Metatranscriptomic Analysis of Multiple Environmental Stresses Identifies RAP2.4 Gene Associated with Arabidopsis Immunity to Botrytis cinerea

Arjun Sham¹, Hibatullah Al-Ashram¹, Kenna Whitley³, Rabah Iratni¹, Khaled A. El-Tarabily¹,²* & Synan F. AbuQamar¹*

In this study, we aimed to identify common genetic components during stress response responsible for crosstalk among stresses, and to determine the role of differentially expressed genes in Arabidopsis-Botrytis cinerea interaction. Of 1,554 B. cinerea up-regulated genes, 24%, 1.4% and 14% were induced by biotic, abiotic and hormonal treatments, respectively. About 18%, 2.5% and 22% of B. cinerea down-regulated genes were also repressed by the same stress groups. Our transcriptomic analysis indicates that plant responses to all tested stresses can be mediated by commonly regulated genes; and protein-protein interaction network confirms the cross-interaction between proteins regulated by these genes. Upon challenges to individual or multiple stress(es), accumulation of signaling molecules (e.g. hormones) plays a major role in the activation of downstream defense responses. In silico gene analyses enabled us to assess the involvement of RAP2.4 (related to AP2.4) in plant immunity. Arabidopsis RAP2.4 was repressed by B. cinerea, and its mutants enhanced resistance to the same pathogen. To the best of our knowledge, this is the first report demonstrating the role of RAP2.4 in plant defense against B. cinerea. This research can provide a basis for breeding programs to increase tolerance and improve yield performance in crops.

Plants frequently have to cope with a wide array of environmental (abiotic and biotic) challenges. In nature, simultaneous or sequential exposure of plants to multiple stress conditions i.e., more than one abiotic and/or biotic stress, occurs more often than to a single individual stress¹,². Plants have evolutionarily developed sophisticated adaptation and defense mechanisms³. Thus, this response cannot be predicted based on a plant response to an individual stress. Depending on the length, intensity and severity of stresses, the complexity of plant response can be orchestrated by the integration of a number of metabolic pathways and the crosstalk between different signal transduction pathways⁴. The specificity of response can be determined by an array of mechanisms, which may crosstalk or diverge, and form complex networks including transcription factors (TFs), kinases and reactive oxygen species that may interact with each other⁵. As a result, plants tune gene expression along with their physiological needs to promote adaptation to short- and long-term environmental changes.

There are a number of global transcriptome analyses investigating various environmental stresses in plants including Arabidopsis thaliana. Although many microarray studies have focused on individual stresses⁶–⁸, there is growing evidence that a unique gene expression can be activated in plants under simultaneous abiotic and/or biotic challenges. Researchers have identified specific and common molecular responses to bacterial, fungal and viral pathogen attacks; others have analyzed plant responses to multiple abiotic stresses using different transcriptomic tools⁹–¹¹. In their efforts to compare between different stress responses, two microarray studies identified the genes and pathways that are commonly induced by the necrotrophic fungal pathogen Botrytis cinerea (biotic) and abiotic (salt, heat, cold, drought, osmotic and oxidative stress) threats¹²–¹³. The effects of simultaneous biotic

¹Department of Biology, United Arab Emirates University, 15551, Al-Ain, UAE. ²School of Veterinary and Life Sciences, Murdoch University, Murdoch, Western Australia, 6150, Australia. *email: ktarabily@uaeu.ac.ae; sabuqamar@uaeu.ac.ae
and abiotic stresses may interact either synergically or antagonistically. Hence, genes and molecular mechanisms involved in regulating plant responses are mainly associated with signal molecules known as plant hormones12,23. Several studies have reported that the major phytohormones, salicylic acid (SA), jasmonates (JA), ethylene (ET) and abscisic acid (ABA), are involved in the regulation of plant response to the adverse effect of biotic and abiotic stresses3,4,15. To lesser extent, other hormones such as auxins, gibberellic acid, cytokinins, brassinosteroids and strigolactones may also play a role in plant defense signalling pathways4,14,16,17. Studies have reported the association of different defense signaling pathways in response to pathogens and described that among the co-expressed genes, a majority is co-induced or -repressed with the treatment of SA and methyl-JA (MeJA)8,17. Plant response to certain insects can also be related to hormone signalling. For example, a response of wounded insects such as Spodoptera exigua (Hübner) and Pieris rapae, the level of JA, JA-isoleucine and ET was increased18. In tomato (Solanum lycopersicum), tomato protein kinase 1b (TPK1b) RNA interference (RNAi) mutant plants showed increased susceptibility to B. cinerea and the herbivorous insect Manduca sexta, and increased sensitivity to 1-aminoacyclopropene-1-carboxylic acid (ACC), the natural precursor of ET19. In addition, ectopic expression of TPK1b in Arabidopsis conferred increased resistance to B. cinerea and Alternaria brassicicola. This suggests that TPK1b and ET as key regulators of insect and pathogen defense responses.

