induced innate immune
 67 response in *Arabidopsis* and Avr9 race-specific defense response in tobacco (Navarro et al., 2004), a
 68 systematic comparison between genes induced during PTI and ETI in *Arabidopsis* is lacking. This
 69 analysis may help to elucidate the convergence and divergence of PTI and ETI at the transcription
 70 level.

71

72 Salicylic acid (SA), jasmonic acid (JA), and ethylene are major phytohormones involved in plant
 73 innate immunity and act downstream of PTI/ETI (Zhang et al., 2018). SA and JA/ET-mediated
 74 signaling pathways are considered to be the backbone of the immune response to biotic invaders
 75 (Koornneef and Pieterse, 2008). In general, SA signaling is important for resistance to biotrophs or
 76 hemibiotrophs (e.g. *Hyaloperonospora parasitica* and *Pto* DC3000), whereas JA/ET signaling is
 77 associated with resistance to necrotrophs (e.g. *Alternaria brassicicola* and *Botrytis cinerea*)
 78 (Koornneef and Pieterse, 2008). However, PAMP-triggered PTI and NLR-mediated ETI have been
 79 shown to reduce both biotrophic and necrotrophic infections in many plant species (Staal et al.,
 80 2008; Lacombe et al., 2010; Newman et al., 2013; L et al., 2014; Na et al., 2020). The exact roles
 81 and interactions of these two antagonistic defense phytohormones in ETI and PTI are confusing. In
 82 previous researches, Tsuda et. al. showed that the four major sectors of defense phytohormone
 83 signaling network (JA/ethylene/PAD4/SA signaling) synergized for bacterial resistance in flg22-
 84 PTI, whereas they strongly compensated each other for bacterial resistance in AvrRpt2-ETI (Tsuda
 85 et al., 2009). Using the network reconstruction strategies, in which the SA/JA/ethylene/PAD4
 86 signaling sectors are virtually abolished and then restored by removing mutations one by one or in
 87 combination, Hillmer et al. showed that flg22-PTI signaling network is highly buffered against
 88 pathogenic perturbations (Hillmer et al., 2017). More recently, Mine et al. proposed that
 89 AvrRpt2/AvrRpm1-ETI is independent of SA/JA/ethylene/PAD4 signaling sectors because when
 90 ETI is triggered by avirulent *Pto* DC3000 expressing AvrRpt2, the *deps* mutant (in which the genes:
 91 *DDE2*, *EIN2*, *PAD4*, and *SID2* were removed) showed the same transcriptomic responses as in
 92 wild-type plants, but with a delay of several hours (Mine et al., 2018). Yuan et al. showed that

AvrRpt2-ETI rapidly elevates and enhances the PTI pathway in an SA-independent manner (Yuan et al., 2020). So, although numerous studies have shown that PTI/ETI is linked to SA/JA pathways, how SA/JA signaling interact to mediate downstream of PTI/ETI signaling remains a mystery.

Genome-wide transcriptional profiling has become an instrumental resource and is widely in plant immunity researches (Attaran et al., 2014; Lewis et al., 2015; Hickman et al., 2017; Hillmer et al., 2017; Mine et al., 2018; Hickman et al., 2019; Zhang et al., 2020a). Many genes related to plant defense are initially identified through traditional differential expression approaches. Although this approach has been very successful, much of the information behind gene expression datasets have been overlooked, such as the distinct interconnection between genes in response to different conditions (de la Fuente, 2010). Network-based systems analysis provides a more nuanced molecular definition of enigmatic plant immune systems than differential expression analysis (DEA) because it provides a natural framework in which genes encoding functionally related proteins or sharing common regulatory mechanisms are assembled into distinct components or modules (i.e., sets of co-expressed genes). These modules can be associated with a phenotype of interest (Le et al., 2010; Delahaye-Duriez et al., 2016; Hickman et al., 2017; Mostafavi et al., 2018; Srivastava et al., 2018; Zhang et al., 2020a). Furthermore, co-expression network approaches offer an unsupervised and context-specific perspective to identify modules as candidate regulators or drivers of disease states, independent of historical bias stemming from specific genes and pathway (Delahaye-Duriez et al., 2016; Mostafavi et al., 2018; Srivastava et al., 2018; Zhang et al., 2020a). Modern approaches, such as SpeakEasy algorithm (Gaiteri et al., 2015) and WGCNA algorithm (Langfelder and Horvath, 2008), can identify modules in which genes are highly co-express across samples. These co-expression approaches have been used to identify new candidate regulatory molecules of disease and plant defense (Mostafavi et al., 2018; Zhang et al., 2020a). Besides, differential co-expression analysis (DCA) is emerging to complement the shortage of traditional DEA (de la Fuente, 2010). Comparing the structure of the two co-expression networks provides insight into context-specific dysregulation underlying the co-expression patterns. Genes with strongly altered connectivity between cases and controls are considered to play key roles in the disease phenotype (de la Fuente, 2010). Also, differentially co-expressed modules (DCM) can be identified (Choi and Kendzierski, 2009).

Here, we performed a gene co-expression network analysis of the *Arabidopsis* genome-wide transcriptional profiles under normal condition (Mock) and unsupervised exploration of the biological processes and pathways perturbed during PTI and ETI activation. We aimed to address some currently unresolved questions, including the genes responsible for convergence and divergence of PTI and ETI, and how SA/JA signaling interplay to mediate downstream of PTI/ETI signaling. Finally, we sought to identify and prioritize co-expression modules and genes that play important roles in PTI and ETI, respectively. Thus, (i) we compared the alterations in differential expression profiling of each co-expression module after treatment with PAMPs (flg22, chitin, nlp20), ETI-eliciting bacterial strains infection (avirulent *Pto* DC3000 expressing AvrRpt2 or AvrRpm1), and virulent *Pto* DC3000 infection; (ii) For *Arabidopsis* Col-0 (wild type) and mutants (combined mutants of dde2, ein2, pad4, and sid2) under Mock and defense-activated conditions, we compared the alterations in differential expression profiling of genes responding to shared and divergent SA/JA signaling; (iii) Four differential co-expression methods were used to identify differentially co-expressed modules (DCMs) and differentially co-expressed genes/links (DCGs/DCLs) between PTI/ETI and Mock. We found that PTI and ETI have nearly identical transcriptomic responses; Most JA-responsive genes are repressed and SA-responsive genes are activated during PTI and ETI; In DCA, some known important regulators of ETI, such as *FLS2*, were identified, while the genes involved in early responses to PTI (e.g. activation of Ga^{2+} signaling, activation of ethylene signaling and activation of WRKY transcription factors (TFs)) and PTI-biomarkers such as *WRKY22* and *WRKY29* were identified. Our results were validated by independent datasets.

145

146 Results

147 Network construction

To unsupervised identify biological processes perturbed in PTI/ETI and to avoid other unknown-potential effects, we selected 71 Mock samples (Col-1 + mock) from 10 bio projects (composed of mutants, Mock, PAMPs treatment, ETI-eliciting bacterial strain infection, virulent *Pto* DC3000 infection; Supplemental Table S1) as a starting point for genome-wide co-expression network analysis. A summary of our analysis is shown in Fig. 1. Briefly, 560 *Arabidopsis* RNA-seq samples from 11 different studies are used for data preparation. After the quality check, the normalized gene expression values are combined into one large gene expression matrix. Surrogate-variable analysis using the ComBat algorithm is performed to eliminate batch effects originating from different

156 studies. Three outlier samples detected by Kolmogorov-Smirnov statistical test and upper quartile
 157 dispersion are removed, as well as the mutant samples described in the original study. Only samples
 158 annotated as Mock (or Col-1 + mock) are used to build gene co-expression networks (modules)
 159 implemented by SpeakEasy consensus clustering algorithm (Gaiteri et al., 2015). As a result, we
 160 identify 67 mutually exclusive modules, of which 42 modules ranged in size from 15 and 648 genes
 161 (mean and median module sizes of 252 and 264 genes, respectively) are retained (Supplemental
 162 Table S2). Co-expression sub-modules ('consensus modules') common in PTI, ETI, and *Pto*
 163 DC3000 infection are then identified within each co-expression module (Supplemental Table S2).
 164 Finally, we evaluate the differential expression and differential co-expression of genes within each
 165 module during PTI, ETI, and *Pto* DC3000 infection compared to Mock conditions.