B. cinerea is considered the second most important plant pathogen, causing significant economic damage on over 200 crops worldwide20. Moreover, B. cinerea has become an important model for studying interactions between plants and necrotrophic pathogens21. Typically, SA, JA and ET play major roles in response to pathogen infections; while ABA is responsible for plant defense against abiotic stresses22,23. Hence, the existence of crosstalk in plant responses to biotic and abiotic stresses involves various signaling hormone pathways. For instance, ET and JA have been found to be associated with elevated expression levels of the plant defense gene PDF1.2, which contributes to resistance to B. cinerea and A. brassicicola22. Arabidopsis and tomato mutants with altered ET and JA signaling pathways are susceptible to B. cinerea infection24. Exogeneous treatment of Arabidopsis with ET or MeJA substantially decreased B. cinerea infection, indicating the crucial function of ET and JA in B. cinerea resistance. Furthermore, a study has confirmed the role of expansin-like A2 (EXL2) gene in defense against B. cinerea and tolerance to abiotic stresses25. In Arabidopsis, the negative regulation of WRKY57 against B. cinerea is dependent on the JA signaling pathway25. Another gene from the WRKY family, WRKY33 is found to be important in B. cinerea resistance26,27. Molecular and genetic studies reveal that ABA negatively influences defense to B. cinerea and affects JA/ET and SA levels. Susceptibility/resistance can be determined by the antagonistic effect of ABA on JA, and this crosstalk requires suppression of WRKY33 at early infection stages28. This indicates that B. cinerea promotes disease by suppressing WRKY33-mediated host defense.

It is important to study plant responses to multiple stress conditions in order to identify commonly regulated genes and pathways altered by these stresses. This will help to facilitate specific target gene manipulation for genetic engineering to enhance multiple stress tolerance in plants. There are several gene expression experiments as well as datasets in publicly available data repositories. Meta-analyses of public datasets can yield more biologically and technically sound information than that of individually analyzed datasets29. In addition, results coming from single stress experiments may be inaccurate or skewed if the compared datasets are not genetically equivalent, which is in contrast to analyzing public consistent datasets. The dynamics of whole-transcriptome profiles of Arabidopsis exposed to sequential double stresses treated with combinations of B. cinerea, P. rapae and drought stress has been analyzed22. The study revealed that around one-third of the differentially expressed genes (DEGs) were shared by at least two single stresses.

In the present study, we analyzed and compared the public transcriptomic data to identify the overlapping stress-regulated genes in response to B. cinerea and other biotic (Pseudomonas syringae pv. tomato DC3000 virulent and avirulent Rpm1 strains, A. brassicicola and P. rapae), abiotic (oxidative stress and wounding) and hormonal (SA, JA, ET and ABA) stresses. These data were further analyzed to sort the up- and down-regulated genes in all stresses. This study revealed the genes uniquely expressed in response to each of these stresses, and those commonly expressed in response to B. cinerea and other stresses. The Arabidopsis Protein-protein Interaction (PPI) network demonstrated the complex regulatory network co-expressed across the multiple stresses. We also developed a transcriptome-verse mapping strategy to compare the interactions between proteins encoded by genes that belong to different expression-profiling clusters. T-DNA insertion mutant lines of Arabidopsis were used to analyze the role of TPK1b and ET as key regulators of insect and pathogen defense responses.

Results

Screening of DEGs in response to individual stresses. We aimed to identify unique and common DEGs among transcriptomic datasets related to environmental and hormonal stresses in Arabidopsis. Similar to previous studies23,30, B. cinerea up-regulated genes (BUGs) and B. cinerea down-regulated genes (BDGs) were identified based on their transcriptional levels in response to B. cinerea at 18 hours post inoculation (hpi). Using publicly available databases30, DEGs were also detected in Arabidopsis plants infected with B. cinerea at 18 hpi (Dataset S1, Supporting Information). For each individual dataset, the complete list of pathogen/pest infection (P. syringae pv. tomato DC3000 and avrRpm1, A. brassicicola or P. rapae), abiotic stress (wounding and oxidative stress) or hormone treatment (SA, MeJA, ACC and ABA) (Fig. S1–S2, Supporting Information) can be found in Datasets S1–S4 (Supporting Information). Microarray analysis showed 1554 BUGs (6.8% of Arabidopsis genome) and 1206 BDGs (5.3%) (Fig. 1A,B). In response to P. syringae pv. tomato DC3000 and avrRpm1, we found 2422 and 1989 genes considered up-regulated, and 2270 and 2085 considered down-regulated, respectively. There were
1902 genes (933 up-regulated; 969 down-regulated) after inoculation with *A. brassicicola*, 2382 genes (1386 up-and 996 down-regulated) were detected as DEGs in *Arabidopsis* plants infested with *P. rapae*.

The least number of DEGs was observed in plants under the two treatments that belong to oxidative stress and wounding (Fig. 1A,B). This could be attributed to the natural adaptation of *Arabidopsis* to the abiotic stress group in comparison to other stress conditions 10,11. In *Arabidopsis*, the gene expression levels upon individual treatments of SA, MeJA, ACC and ABA were altered for 3,051, 2,918, 2,590 and 3,231 transcripts, respectively, from which 1,340 (43.9%), 1,397 (47.9%), 1,328 (51.3%) and 1,564 (48.4%) genes were stress-induced genes. On average, most FC of the DEGs in all stresses ranged between two-fold or three-fold. Interestingly, we noticed that some genes were induced >10 fold or repressed <10 fold. Although each dataset may have its unique DEGs, this does not rule out the possibility that common genes can be identified across the categories of stresses.

To visualize the gene expression data of *B. cinerea* and other examined stresses, a heatmap of the top 50 BUGs and BDGs was generated (Fig. 1C). The heatmap was combined with clustering methods, displaying a group of genes/samples together based on the similarity of their gene expression pattern. Together, this can be the first step for identifying genes that are commonly regulated or biological signatures that are associated with multiple conditions.

**Highly conserved expression of common DEGs to multiple stress responses.** A scatter plot was constructed to compare the transcript level of the DEGs of each dataset with that altered by *B. cinerea* infection (Fig. 2). Clearly, our results demonstrated similar patterns of gene expression levels between *Arabidopsis* plants infected with *B. cinerea* and any individual stress at the time of treatment. This suggests that the mode of action of some DEGs common to multiple stresses may contribute in functions or processes that are common among responses to stress acclimation. We also determined the number (and percentage) of genes that were co-regulated in response to *B. cinerea* and single treatments of each stress type. In response to both the virulent and avirulent strains of *P. syringae*, more than 2/3 and 1/2 of the genes were also induced and repressed, respectively, to *B.
Upon inoculation with *B. cinerea*, 40–50% of the genes were expressed by either the fungal pathogen *A. brassicicola* or the herbivory insect *P. rapae*. We also noticed that between 443 (28.5% in ACC) and 562 (36.2% in ABA) of the induced, and 429 (35.6% in ACC) and 532 (44.1% in SA) of the repressed genes in response to single hormonal stresses were co-regulated with infections by *B. cinerea*. To lesser extent, 193 (12.4%) and 69 (4.4%) of the induced and repressed genes in response to oxidative stress, and 69 (4.4%) and 47 (3.9%) of the induced and repressed genes in response to wounding were co-regulated with infections by *B. cinerea*.