166
 167 We then investigate whether these 42 modules are reproducible in gene expression datasets treated
 168 by PAMPs, Effectors, and *Pto* DC3000, or whether they are reproducible in independent gene
 169 expression datasets treated by Mock. To this end, we get expression datasets treated by PAMPs,
 170 effectors, and *Pto* DC3000 (see Supplemental Table S1) as well as two independent publicly
 171 available Mock-treated expression datasets (PRJNA224133 and SRP041507), and then evaluate
 172 modules' conservation using the Z_{summary} value (Langfelder et al., 2011). 32 (76%) of the 42
 173 modules are at least moderately preserved ($Z_{\text{summary}} > 5$) under at least three conditions, and nearly
 174 half of modules are significantly preserved ($Z_{\text{summary}} > 10$) under at least three conditions
 175 (Supplemental Figure S1). In summary, considering the overlap of modules preserved under
 176 different conditions, we suggest that most of the co-expression modules constructed under Mock
 177 condition are reproducible.

178
 179 The biological terms and canonical pathways enrichment analysis revealed that 42 modules are
 180 generally enriched for specific/unique functional terms (Supplemental Figure S2-5). The complete
 181 results of the functional enrichment analysis for each module are reported in and Supplemental
 182 Table S3. Among the enriched functional terms, the defense-related process (e.g. GO biological
 183 process: defense response, response to JA, response to SA, response to ethylene, plant-type
 184 hypersensitive response, systemic acquired resistance, induced systemic resistance; KEGG pathway:
 185 plant-pathogen interaction, MAPK signaling pathway – plant) predominantly present in co-
 186 expression modules M1/2/3/5/6/7/8/9.

187

188 By performing gene set enrichment analysis on TF families, R genes, RLPs, RLKs, we found that
189 WRKY, ERF, C3H TFs, R genes, RLPs, RLKs are mainly presented in co-expression modules
190 M1/2/3/5/6/7/8/9/40/41/51/53 (Supplemental Table S3 and Figure S6). A more detailed examination
191 reveals 13 WRKY TFs, 16 R genes, 20 RLKs, and six RLKs among 520 genes in M5; 27 R genes
192 and 12 RLPs among 392 genes in M6; and 11 R genes among 316 genes in M7 (Supplemental Table
193 S2). There are 72 WRKY TFs, 57 RLPs, 223 RLKs and 167 NBS-encoding R genes in *Arabidopsis*
194 (Dong et al., 2003; Yu et al., 2014). In summary, about 32% R genes, 34% WRKY TFs, 31% RLPs,
195 11% RLKs are found in M5/6/7.

196

197 TFs exert their activity in a plant cell by activating their respective regulons, which consist of
198 transcriptionally co-regulated operons. We are inspired by the VIPER and aREA algorithms. These
199 algorithms perform gene set enrichment analysis on regulons (constructed by reverse engineering
200 gene networks) to infer whether a protein is activated (Margolin et al., 2006; Alvarez et al., 2016),
201 rather than directly measuring protein activity. Here, we perform regulon enrichment analysis using
202 hypergeometric tests (see methods). As results shown in Supplemental Figure S7, 42 modules are
203 presumed to be regulated by specific/unique regulators. The gene regulated by WRKY TFs are
204 predominantly found in M2/3/5/6/7/8. Notably, among these modules, the genes in M5 are regulated
205 by 16 WRKY TFs (Supplemental Table S3). Taken together, the genes in M1/2/3/5/6/7/8/9 may play
206 important roles in plant defense. These also provide a global view of the functional and regulatory
207 structural framework of *Arabidopsis* under normal conditions.

208

209 **Transcriptional reprogramming during PTI and ETI was qualitatively similar**

210 As the co-expression modules are constructed base on Mock samples, gene expression patterns may
211 not be consistent across PTI, ETI and *Pto* DC3000 infection. Therefore, we use consensus-weighted
212 gene co-expression network analysis to identify consensus co-expression sub-modules common in
213 each module under these three conditions to resolve this problem (Supplemental Table S2). To
214 obtain a global overview of the similarities and differences in differential gene expression during
215 PTI, ETI, and *Pto* DC3000 infection, we demonstrate the temporal dynamics of differential
216 expression under these conditions on a range of datasets (Supplemental Figure S8). Consistent with
217 previous analyses, transcriptional reprogramming in PTI is rapid (starting within half an hour) and

transient, whereas transcriptional reprogramming in ETI is relatively slow (starting after 4 hours) but long-lasting. However, the reprogramming of induced and repressed genes is consistent during PTI, ETI, and *Pto* DC3000 infection. In addition, genes in M5:Brown, M7:Yellow appear to be activated only during PTI, whereas genes in M65:Turquoise, M66:Turquoise, and M67:Turquoise are activated only during ETI. Overall, this analysis revealed that transcriptional reprogramming during PTI and ETI are qualitatively similar, but temporally distinct.

However, the above results are only qualitative analyses. In order to quantitatively estimate the commonalities and differences during PTI and ETI, the mroast function in Limma R package (Wu et al., 2010) and the RKStest function in GSAR R package (Rahmatallah et al., 2017) are used to detect significant differentially expressed gene sets/modules. Given that both methods are only compatible with at least three sample datasets, only datasets from PRJNA379910, PRJNA412447, PRJNA491484, PRJNA549850, PRJNA348676, PRJNA313379, GSE56094, and PRJNA383861 are used for this analysis. Although two methods show similar results, only mroast results are retained because it not only perform a statistically-rigorous geneset test but also test for uni- or bi-direction regulation (whereas RKStest does not test for regulatory direction) (Wu et al., 2010; Rahmatallah et al., 2017). This result confirms the qualitative analysis (Fig. 2). In addition, to further confirm the above results, we perform the same analysis on independent datasets, including GSE56094, PRJNA313379, PRJNA383861, part of PRJNA348676 (Fig. 2). In GSE56094 (a microarray dataset), transcriptional reprogramming during infection of *Pto* DC3000 and *Pto* DC3000 *hrpA*- (which can not form a type III secretion system to deliver the suite of effectors into plant cells but triggers PTI (Lewis et al., 2015)) is nearly identical to the results of the RNAseq platform. In PRJNA348676, the flg22-treated *fls2* mutants are used as Mock samples because there is no true Mock sample (Col-1+ no treatment) and the *fls2* mutants lack specific perception of flg22 (Chinchilla et al., 2006; Hillmer et al., 2017). Here, upon flg22 treatment, the differential expression dynamics between Col-1 plants and *fls2* mutants were in line with above observation. We also observe that *rrsc* (*rpm1 rps2*) (a mutant lacking the AvrRpm1 and AvrRpt2 cognate receptors (Mine et al., 2018)) have no significant transcriptome response during ETI, except for genes in M65:turquoise, M66:turquoise, M67:turquoise, and M50:blue. Thus, the genes in M65:turquoise, M66:turquoise, and M67:turquoise are potentially independent of *Rpm1* or *RPS2* during ETI. Taken together, these results confirm that transcriptional reprogramming during PTI and ETI is qualitatively similar.