**Table 1.** Common regulation of *Botrytis cinerea*-regulated genes with different treatments. Percentages of *B. cinerea* up-regulated genes (BUGs) and *B. cinerea* down-regulated genes (BDGs) that were also at least twofold increased or decreased by the biotic, abiotic and hormonal stresses. Percentage = (Count of commonly up- or down-regulated genes of the treatment/count of BUGs (1554 genes) or BDGs (1206 genes)) *100%. 

**Figure 2.** Comparison of gene expression in plants infected with *Botrytis cinerea* vs. challenged with environmental and hormonal stresses. Normalized expression value for each probeset in wild-type plants infected with *B. cinerea* at 18 hpi (*Bc−18*) is plotted on X-axis versus the expression value in wild-type plants after treatment with biotic, (A) *Pseudomonas syringae* pv. *tomato* DC3000 (*Pst-24*), (B) *P. syringae* pv. *tomato* avrRpm1 (*PstavrRpm1-24*), (C) *Alternaria brassicicola* (*Ab-24*), and (D) *Peris rapae* (*Pr-24*); abiotic, (E) oxidative stress (*Ox-24*), and (F) wounding (*W-24*); and hormonal (G) salicylic acid (SA-3), (H) methyl-jasmonate (MeJA-3), (I) 1-aminoacyclopropane-1-carboxylate (ACC-3), and (J) abscisic acid (ABA-3) stresses. hpi, hours post inoculation.
Stress-induced responses to pathogen stress are frequently considered to be distinct from those to abiotic and hormone challenges. By comparing the expression of insecticide-treated group, there were 21 commonly up-regulated genes and 30 commonly down-regulated genes (Fig. S3, Supporting Information). The overlapping DEGs in response to B. cinerea and abiotic stress-treated group, there were 21 commonly up-regulated genes and 30 commonly down-regulated genes, respectively. Similarly, 211 genes were induced by B. cinerea-down-regulated genes, respectively. Comparing B. cinerea and abiotic stress-treated group, there were 380 and 218 commonly up- and down-regulated genes, respectively. Only one gene was induced, and three genes were repressed in the rest of single stresses can be found in Datasets S5–S7 (Supporting Information).

The GO annotation was established on commonalities of stress groups were obtained from Dataset S8 (Supporting Information). The three altered expressed genes by the 11 tested stresses, were also up- and down-regulated by paraquat (oxidative stress) and wounding treatments, respectively. Only one gene was induced, and three genes were repressed in response to all individual stresses (Table 1). The list of identified DEGs that were co-regulated by B. cinerea infection and/or other biotic, abiotic and hormonal stress groups in Arabidopsis. A list of identified DEGs that were co-regulated by B. cinerea infection and/or other biotic, abiotic and hormonal stress groups in Arabidopsis. A twofold difference in expression level between B. cinerea-infected and non-infected samples was set for considering a gene to be up-regulated and down-regulated in response to all individual stresses (Table 1). The list of identified DEGs that were co-regulated by B. cinerea infection and/or other biotic, abiotic and hormonal stress groups in Arabidopsis. A twofold difference in expression level between B. cinerea-infected and non-infected samples was set for considering a gene to be up-regulated and down-regulated in response to all individual stresses (Table 1). The list of identified DEGs that were co-regulated by B. cinerea infection and/or other biotic, abiotic and hormonal stress groups in Arabidopsis. A twofold difference in expression level between B. cinerea-infected and non-infected samples was set for considering a gene to be up-regulated and down-regulated in response to all individual stresses (Table 1).

**Common DEGs and gene ontology (GO) functional enrichment analyses.** Venn diagrams detected large overlaps in gene expression among the stress response treatments (Fig. S3, Supporting Information). By comparing B. cinerea-inoculated and abiotic-stressed group, there were 380 and 218 commonly up- and down-regulated genes, respectively. Similarly, 211 genes were induced by B. cinerea infection as well as by all other biotic, abiotic and hormone challenges. Based on the functional similarities of their encoded proteins, BUGs or BDGs were grouped with those up- or down-regulated belonging to other stress classes. According to AGI locus identifiers, 45 functional categories were classified into three major categories upon their biological processes, molecular functions and cellular components (Fig. S4, Supporting Information).

**Table 2.** Changes in expression of top 15 up- and down-regulated genes encoding putative proteins during Botrytis cinerea infection and/or other biotic, abiotic and hormonal stress responses in Arabidopsis. *a* Fold change (FC) was calculated by dividing the expression of B. cinerea-infected by that of non-infected samples. A twofold difference in expression level between B. cinerea-infected and non-infected samples was set for considering a gene to be up-regulated and down-regulated in response to all individual stresses (Table 1). The list of identified DEGs that were co-regulated by B. cinerea infection and/or other biotic, abiotic and hormonal stress groups in Arabidopsis. A twofold difference in expression level between B. cinerea-infected and non-infected samples was set for considering a gene to be up-regulated and down-regulated in response to all individual stresses (Table 1).
Our analysis revealed that the general "response to stresses" was the category of most up-regulated clustered genes when plants were inoculated with *B. cinerea* and abiotic stress-affected group. The dominant subcategory 'signal transduction' was highly associated with plant defense against pathogens and abiotic cues. The ABA insensitive 1 (ABI1) was up-regulated by *B. cinerea* and bacterial pathogens (Dataset S5, Supporting Information) as well as the SA and ACC hormones (Dataset S7, Supporting Information). This suggests that plant hormones are tightly associated with defense against *B. cinerea* and other environmental stresses. Enzymatic activities including kinases were also among the dominant subcategories in *BUG* and other groups (Fig. S4, Supporting Information). In addition, "cell wall" term within the cellular component was highly up-regulated in the tested groups; and the wall-associated kinase 1 (WAK1) was also induced by multiple stresses (Datasets S5 and S7, Supporting Information).

Different GO terms of *BDG* encoding for structural activity, receptor binding/activity or enzymatic activity proteins were highly down-regulated in response to biotic, abiotic or hormonal stresses, respectively (Fig. S4, Supporting Information). The "electron transport/energy pathways" was the category that most down-regulated genes belonged in response to *B. cinerea* /biotic stress group and *B. cinerea* /hormonal stress group; whereas, "plastids" and "ribosomes" were the dominant subcategories in the cellular component. Consistent with previous findings, our data suggest rapid metabolic repression of photosynthetic proteins when plants are under stress including infection with *B. cinerea* (Fig. S4, Supporting Information). Many reports have shown that *B. cinerea* induces/represses several genes particularly those encoding developmental, structural and regulatory proteins in Arabidopsis. Our knowledge of biological mechanisms related to *B. cinerea* infection is still meager. Therefore, we constructed interactome networks of commonly regulated genes in silico. Arabidopsis PPI network of DEGs after exposure to groups that belong to multiple environmental challenges and hormonal treatments were annotated by calculating their interactive degrees obtained from the STRING database. The interactive networks displayed clique in the sub-network, suggesting that the hub proteins may form a super complex to play important roles in stress response. As a result, a total of 258 nodes were demonstrated to be involved in network construction and 250 edges were established in the network. Key DEGs, such as those encoding RAP2.4 protein possessed degrees of 10 were markedly more compared with those of other proteins (Fig. 3; Dataset S9, Supporting Information); and functional analysis of RAP2.4 was further studied. Together, this indicates that the dynamic interactive networks between commonly regulated genes may help decide whether certain network topologies can explain experimental observations.

**qRT-PCR validation for the microarray results.** To confirm the changes in gene expression revealed by the microarray analyses, 13 DEGs were selected based on the microarray data for verification tests (Fig. 4).
We performed qRT-PCR on Arabidopsis leaves infected with B. cinerea at 0 and 18 hpi. The transcript levels of the DEGs (9 up-regulated; 4 down-regulated) with altered expression in response to various stress treatments (Table 2), were quantified and compared with the obtained microarray analyses. Although the FC values observed in the two expression methods differed somewhat, the tested DEGs displayed comparable patterns in transcript accumulation in the analyses of the two approaches (Table 3; Fig. 4). In response to B. cinerea, for example, the transcript levels of the nine BUGs (CCR2, CYP71A13, α-DOX1, PDF1.2, At1g754300, At3g51660, NATA1, SRG1 and ELI3–2) were increased (Fig. 4), similar to that of the same set of genes identified in the microarray analyses. On the other hand, RAP2.4, DIR-Like, At1g5490 and HAD, which were considered as BDG s, were also repressed by B. cinerea at 18 hpi. In general, a similar trend of expression was found in both the microarrays and the qRT-PCR.