250

251 Genes uniquely activated during PTI are then examined in detail. The genes in M5:Brown are
 252 significantly upregulated by flg22, chitin, *Pto* DC3000 *hrpA*-, show no respond to *Pto* DC3000 and
 253 *Pto* AvrRpt2 infection (Fig. 2 and Supplemental Figure S8). Five (*FLS2*, *CRK29/34/41*,
 254 *AT1G66920/LRK10L-2.4*) out of 43 genes in this module are annotated as receptors (Supplemental
 255 Table S2). *FLS2* can specifically percept and bind bacterial flagellin (e.g. flg22), which then trigger
 256 PTI (Chinchilla et al., 2006). Consistently, Ngou et al. showed no significant change in the
 257 transcriptional level of *FLS2* during ETI triggered by ED in *Col-0/DEX::avrRpt2*, or *Col-*
 258 *0/DEX::avrRpm1* transgenic plants (Ngou et al., 2020). Although gene expression of *FLS2* differed
 259 in response to immune responses triggered by different effectors (Ngou et al., 2020), these results
 260 may suggest that *FLS2* is specifically activated in PTI rather than AvrRpt2-ETI. In addition,
 261 *AT5G18360/BARI* (a R gene), *AT5G49780* (a RLK), *AT1G63580/CRRSP6*, and *AT2G43610* are
 262 presented in this module and are involved in transmembrane receptor protein/kinase signaling
 263 pathway. These membrane-associated proteins (e.g. *FLS2* and *CRRSP6*) may play important roles
 264 in the transduction of early immune signals triggered on the external side of the plasma membrane
 265 into plant cells, as well as in cell-to-cell communication (Chinchilla et al., 2006; Göhre et al., 2008;
 266 Thomas et al., 2008). Recently, *BARI* was found to be required for recognition of HopB, a *Pto*
 267 DC3000 Type III Effector (Laflamme et al., 2020). *AT2G43610* is annotated as chitinase and
 268 contains a chitin-binding type-1 domain.

269

270 In M7:Yellow, 8 (*CW9*, *BNT1/AT5G11250*, *RDL5*, *PBL1*, *ATMORC7*, *NDR1*, *ERF114*, *ATLP-3*) out
 271 of 26 genes in this module are related to plant defense. Three (*CW9*, *BNT1/AT5G11250*, *RDL5*) of
 272 them are R genes. *PBL1* encodes the receptor-like cytoplasmic kinase and is required for flg22-
 273 induced calcium signaling and downstream-defense events (Ranf et al., 2014). *ATMORC7* appears
 274 to be co-expressed with multiple disease resistance genes and positively modulate *Arabidopsis*
 275 against the oomycete pathogen, *Hyaloperonospora arabidopsidis* (Harris et al., 2016). *NDR1* is
 276 required for the immune response mediated by R proteins containing coiled-coil domain (e.g. RPS2,
 277 RPS5, RPM1) by directly interacting with RIN4 (Day et al., 2006) (Knepper et al., 2011). These
 278 suggest that while these genes respond uniquely to PTI at transcription levels, these genes may be
 279 involved in ETI at post-transcription levels.

280

281 **Transcriptional reprogramming during ETI and PTI independent of the defense** 282 **phytohormone signaling networks**

283 In previous works, Tsuda et al. showed that the flg22/AvrRpt2-triggered *Arabidopsis* immune
284 network is almost entirely controlled by the defense phytohormone signaling network (mediated by
285 SA, JA, ET and *PAD4*) (Tsuda et al., 2009). In general, *DDE2*, *EIN2*, and *SID2* are essential
286 components of JA, ET, and SA signaling, respectively (Tsuda et al., 2009). Fg22/AvrRpt2-induced
287 resistance to *Pto* DC3000 is essentially intact in plants mutated in any of these four genes and
288 severely impaired in the quadruple mutant (*deps*) (Tsuda et al., 2009). Here, in both the *deps* and
289 *pad4 sid2* mutants, we found that defense module genes (e.g., M1/2/3/5/8) are down-regulated by
290 *Pto* DC3000 but up-regulated by the treatment of PAMPs and Effectors (Fig. 2 and Supplemental
291 Figure S8). In both the *pad4* and *sid2* mutants, these genes are up-regulated after *Pto* DC3000
292 infection, but with a delay of several hours (Supplemental Figure S8). These may indicate that an
293 intact phytohormone network is required for plant defense against *Pto* DC3000 and the rapid
294 establishment of robust resistance in the early stages of bacterial infection.

295

296 In addition, susceptible plants including *dde2*, *ein2*, *pad4*, *sid2*, *dde2 ein2*, *dde2 pad4*, *dde2 sid2*,
297 *ein2 pad4*, *ein2 sid2*, *pad4 sid2*, *dde2 ein2 pad4*, *dde2 ein2 sid2*, *dde2 pad4 sid2*, *ein2 pad4 sid2*,
298 *deps*, showed almost the same transcriptomic responses as the *fls2* mutants upon flg22 treatment
299 (Fig. 2). Indeed, ETI here is triggered by *Pto* DC3000, which carried “avirulent” effector genes.
300 These strains elicit ETI while also inevitably delivering PAMPs to the plant, thereby inducing PTI
301 (Hatsugai et al., 2017). Therefore, it is possible that PTI and ETI act simultaneously during this
302 “avirulent” *Pto* DC3000 strains infection. To rule out the possibility that the PTI signal affects ETI
303 signaling, we evaluate the above results using PRJNA383861 as a validation dataset where only the
304 ETI signaling could be activated by expressing *AvrRpt2* in *planta* (Hatsugai et al., 2017). As shown
305 in Fig. 2, most of genes in defense modules (e.g. M1/2/3/5/6/7) show significantly up-regulation
306 during ETI, PTI, and PTI+ETI (treated by flg22+ED) compared with Mock-treated *deps* mutants
307 (Fig. 2). So, these suggest that transcriptional reprogramming during ETI and PTI may be
308 independent of *DDE2*, *EIN2*, *PAD4*, or *SID2*-mediated phytohormone signaling network.

309

310 **SA signaling contributes significantly to PTI and ETI compared to JA signaling**

311 In general, JA and SA signaling are functional antagonistic and act downstream of PTI and ETI.
 312 However, their precise roles and interactions in PTI and ETI remain elusive. To investigate their
 313 interplay in PTI and ETI, we do the same analysis described above for the genes responsible for the
 314 divergence, convergence, and antagonism of JA/SA, which identified in previous work (see
 315 Methods for details) (Zhang et al., 2020b). Firstly, consensus sub-modules are identified using gene
 316 expression matrices from the datasets treated by PAMPs, effectors, and *Pto* DC3000 infection. Then,
 317 DEA and differentially expressed gene set/module analyses are performed on the PRJNA379910,
 318 PRJNA412447, PRJNA491484, PRJNA549850, PRJNA348676, PRJNA313379, GSE56094,
 319 PRJNA383861, and PRJNA224133 datasets. These results are summarized in Fig. 3 and
 320 Supplemental Figure S9. In wild-type plants, almost all genes activated by SA remain activated
 321 during PTI, ETI, and *Pto* DC3000 infection, whereas most of the genes uniquely activated by JA or
 322 activated by JA but repressed by SA are down-regulated during these processes; almost all genes
 323 uniquely repressed by SA or co-repressed by both phytohormones are also down-regulated during
 324 these processes, whereas some genes uniquely repressed by JA are up-regulated by PAMPs or
 325 effectors. In addition, almost all defense module genes (e.g. M1/2/3/5/6/7) are significantly up-
 326 regulated by SA and some are down-regulated by JA (Fig. 2). In conclusion, SA-responsive genes
 327 are major contributors to PTI and ETI compared to JA-responsive genes.

328

329 Here, we also found that in *pad4*, *sid2*, *sid2 pad4*, and *deps* mutants, SA-induced genes are
 330 repressed but up-regulated during PTI and ETI (Fig. 3 and Supplemental Figure S9). Since JA has
 331 an antagonistic effect on SA, there is no doubt that in the SA signaling-impaired mutants *pad4*, *sid2*,
 332 *sid2 pad4*, genes activated by SA and repressed by JA are up-regulated. At the meanwhile, in these
 333 signaling-impaired mutants, genes activated by SA but repressed by JA are upregulated during PTI,
 334 ETI and *Pto* DC3000 infection. These support the notion again that transcriptional reprogramming
 335 during ETI and PTI is independent of *DDE2*, *EIN2*, *PAD4*, or *SID2*-mediated phytohormone
 336 signaling networks. Notably, in *pad4*, *sid2 pad4*, *deps* mutants, genes uniquely activated by JA
 337 (except in JAup:salmon/Red) are repressed, but these genes are activated during the infections of
 338 *Pto* DC3000, “avirulent” *Pto* DC3000 strains and repressed during the treatment of PAMPs. This
 339 seems to indicate that the transcriptional reprogramming during PTI and ETI is no longer similar in
 340 these mutants. However, the gene in JAup:Black remained repressed during ED-induced ETI in
 341 *deps/DEX::avrRpt2 planta* (Fig. 3). We suggest that these apparent differences between PAMPs-PTI
 342 and “avirulent” *Pto* DC3000 strains-ETI in defense-deficient mutants may be caused by other
 343 inducers expressed by *Pto* DC3000, such as coronatine, a JA analog (Attaran et al., 2014).

344

345 **Prioritizing genes in module M2 and M5**

346 Here, we found that almost identical gene sets are induced during PTI and ETI. However, it has
 347 long been observed that the immune responses in ETI are more prolonged and robust than in PTI
 348 (Tsuda and Katagiri, 2010). Thus, plants appear to use different regulatory mechanisms to regulate
 349 common gene sets in different types of plant defense. Recently, DCA has been applied to explain
 350 differential regulatory processes among different diseases (Xu et al., 2015; Delahaye-Duriez et al.,
 351 2016). Under the premise that highly co-expressed genes are more likely to be co-regulated,
 352 significant changes in co-expression patterns between case and control may indicate changes in
 353 regulation. To explore the mechanisms of differential regulation between different types of plant
 354 defense, we perform DCAs in each module during PTI, ETI, *Pto* DC3000 infection, respectively.
 355 These DCAs are implemented by GSNCA, GSCA, DiffCoEx and diffcoexp methods (Choi and
 356 Kendzierski, 2009; Tesson et al., 2010; Rahmatallah et al., 2014; Wei et al., 2018). The results are
 357 summarized in Fig. 4. The GSNCA and GSCA methods display that the genes in almost all modules
 358 show significantly differential co-expression during PTI and ETI. These suggest that the
 359 *Arabidopsis* gene network is extensively rewired during the transition from vegetative growth to a
 360 defensive state. However, because the GSNCA and GSCA methods reveal so many DCMs, it is
 361 difficult for these two methods to determine which modules will play an important role in
 362 regulating plant defense. To this end, we focus on the results of the DiffCoEx and diffcoexp
 363 methods. These two methods are useful tools to identify DCGs with significantly more DCLs than
 364 by chance (Tesson et al., 2010; Wei et al., 2018). As shown in Figure 4, the number of DCGs
 365 identified by both methods have the same trend across modules, with M2/5 having the highest
 366 number of DCGs. Although the DiffCoEx method identified more DCGs than the diffcoexp method,
 367 the DCLs identified by the diffcoexp method was statistically significant. In addition, the DCLs
 368 identified by diffcoexp method could be divided into three categories: diff signed, same signed, or
 369 switched opposites (see detail definition in methods). These results are summarized in Fig. 5 and
 370 Supplemental Table S4. Our results show that nearly 54% of the DCLs are diff signed, 44% are
 371 same signed, and 2% DCLs are reversed associations. The total number of DCLs in PTI is nearly
 372 half that of ETI or *Pto* DC3000. This suggested that the *Arabidopsis* regulatory network is more
 373 extensively rewired during ETI/*Pto* DC3000 infection than during PTI. The most striking difference
 374 between PTI and ETI/*Pto* DC3000 infection is that only a small number of DCLs were significantly
 375 reversed associations during PTI. DCLs that are reversed association during ETI and *Pto* DC3000
 376 infections are predominantly found in M5. Besides, M5 is also the module that contains the highest

number of DCLs of all modules under effector and *Pto* DC3000 infection treatment. On the other hand, M2 is the module that contains the highest number of DCLs of all modules under PAMPs treatment. Thus, DCGs in M5 and M2 may play vital roles in modulating the transition from vegetative growth to a defensive state during ETI and PTI, respectively.

To show the reorganization of the network of plants shifting from growth to defense in M5 and M2, a direct comparison of the corresponding topological matrices under Mock or PTI/ETI/*Pto* DC3000 infection conditions are presented in Fig. 6. These demonstrate that defense activation reconfigures specific portions of the molecular interaction structures. We observe that most of the DCGs identified by the diffcoexp method are negatively correlated with other genes (non-DCGs) under the corresponding conditions compared to the Mock conditions. This further confirms the results of diffcoexp method. Notably, the DCGs during ETI and *Pto* DC3000 have a great overlap and mainly presented in M5:Brown, while the DCGs during PTI are mainly presented in M2:Blue. We find that DCGs during PTI have different distribution and few overlaps with those during ETI and *Pto* DC3000 (Fig. 6). These may provide clues to explore the distinct regulatory mechanisms during PTI and ETI.

During ETI, 28% (148 genes) of M5 are identified as DCGs. These effector-induced DCGs are enriched in ontologies capturing protein phosphorylation, response to bacterium, innate immune response, glucosinolate catabolic process, defense response. Notably, these effector-induced ontologies also capture cell surface receptor signaling pathway. A more detailed examination of these DCGs reveals several that encode known regulators of the plant immunity: *PDF1.2*, *PDF1.2c*, *PR1*, *FLS2*, *LYK4*, *SOBIR1*, *IOS1*, *ZAR1*, *SGT1A*, *WRKY53*, *BGLU26/PEN2*. *PDF1.2* and *PR1* are known biomarkers of JA- and SA-mediated defense response, respectively (Koornneef and Pieterse, 2008). *FLS2* and *LYK4* play vital roles in flg22 and chitin recognition, respectively, and mediate the PTI (Cao et al., 2014; Ranf et al., 2014). *SOBIR1* encodes a receptor-like kinase and functions in parallel with *PAD4* to modulate cell death and plant defense responses (Gao et al., 2009). *IOS1* encodes another receptor-like kinase and is critical for *BAK1*-dependent and *BAK1*-independent PTI (Yeh et al., 2016). *ZAR1* encodes a CC-NB-LRR receptor-like protein and mediated plant immunity independent R gene-mediated signaling genes including *EDS1*, *PAD4*, *EDS16*, *SID2*, *RBOHD/F*, *SGT1a*, *SGT1b*, *NDRI*, or *RARI* (Lewis et al., 2010). The TF encoded by *WRKY53* plays a vital role in defending against *Pto* DC3000 (Murray et al., 2007). Atypical myrosinase encoded by *PEN2* is

involved in glucosinolate metabolism and is an essential component of cell wall defense (Clay et al., 2009). The bacterial effectors can target FLS2, SOBIR1, and suppress host defense signaling (Göhre et al., 2008; Wu et al., 2018). Five of cysteine-rich receptor-like kinases (CRK5/14/15/29/41) are also identified as DCGs in this module. These CRKs are suggested to play important roles in the modulation of pathogen defense and programmed cell death (Wrzaczek et al., 2010). Besides, *AT5G18360*, *FLS2* and *CRK29* are the genes with the top 10 highest number of DCLs during ETI (Fig. 6 and Supplemental Table S4). *AT5G18360* was an R gene. Therefore, the DCGs we identified in this module may play important roles in ETI, while unknown functional DCGs may provide candidate genes for a better understanding of ETI. Here, we also find two negative regulators (AHL27, DMR6) of plant innate immunity and one growth inhibitors (RAV1). AHL27 negatively regulate PTI, and plants overexpressing *AHL20* show greater sensitivity to *Pto* DC3000 (Lu et al., 2010). *DMR6* suppresses plant immunity, and *dmr6* mutants are resistant to the bacterium *Pto* DC3000, the oomycete *Phytophthora capsici*, and the downy mildew *Hyaloperonospora arabidopsidis* (Zeilmaker et al., 2015). *RAV1* functions as a negative regulator during plant development and is down-regulated by brassinosteroid (Hu et al., 2004). These may provide clues for exploring the mechanism by which ETI triggered PTI inhibition and plant growth inhibition.