Mutations in RAP2.4 enhanced resistance to B. cinerea. From the microarray data, RAP2.4 was selected on the basis of its expression profiles for further analysis. Two mutant alleles in RAP2.4 gene displayed very low basal and repressed RAP2.4 expression compared to wild-type plants (Fig. 5A). The rap2.4-1 showed the lowest transcript levels of RAP2.4 at 18 hpi with B. cinerea; and therefore, this T-DNA insertion line was used in further experiments. We assumed that RAP2.4 more likely plays a role in defense. Two homozygous T-DNA insertion lines were inoculated with the fungal pathogen B. cinerea, evaluated for disease resistance and compared with inoculated Arabidopsis wild-type (Col-0) plants – a relatively resistant ecotype.

After inoculations, disease lesions remained restricted in both T-DNA insertion mutant lines of RAP2.4 and exhibited a more resistant phenotype than the wild-type disease phenotype at all time points of infection (Fig. 5B). In wild-type plants, lesions expanded until 4 days post inoculation (dpi), with chlorosis surrounding them. Obviously, when we measured the diameter of the lesions, the rap2.4-1 and rap2.4-2 mutants demonstrated smaller lesions than the wild-type plants (Fig. 5C), confirming that in the observed disease phenotype. To measure disease development more precisely, fungal biomass accumulation of B. cinerea ActinA (Be ActinA) per leaf...
SA-mediated defense-associated genes, PR1, of B. cinerea contributes to plant immunity toward B. cinerea rap2.4 mutants (Fig. 5D). This suggests that clear decrease in the growth of fungal biomass in the was measured at 3 and 6 dpi for each of the mutant lines. Compared to growth in wild-type plants, there was a
insertion mutant plants after drop-inoculation with B. cinerea using qRT-PCR; (D) fungal growth in leaves of B. cinerea (Fig. 5).

Figure 5. Reduced RAP2.4 transcript levels enhanced resistance to Botrytis cinerea. (A) Relative expression after infection (Fig. 6J). The expression of genes that were responsive to the cyclopentenones, phytoprostanes

Down-regulation of RAP2.4 alters the expression of defense-regulated genes in response to B. cinerea. In order to link RAP2.4 function in defense to specific pathway(s), we assessed the response of B. cinerea-infected tissues to molecular markers of different signaling pathways. The transcript levels of the SA-mediated defense-associated genes, PR1, β-1,3-glucanase (BGL2/PR2) and phytoalexin deficient 4 (PAD4) were determined in rap2.4-1 mutant. The basal expression levels of all SA-associated genes in uninfected plants revealed significant reduction in rap2.4 compared to wild type (Fig. 6A–C). Although the transcript of the SA marker gene, PR-1, increased at 18 hpi with B. cinerea in wild-type plants, the expression was significantly higher in rap2.4 (Fig. 6A). This suggests that rap2.4 plants respond faster in terms of PR-1 expression; thus, the increased PR-1 levels may be an indirect consequence of the decreased rate of fungal growth in rap2.4 plants. After B. cinerea inoculation, PR2 and PAD4 showed a similar pattern of expression between rap2.4 and wild type plants infected with B. cinerea although the repression was much lower in the mutant plants (Fig. 6B,C). This suggests that the basal expression level of SA-related defense genes might be dependent on RAP2.4.

At 18 hpi with B. cinerea, the transcript levels of JA/ET pathway defense marker genes, PDF1.2, PR3 (β-chitinase) and PR4 (hevein-like) were significantly induced in both wild-type and rap2.4 mutant plants (Fig. 6D–F). The increase in the expression of PR3 and PR4, but not PDF1.2, was comparable in wild-type and rap2.4 plants. Hence, these B. cinerea-induced genes which are activated by the oxylipins, JA and/or OPDA, are CO11-dependent and are negatively regulated by the TF, MYC2. In wild-type plants, there was repression in VSP2 in response to B. cinerea; thus, the transcript levels of VSP2 were markedly increased in rap2.4 after inoculation (Fig. 6G). Similar to VSP2, healthy rap2.4 plants expressed lower basal levels of MYC2, but the transcript accumulated to high levels within 18 hpi (Fig. 6H). This is in contrast to the suppression of MYC2 in B. cinerea-inoculated wild-type plants. This suggests that the down-regulation of RAP2.4 may require oxylipins during pathogen infection.

There was an increase in transcript levels of 12-oxophytodienoate reductase 1 (OPR1) when wild-type and rap2.4 plants were challenged with the necrotrophic fungal pathogen B. cinerea (Fig. 6I). The induction was significantly increased in rap2.4 mutant. On the other hand, the expression of OPR3 was altered in rap2.4 mutant plants after infection (Fig. 6I). The expression of genes that were responsive to the cyclopentenones, phytoprostanes.
Arabidopsis detoxification-related genes, GST6 and GSTU19, were up-regulated in wild-type-infected plants (Fig. 6K,L). No induction of the same genes was observed in rap2.4 mutant after infection, indicating that there is a common regulation between electrophilic oxylipins and B. cinerea, and that RAP2.4 plays a major role in this regulation. Overall, these results suggest that the down-regulation of RAP2.4 gene alters the expression of JA- and/or cyclopentenone-mediated response genes, which may result in the increased resistance of rap2.4 mutants to the necrotrophic pathogen B. cinerea.

Discussion

Plants are often affected by diseases mainly caused by pathogens, with a consequent serious reduction in plant growth, development and productivity. Abiotic stress conditions have a direct impact on plant-microbe interactions, and alternation of plant physiology and defense responses. Plants retain a range of defense mechanisms to combat stress conditions, which involve a variety of metabolic reprogramming events and cellular pathways. Once sensing a single or multiple stress(es), plants show an immediate and evoking response to initiate a complex stress-specific signaling by synthesizing hormones and accumulating phenolic compounds. In this work, we performed a meta-analysis of transcriptomic data related to environmental challenges in Arabidopsis publicly available at present to explore the complexity of the transcriptional changes of Arabidopsis. For this reason, we analyzed the transcriptome of Arabidopsis at one time point after pathogen infection, insect infestation or abiotic stress using Affymetrix ATH1 whole-genome GeneChips. We aimed to determine which genes, pathways, gene set categories and predicted PPI networks may play a key role in specific responses to environmental stresses (pathogen infections and abiotic cues). Our focus in the current study was not only on plant responses to the necrotrophic pathogen B. cinerea, but also to the virulent and avirulent bacterial strains of P. syringae pv. tomato, the other fungal pathogen A. brassicicola and the herbivore insect P. rapae. We also extended our analysis to include oxidative stress and wounding on Arabidopsis. Because alterations in the level of phytohormones have some prominent responses against environmental stresses in planta, we studied the effect of SA, MeJA, ACC and ABA treatments.

There is an increasing demand for more meta-analysis of transcriptomic studies, which can be featured for many reasons. First, the transcript levels are highly affected by changing environmental conditions. Second, the inconsistency in the generated results, coming from field studies, can be affected by other disturbing factors.
Third, the integration of high-throughput technologies (transcriptomics, proteomics and metabolomics) is of a concern nowadays due to the high costs and qualified experts in “omic” analyses. Moreover, the commonalities of independent studies will identify genes strongly associated with the studied stresses in order to focus on the functional analysis of these common DEGs. Here, the well-defined analysis pipelines, standardized approaches for data analysis in addition to the availability of datasets to public were among the reasons that microarrays were selected over any other transcriptomic approaches.