425

23% (145 genes) of genes in M2 are identified as DCGs during PTI. These PAMPs-induced DCGs are enriched in ontologies capturing the response to oxygen-containing compound, response to chemical, response to chitin, cation transport, negative regulation of brassinosteroid mediated signaling. Upon sensing flg22 by a surface-localized pattern-recognition receptor, a cascade of signaling events including cytosolic calcium (Ca^{2+}) influx, generation of ROS through the activation of plant NADPH oxidases, activation of mitogen-activated protein kinases, activation of WRKY TFs will occur within a time scale of minutes to an hour (Spallek, 2011). Ethylene Signaling is activated in response to flg22 and is required for the ROS burst in the early stages of PTI (Mersmann et al., 2010). Here, four gene (*ACA12*, *CAX7/CCX1*, *CCX2*, *SOS3*) involved in calcium signaling, six genes (*EFE*, *ACS11*, *RAP2.1*, *ERF53*, *ERF103*, *TEM1*) related to ethylene signaling and four WRKY TFs (*WRKY11*, *WRKY18*, *WRKY22*, *WRKY29*) are identified as DCGs in this module during PTI. Auto-inhibited Ca^{2+} -ATPases (ACA) are essential in defining the shape of calcium transients and triggering plant responses to various stimuli (Limonta et al., 2014). *CCX1* and *CCX2* are two out of five members of cation/ Ca^{2+} exchanger (CCX). CCX1 regulates ROS production and the initiation of leaf senescence in *Arabidopsis* (Li et al., 2016). CCX2 is directly related to the control of Ca^{2+} fluxes between the endoplasmic reticulum and the cytosol, thus

modulating Ca^{2+} homeostatic in the cytosol (Corso et al., 2018). *SOS3* encodes a Ca^{2+} sensor that is required for salt stress response (Sánchez-Barrena et al., 2005). *EFE* and *ACS11* are key factors in ethylene biosynthesis (Ma et al., 1993; T et al., 2003). *RAP2.1*, *ERF53*, *ERF103* and *TEM1* are ethylene-responsive TFs. *WRKY11*, *WRKY18*, *WRKY22*, *WRKY29* are activated by chitin and may play important roles in plant defense (Libault et al., 2007). *WRKY22* and *WRKY29* are also canonical marker genes of flg22-induced PTI pathway (Asai et al., 2002). These suggest that the DCGs in this module might provide potentially molecular connections between the PRR immune complexes and early downstream signaling. Another distinctive feature of these DCGs is three genes (*CYP94B3*, *AT5G05600*, *JRG21*) that negatively regulate JA signaling by inactivating JA or JA-Ile (Koo et al., 2011; Smirnova et al., 2017). These may explain the above observation that some of JA responsive genes are down-regulated or non-responsive during PTI (Fig 2 and Fig. 3). Besides, growth inhibitors are also identified as DCGs during PTI, including *RGL3*, *BZS1/BBX20*, *PP2A*. *RGL3* encodes an *Arabidopsis* DELLA protein that is a negative regulator of the gibberellin response (Wild et al., 2012). *BZS1/BBX20* encoded a B-box zinc finger protein that is a negative regulator of the brassinosteroid (BR) response (Fan et al., 2012). Cytoplasmic PP2A dephosphorylates BRI1 (which is a BR receptor) and represses the BR response, while nuclear PP2A dephosphorylates BZR1 and activates the BR response (Wang et al., 2016). BR promotes plant growth by inducing dephosphorylation of BZR1. BZR1 can transcriptionally repress *BZS1/BBX20* (Fan et al., 2012). However, de-phosphorylated BZR1 can not be detected after treatment with flg22 (Jiménez-Góngora et al., 2015). The transcriptional levels of BR biosynthetic marker genes *CPD* and *BR6ox2* are inhibited by flg22 (Jiménez-Góngora et al., 2015). Therefore, we suggest that flg22 may inhibit plant growth by suppressing BR/gibberellin signaling at the transcriptional level. These may provide clues for exploring the mechanism by which PTI triggers growth inhibition.

466

467 Discussion

It has long been observed that PTI and ETI broadly share plant immune responses such as ROS burst, protein phosphorylation, hormonal changes, but the kinetics and amplitude of these responses are different (Tsuda and Katagiri, 2010). The difference between PTI and ETI remains elusive, and both immune reactions can be considered as a continuum of signaling mechanisms (Thomma et al., 2011). Systematic exploration of the alteration in immune signaling networks from the Mock condition toward PTI or ETI will provide a bird-view to the difference between different types of

474 plant defenses. Here, based on two complementary methods, DEA and DCA, half of the genes in
475 the *Arabidopsis* genome are used to investigate these differences. Although there are a few genes
476 uniquely response to PTI or ETI, transcriptional reprogramming during PTI and ETI is qualitatively
477 similar. In spite that SA and JA/ET play important roles in plant immune systems, the expression of
478 defense-related genes during ETI and PTI is independent of the JA/ethylene/PAD4/SA network, but
479 the phytohormone network is required for defense against *Pto* DC3000 infection. DCAs reveal that
480 the co-expression patterns during PTI are different from those during ETI. This suggests that plants
481 employ different regulatory strategies to modulate the same genes during PTI and ETI. The DCGs
482 with the highest number of DCLs can provide candidate genes that play important roles in PTI or
483 ETI, such as *FLS2*, *LYK4*, *SOBIR1*, *PDF1.2*, *PR1*, *WRKY18*, *WRKY22*, *WRKY29*.

484

485 By evaluating additional defense responses that reduced *Pto* DC3000 infection performance by pre-
486 treating *Arabidopsis* (wild-type plants or combined mutants of *dde2*, *ein2*, *pad4*, and *sid2*) with
487 "avirulent" *Pto* DC3000 strains or *flg22*, Tsuda et al. found that perturbing each sector of the plant
488 hormone network separately had a limited effect on plant defense, but perturbing all four signaling
489 sectors simultaneously greatly attenuated *flg22*-PTI and *AvrRpt2*-ETI (Tsuda et al., 2009). However,
490 when two successive stresses are applied, the transcriptome profiles of the plants are very similar to
491 those of the second stress and are independent of the nature of the first stress, but the signatures of
492 the first stress can be recognized in the successive stress profiles (Coolen et al., 2016). The
493 bioassays with successive stress treatments exhibit that the first stress treatment has no strong effect
494 on the performance of the cabbage white butterfly *P. rapae* (Davila Olivas et al., 2016). These
495 suggest that plants shift their response to the most recent stress applied. So the significant signatures
496 of pre-treatment with *flg22* or avirulent strains may be dependent on *DDE2/EIN2/PAD4/SID2*-
497 mediated signaling and contribute to the reduced performance of *Pto* DC3000 infection. Here, we
498 show that in *deps* mutants, the defense module genes are activated by PAMPs and effectors, but
499 repressed by *Pto* DC3000 infection. Whereas in wild type plants, these genes can be activated by
500 PAMPs, effectors, and *Pto* DC3000 infection. Mine et al. suggest that *DDE2/EIN2/PAD4/SID2*-
501 mediated signaling was required for ETI to achieve a higher and faster transcriptional response in
502 the early stages of activation (Mine et al., 2018). So, the transcriptional reprogramming during
503 effector/*flg22*-triggered immunity is independent of the defense phytohormone signaling network.
504 Here, we also observe that almost all SA-responsive genes are upregulated during PTI and ETI,
505 while most JA-responsive genes are downregulated during PTI and ETI. Furthermore, most of the
506 genes in the defense module are also down-regulated by JA analogs but up-regulated by SA analogs.

Thus, SA signaling is a major contributor to PTI and ETI compared to the JA signaling. In the past decades, pathogens carrying effector genes are used to study ETI. But during infection of these pathogens, both PTI and ETI are at play. So, experimental design for the unique activation of ETI or PTI will be necessary.

Recently, PTI and ETI are found to mutually potentiate to activate a strong defense against pathogens and to prolong the duration of the immune response (Ngou et al., 2020; Yuan et al., 2020). The *Arabidopsis* PRR/co-receptor mutants, *fls2/efr/cerk1* and *bak1/bkk1/cerk1* mutants, are unable to mount an effective ETI against incompatible *Pto* DC3000 bacteria (Yuan et al., 2020). This suggests that ETI increases defense strength through PTI-specific receptors (Yuan et al., 2020). Here, the genes (e.g. *FLS2*, *LYK4*, *SOBIR1*, *IOS1*, *ZAR1*) encoding proteins related to PAMP-receptors are identified as DCGs in the module containing the highest number of DCLs during ETI. Moreover, some of these proteins are also targeted by effectors. For instance, *FLS2* can be directly degraded by the bacterial ubiquitin ligase *AvrPtoB* (Göhre et al., 2008). The *Pto* DC3000 effector *AvrPto* interacts with *SOBIR1*, resulting in the impairment of *Avr4*-triggered defense responses mediated by *SOBIR1* (Wu et al., 2018). *SOBIR1* and *ZAR1*-mediated immune responses are independent *PAD4*- and *SID2*-mediated signaling (Gao et al., 2009; Lewis et al., 2010). The DCGs identified here may play important roles in modulating ETI response and further support the point that ETI is independent of the *PAD4*/SA network. The production of ROS is a common immune output during PTI and ETI. Interestingly, in *AvrRpt2*-expressing *planta*, ED-triggered ETI did not induce ROS production within 24 hours, whereas co-treatment with *flg22* and ED triggered much stronger ROS accumulation than *flg22* treatment alone (Ngou et al., 2020). These suggest that PTI-induced ROS production can be enhanced by co-activation of PTI and ETI, and that ROS bursts are specific outputs of PTI. Here, many genes involved in the regulation of ROS during PTI are identified as DCGs in the module containing the largest number of DCLs. Moreover, genes involved in the early response to PTI (e.g. the activation of Ga^{2+} signaling, the activation of ethylene signaling and the activation of WRKY TFs) are presented in these DCGs. These genes may play important roles in modulating the response during PTI.

DEA has been extensively used in many gene expression studies to elucidate meaningful differences between experimental conditions. The main task of DEA is to identify genes that differ in abundance of biological molecules (e.g., RNA) under different conditions. DEA is useful in

539 prioritizing genes that may be dysregulated in specific contexts. Differential expression genes can
540 serve as potential candidate genes in response to specific stimuli, such as pathogen infections.
541 However, DEA only considers each gene individually, ignoring the potential interactions between
542 genes. Biomolecules such as genes, RNAs and proteins do not function in isolation. They
543 coordinate as functional modules to perform specific functions and may have similar expression
544 patterns. Co-regulated genes often have similar expression patterns. Co-expression network analysis
545 can capture the modular nature of biological systems. Interactions between molecules are not static
546 and can change dramatically depending on different conditions. Not only can DCA detect modules
547 that are differentially co-expressed under a variety of conditions, but it can also identify DCGs and
548 DCLs. These DCMs, DCLs, and DCGs can provide insights into altered regulatory mechanisms
549 between categories, such as cases and controls. Furthermore, as a complement to DEA, DCA is
550 useful in identifying genes that may not show significant changes in transcription levels. Here, to
551 systematically investigate differences before and after plant defense activation, we perform DCE
552 and DCA on half of the genes in the *Arabidopsis* genome based on publicly accessible datasets
553 treated by Mock, PAMPs, effectors, *Pto* DC3000 infection. We find that genes in consensus sub-
554 modules still show similar expression patterns before and after the plant defense activation, but no
555 longer exhibit the same co-expression pattern as genes in the other sub-modules. Genes involved in
556 the early defense response or essential for immune activation (e.g. *FLS2*, *LKY5*) or biomarkers of
557 the defense response (e.g. *PR1*, *PDF1.2*, *WRKY22*, *WRKY29*) are identified as DCGs in the module
558 containing the highest number of DCLs during PTI and ETI. An interesting hypothesis is that under
559 normal/Mock conditions, a small subset of genes encoding proteins that interact directly with the
560 inducer, are tightly co-expressed with other sub-module genes within the same larger co-expression
561 module, and when plant cells sense the inducer, they no longer exhibit the same expression pattern
562 as other sub-module genes due to signal decay over time and transmission along the signaling
563 pathways. Unexpectedly, we find that DCAs identified by the diffcoexp method before and after
564 defense activation are predominantly presented in one or two sub-modules and showed reverse
565 association with other sub-module genes within the same Mock module, but are still co-expressed
566 with each other (Fig. 6). On this premise, DCA may be an effective tool for capturing genes
567 encoding proteins that interact directly with the inducer. Furthermore, Srivastava P K et al. have
568 shown that DCA is a systems-level framework for drug target discovery (Srivastava et al., 2018).
569 Notably, post-transcriptional modifications such as ubiquitination, methylation and glycosylation
570 can affect gene interactions without altering gene expression. A comprehensive multi-omics
571 analysis will enhance the understanding of the mode of action of inducers.

572

573 **Conclusions**

574 In conclusion, our results indicate that the JA/ethylene/PAD4/SA signaling network is required for
575 resistance to *Pseudomonas syringae* but not for the activation of PTI and ETI; PTI and ETI have
576 similar differential expression patterns but differential co-expression patterns. These may deepen
577 our understanding of plant immune mechanisms.

578

579 **Methods**

580 **Data collection and preprocessing**

581 The *Arabidopsis* RNA-seq data used in this study were obtained from NCBI GEO database
582 (GSE88798 , GSE56117 , GSE133053/ PRJNA549850 , GSE120068 , GSE81366 , GSE127879,
583 GSE40044 , GSE85923, PRJNA379910, PRJNA412447, PRJNA434451). All .sra files were
584 downloaded from each bio project and converted to fastq files. A total of 560 samples were
585 obtained. RNA-seq reads were aligned to TAIR10 genome using Hisat2 v.2.0.4 (Kim et al., 2015)
586 with default parameters. Unique mapping reads for each representative gene model were quantified
587 using FeatureCounts (Liao et al., 2014). The RNA-seq counts were normalized by the voom method
588 in limma v.3.32.8 (Ritchie et al., 2015). Batch effects originating from each bio project were
589 corrected via ComBat method in sva v.3.32.1. Then, three outlier samples (SRR9331163,
590 SRR9331162, SRR9331201 of GSE133053/PRJNA549850) detected based on Kolmogorov-
591 Smirnov statistical testing and upper quartile dispersion (75%) were discarded. Finally, the top 35%
592 most variable genes (in terms of expression) remained for co-expression network analysis.

593

594 **Co-expression network analysis**

595 The samples annotated as Mock were used to constructing a co-expression network. Firstly, the
596 expression matrix was transformed into a disorganized adjacency matrix, implemented by biweight
597 midcorrelation method in WGCNA R package (Langfelder and Horvath, 2008). We chose to use
598 this correlation method because it is more tolerant of outliers than Pearson correlation method. Then,
599 the Speakeasy algorithm (Gaiteri et al., 2015) was used to derive gene modules from the adjacency
600 matrix with the following parameters: [cluster_codes clusters_in_cells convenient_node_ordering]

601 = layer_SpeakEasy(3, 10, adjacency_matrix, 20). This produced 76 modules, of which 42
602 containing at least 15 gene members (10, 588 genes) were retained for downstream analysis. We
603 chose to use SpeakEasy because it determines cluster assignments that overlap significantly with
604 those proposed by the WGCNA algorithm, as well as its state-of-the-art performance on benchmark
605 and real-world datasets (Gaiteri et al., 2015; Mostafavi et al., 2018).

606

607 Validation experiment and replication of gene modules

608 To evaluate the preservation of a given module in the second (validation) dataset, the module
609 preservation statistics test (called ‘z-summary’) was implemented by modulePreservation function
610 in WGCNA R package (Langfelder et al., 2011) with the following parameters: networkType =
611 "signed", corFnc = "bicor", and nPermutations = 10000. We employed five datasets as validation
612 datasets: (i) the gene expression data from the above-mentioned normalized gene expression matrix
613 annotated with PAMPs, Effectors (ii), and *Pto* DC3000 (iii); the Mock samples from two
614 independent dataset PRJNA224133 (iv) and SRP041507 (v). We summarize the results of this
615 analysis and showed them in a heatmap implemented with the ComplexHeatmap R package (Gu et
616 al., 2016).

617

618 To validate the results from DEA, we used the datasets from GSE56094 (Mock, Mock + *Pto*
619 DC3000, Mock + *Pto* DC3000 *hra*-), PRJNA313379 (Col-1 + flg22, *fls2* + flg22, 15 mutants
620 (“*dde2.2*”, “*ein2.1*”, “*pad4.1*”, “*sid2.2*”, “*dde2.2 ein2.1*”, “*dde2.2 pad4.1*”, “*dde2.2 sid2.2*”, “*ein2.1*
621 *pad4.1*”, “*ein2.1 sid2.2*”, “*pad4.1 sid2.2*”, “*dde2.2 ein2.1 pad4.1*”, “*dde2.2 ein2.1 sid2.2*”, “*dde2.2*
622 *pad4.1 sid2.2*”, “*ein2.1 pad4.1 sid2.2*”, “*deps*”) + flg22), PRJNA383861 (*deps AvrRpt2* + flg22,
623 *deps AvrRpt2* + estradiol (ED), *deps AvrRpt2* + flg22 + ED), and PRJNA348676 (5 mutants (“*rrsc*”,
624 “*pad4.1*”, “*sid2.2*”, “*pad4.1 sid2.2*”, “*deps*”), 5 mutants + *Pto* DC3000, 5 mutants + *Pto* DC3000
625 *AvrRpt2*, 2 mutants (“*rrsc*”, “*deps*”)+ *Pto* DC3000 *AvrRpm1*). The raw gene expression matrix for
626 GSE56094 and PRJNA313379, which belonged to microarray datasets, were download from the
627 NCBI GEO database. The .sra files for PRJNA348676 , PRJNA383861, PRJNA224133, and
628 SRP041507 were download from NCBI GEO database and were processed using the above-
629 mentioned methods.

630

631 Differential expression analysis (DEA)

632 Differential gene expressions were evaluated by calculating the difference in normalized expression
 633 between the matched treatment and Mock (wild type/Col-1+ mock) samples. However, there were
 634 no Mock samples in validation datasets PRJNA383861 and PRJNA313379. So we assigned the
 635 treatment *depsAvrRpt2*+Mock and *fls2*+flg22 to Mock, respectively. *fls2* mutants, which had null-
 636 mutations in the flg22 receptor gene, block the specific perception of flg22 in *Arabidopsis*
 637 (Chinchilla et al., 2006). To investigate whether the genes in a module tended to have higher-than-
 638 average down-regulation or up-regulation during different types of defense as well as whether there
 639 is higher-than-average misregulation in either direction, gene set enrichment analysis was
 640 performed using the mroast function in Limma R package with 10,000 rotations (Wu et al., 2010).
 641 This function evaluates a set of genes as a unit and tests whether a set of genes are differentially
 642 expressed. This method is also sensitive to gene sets that contain a small fraction of differential
 643 expressed genes within them. Modules with P -value < 0.05 are considered as significantly
 644 differential expression modules. The RKStest function in GSAR R package (Rahmatallah et al.,
 645 2017) was also used to identify differential expression modules.

646

647 Differential co-expression network analysis

648 To find whether a co-expressed gene cluster under Mock conditions shows different correlations
 649 during PTI, ETI, or *Pto* DC3000 infection, DCA was implemented by GSNCA (Rahmatallah et al.,
 650 2014; Rahmatallah et al., 2017), GSCA (Choi and Kendzierski, 2009), DiffCoEx (Tesson et al.,
 651 2010) and diffcoexp (Wei et al., 2018). Of these tools that measure co-expression using Spearman
 652 correlation coefficients, GSNCA and GSCA were used to identify DCMs, while DiffCoEx and
 653 diffcoexp were used to identify DCGs and DCLs. In 10,000 permutations, modules with P -value $<$
 654 0.05 were considered as significant DCMs. In DiffCoEx method, genes that were not part of any
 655 differential co-expression module were assigned as "gray" modules (Tesson et al., 2010); In the
 656 results of diffcoexp method, genes with P -value < 0.05 were considered as significant DCGs, and
 657 the DCLs are divided into three categories: diff signed, same signed, or switched opposites (Wei et
 658 al., 2018). Diff signed indicated that the gene pair was observed oppositely correlation coefficients
 659 under case and control conditions and only one of them meets two criteria: the absolute Spearman's
 660 correlation coefficient is greater than 0.5 and adjusted p-value less than 0.1; Same signed indicates
 661 that the gene pair has same signed Spearman's correlation coefficient under both case and control;
 662 Switched opposites indicates that the gene pair has oppositely signed Spearman's correlation

coefficient under both case and control and both of them meet two criteria: the absolute Spearman's correlation coefficient is greater than 0.5 and adjusted p-value less than 0.1. Besides, if the variance of gene expression distributions in case samples are drastically higher than in the Mock samples, the regulatory relationships in Mock might have been lost (de la Fuente, 2010).

Consensus co-expression network analysis

For each module, the blockwiseConsensusModules function in WGCNA R package (Langfelder et al., 2013) was used to identify co-expression sub-modules common in PTI, ETI and *Pto* DC3000 infection based on corresponding datasets with following parameters: $\beta = 7$, minModuleSize = 15, corType = "bicor", deepSplit = 2, networkType = "signed", mergeCutHeight = 0.25, minBlockSize = 20, 000. The genes that did not belong to any co-expression sub-modules were assigned as “grey” modules

Enriched Regulon analysis

A set of genes controlled by a given TF formed a regulon. A complete set of regulons and the connections among them form a transcriptional regulatory network. Here, we split PlantRegMap (Tian et al., 2020) consisting of genome-wide regulatory relationships curated from literature or deduced from combining TF-binding groups and regulatory elements into a set of regulons. A regulon was named by the regulator/TF's gene id. We also added putative regulatory relationships between known TFs and their targets determined using FIMO with a P -value $\leq 10^{-4}$ (Grant et al., 2011) to complement PlantRegMap. The sequences of 500 bp upstream of the transcription start site were used for promoter motif analysis. Overrepresentation of the members of regulons in the genes of each detected module was performed using hypergeometric tests. P values were corrected with the Bonferroni method for multiple tests.

Functional enrichment analysis

For each module, we computed hypergeometric P values for the enrichment of any Gene Ontology (GO) category and KEGG pathway terms, and then only retained Bonferroni-adjusted $P < 0.05$ for functional terms.

692

693 **Abbreviations**

694 BR: Brassinosteroid

695 Ca²⁺: Calcium

696 DCA: Differential co-expression analysis

697 DCG: Differentially co-expressed genes

698 DCL: Differentially co-expressed links

699 DCM: Differentially co-expressed modules

700 DEA: Differential expression analysis

701 *deps: dde2.2 ein2.1 pad4.1 sid2.2*

702 Ed: Estradiol

703 ETI: Effector-triggered immunity

704 GO: Gene ontology

705 JA: Jasmonic acid

706 NLR: Nucleotide-binding/leucine-rich-repeat

707 PTI: Pathogen-associated molecular patterns (PAMP)-triggered immunity

708 R: Resistant genes

709 RLK: Rceptor-like kinases

710 RLP: Receptor-like proteins

711 *rrsc: rpm1 rps2*

712 ROS: Reactive oxygen species

713 SA: Salicylic acid

714 TF: Transcription factor

715

716 **Supplemental Data**

717 **Supplemental Figure S1.** Module preservation and replication. Module preservation (Z_{summary} , x-
718 axis) is assessed in the 5 test data-sets. Each row indicates the preservation of a module in 5 datasets.
719 The gold module is a random sample representing the entire network as a single module. Module
720 conservation is assessed using the Z_{summary} value. Strong preservation of co-expression is indicated
721 by a Z_{summary} score of >10) and moderate preservation by a Z_{summary} score >5 . (TIF 608 KB)

722

723 **Supplemental Figure S2.** Module gene GO biological process enrichment analysis. The figure
724 shows all significant ($P < 0.05$) GO biological processes enrichments for each module. The p -value
725 for each term is represented by the color scale - the darker the color the more significant the result.
726 The green rectangle represents defense-related modules. (TIF 4.0 MB)

727

728 **Supplemental Figure S3.** Module gene GO cellular component enrichment analysis. The figure
729 shows all significant ($P < 0.05$) GO cellular components enrichments for each module. The p -value
730 for each term is represented by the color scale - the darker the color the more significant the result.
731 The green rectangle represents defense-related modules. (TIF 1.4 MB)

732

733 **Supplemental Figure S4.** Module gene GO molecular function enrichment analysis. The figure
734 shows all significant ($P < 0.05$) GO molecular functions enrichments for each module. The p -value
735 for each term is represented by the color scale - the darker the color the more significant the result.
736 The green rectangle represents defense-related modules. (TIF 3.0 MB)

737

738 **Supplemental Figure S5.** Module gene KEGG pathway enrichment analysis. The figure shows all
739 significant ($P < 0.05$) KEGG pathway enrichments for each module. The p -value for each term is
740 represented by the color scale - the darker the color the more significant the result. The green
741 rectangle represents defense-related modules. (TIF 734 KB)

742

743 **Supplemental Figure S6.** TF, RLP, RLK, R gene enrichment analysis. The figure shows all
744 significant ($P < 0.05$) gene sets for each module. The p -value for each term is represented by the
745 color scale - the darker the color the more significant the result. The green rectangle represents
746 defense-related modules. (TIF 197 KB)

747

748 **Supplemental Figure S7.** Regulon enrichment analysis. The figure shows all significant ($P < 0.05$)
749 regulators for each module. The p -value for each term is represented by the color scale - the darker
750 the color the more significant the result. The green rectangle represents defense-related modules.
751 (TIF 814 KB)

752

753 **Supplemental Figure S8.** Comparative analysis of transcriptome dynamics in *Arabidopsis* during
754 different types of plant defense. Blue indicates that the genes are negatively regulated under the
755 corresponding condition, and red indicates positive regulation. The name of each module and the
756 number of genes contained within it are given on the horizontal axis of each panel. The vertical axis
757 on the left gives the access ID of the corresponding data set and the corresponding processing
758 conditions. The consensus sub-modules are represented by the colored bars. The 'grey' bars in the
759 figure represent genes in the module that do not have a consensus co-expression pattern when
760 treated with PAPMs, virulent *Pto* DC3000, or avirulent *Pto* DC3000. (PDF 18M)

761

762 **Supplemental Figure S9.** Comparative analysis of the contribution of JA/SA responsive genes
763 during different types of plant defense. See also the legend of Figure S8. (PDF 6.6 MB)

764

765 **Supplemental Table S1.** Expression datasets used in this study. Description of GEO series and
766 samples. (DOCX 20 KB)

767

768 **Supplemental Table S2.** List of co-expression modules found in different gene sets. The top 35%
769 most variant genes (in terms of expression across Mock, PTI, ETI, *Pto* DC3000 infection conditions)
770 were used for constructing a co-expression network. The SpeakEasy (SE) algorithm was used to
771 derive gene modules from normalized gene expression data under Mock conditions. Then

772 consensus Weighted Gene Co-expression Network Analysis (WGCNA) was used to find consensus
773 sub-modules common across PTI, ETI, *Pto* DC3000 infection conditions in genes within each
774 module as well as the gene sets responsible for the divergence, convergence, and antagonism of
775 JA/SA signalings in *Arabidopsis*. (XLSX 1.5 MB)

776

777 **Supplemental Table S3.** The results of functional and regulon enrichment analysis for each module.
778 (XLSX 146 KB)

779

780 **Supplemental Table S4.** The DCGs and DCLs identified by diffcoexp method in each module
781 during different types of plant defense. (XLSX 17 MB)

782

783 *Availability of data and materials*

784 All data generated or analyzed during this study are included in this published article [and its
785 Additional files].

786

787 *Competing interests*

788 The authors declare that they have no competing interests

789

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Figure 1. Schematic summary of study design. We hypothesize that during the transition from vegetative growth to a defensive state, the *Arabidopsis* gene regulatory networks under normal/Mock will be reprogrammed in PTI, ETI, *Pto* DC3000 infection. These alterations are informative for molecular processes involved in different types of plant defense. In combined mutants of *dde2*, *ein2*, *pad4*, and *sid2*, the alterations of the gene regulatory networks are informative for the interplay of SA and JA in different types of plant defense. We used samples annotated as normal/Mock (wild type) from different studies to construct gene co-expression networks (using SpeakEasy methods), and in these co-expression modules we constructed "consensus" sub-modules based on gene expression matrices of samples annotated as PAMPs, Effector, and *Pto* DC3000 (using WCGNA consensus Methods). To explore commonalities and

differences among different types of plant defense, we estimated differential expression of individual genes by calculating differences between matched treatments or mutants and Mock samples, and then visualized differential expression patterns in heat maps. The mroast and RKStest functions were used to quantitatively assess the differences at the gene set/module levels during PTI and ETI. GSNCA, GSCA, DiffCoEx, and diffcoexp were used to identify modules, associations/links, and genes that were differentially co-expressed between matched treatments and Mock conditions.

Figure 2. Comparative analysis of transcriptome dynamics in *Arabidopsis* during different types of plant defense. Heat maps showing differential expression modules in which the genes show significantly differential expression between Mock (Col-0 + no treatment) and samples treated

837 with PAPMs, virulent *Pto* DC3000, or avirulent *Pto* DC3000 for Col-0 or the combinatorial mutants
 838 of *dde2*, *ein2*, *pad4*, and *sid2*. In PRJNA383861 and PRJNA313379, we assigned the
 839 *depsAvrRpt2*+Mock and *fls2*+flg22 as Mock, respectively. Blue indicates that the submodule is
 840 negatively regulated under the corresponding condition, and red indicates positive regulation. The
 841 name of each module is given on the horizontal axis. The left and right vertical axes give the access
 842 ID of the corresponding data set and the corresponding processing conditions, respectively.

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Figure 4. Comparative analysis of differential co-expression dynamics during different types of plant defense. GSNCA test and GSCA method were used to identify DCMs (p -value < 0.05), while DiffCoEx and diffcoexp methods were used to identify DCGs. Colored symbols indicate the number of DCGs in each module.

890 **Figure 5. Comparative analysis of differential-co-expression associations during different**
 891 **types of plant defense.** The module names were given on the left vertical axis. The colored
 892 symbols indicate different types of DCLs. The total number of DCLs in each module is shown on
 893 the horizontal axis.

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906 **Figure 6. Correlation patterns of co-expression modules M5 and M2.** The figure summarizes
 907 the co-expression patterns of the module M5 (A) and M2 (B) between Mock (left panel) and
 908 different types of plant defense (right panel). The consensus sub-modules are represented by the
 909 colored bars. The 'grey' bars in the figure represent genes in the module that do not have a
 910 consensus co-expression pattern when treated with PAPMs, virulent *Pto* DC3000, or avirulent *Pto*
 911 DC3000. Spearman's correlation coefficients are exhibited in the form of a color scale with
 912 negative correlations shown in blue and positive correlations shown in red. The color bands named
 913 "P", "E", and "D" represent the location of the DCGs in each sub-module. "P" indicates the DCGs
 914 response to PAMPs, "E" for *Pto* DC3000 carrying "avirulent" effector genes, and "D" for virulent
 915 *Pto* DC3000. The solid black lines and the genes indicate the top 10 DCGs containing the highest
 916 number of DCLs in PTI, ETI and *Pto* DC3000 infection. Colored symbols indicate the number of
 917 DCLs possessed by each DCGs.

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