In Arabidopsis, we found 6.8% and 5.3% of the transcriptome was considered up- and down-regulated in response to B. cinerea, respectively, which is in agreement with previous data in a number of studies. The time point for global expression profiling was set to 18 hpi with B. cinerea, due to the fact that most changes in gene expression occur between 18 and 30 hpi. In addition, the findings of our microarray meta-analyses on Arabidopsis plants individually treated with a tested single biotic stress, abiotic stress or hormone, are comparable to those in the literature. It must be noted that in all Arabidopsis-stress combinations, many genes showed a more than twofold change in expression at the time point tested. Although several studies have reported meta-transcriptomic analysis combining different environmental stresses, this report linked B. cinerea-infected plants with other biotic and abiotic stresses at the transcriptional level. More than 50% of BUGs were also induced by any of the P. syringae pv. tomato strains. In addition, 40–50% of all consistent changes elicited by A. brassicicola and P. rapae were consistently triggered by B. cinerea. This suggests that these genes are commonly activated or repressed during these Arabidopsis-attacker interactions.

On the other hand, the number of co-regulated genes in response to B. cinerea and the tested Arabidopsis-abiotic challenge combinations was much lower. We also investigated the role of hormones in the regulation of the overlapping gene sets of Arabidopsis-B. cinerea interaction. We identified probesets representing individual hormone-responsive genes among the selected B. cinerea-responsive genes. Comparison of the hormone-responsive genes with that of B. cinerea-responsive probesets revealed that 34%, 33%, 29% and 36% of the BUGs are responsive to SA-, JA-, ACC- and ABA, respectively (Table 1). The percentages of all hormonal responsive genes with the B. cinerea-repressed changes were even higher, indicating that hormones play a dominant role in the transcriptional reprogramming of Arabidopsis in response to B. cinerea infection. This is confirmed by another study of which the expression of BUGs is also affected by ethylene-sensitive 2 (ein2), coronatine insensitive 1 (coi1) and SA-deficient (nahG) mutations, suggesting a regulatory role of hormones in mediating gene expression by B. cinerea which may have effects on disease responses. It has been reported that a number of P. syringae-, A. brassicicola-, and P. rapae-induced genes are also considered as JA-responsive genes. Although pathogen/insect attackers with very different modes of action (e.g., B. cinerea, A. brassicicola, P. syringae and P. rapae) may induce similar sets of responsive genes, the majority of these genes are affected by a specific attacker. This suggests that hormones and other factors may enable fine-tuning the transcriptional machinery of defense response.

Interestingly, the eleven stress treatments induced only one gene and repressed three genes that could be considered as common regulators of the overlapping gene sets. The comparative microarray data analysis demonstrated that COR13/JR2 was commonly up-regulated and RAP2.4, At1g72060 and At2g20670 were found to be commonly down-regulated (Fig. S3, Supporting Information). The COR13/JR2 gene encoding cystine lyase is an enzyme that generates an ET precursor, and has been previously reported to be induced by B. cinerea, cold, drought and oxidative stresses, P. syringae-, A. brassicicola-, and P. rapae-induced genes are also considered as JA-responsive genes. Although pathogen/insect attackers with very different modes of action (e.g., B. cinerea, A. brassicicola, P. syringae and P. rapae) may induce similar sets of responsive genes, the majority of these genes are affected by a specific attacker. This suggests that hormones and other factors may enable fine-tuning the transcriptional machinery of defense response.

To get an insight into the function of commonalities of B. cinerea and other stress groups, we categorized the biological function according to the GO tool. Some of these functional categories covered a relatively large proportion of the Arabidopsis genome e.g. "electron transfer/energy pathways", representing most of the annotated genes commonly regulated by B. cinerea-biotic stresses and B. cinerea-hormonal stresses, but not B. cinerea-abiotic stress group. Thus, most of B. cinerea-abiotic stress group belonged to the functional category "biological processes" and "response to stress/stimulus". The number of up-regulated genes predicted to be involved in "response to biotic and abiotic stress" in all Arabidopsis biotic stress combinations tested was similar to observations done by the four biotic stresses of P. syringae, A. brassicicola, P. rapae and the feeding thrips (Frankliniella occidentalis). This distribution of the identified DEG sets over the various functional classes within the genome makes it possible to better understand the importance of the functional category in plant response. Analysis of GO data revealed that several B. cinerea- and abiotic stress-induced genes were in favor in "receptor binding" and "TF activity". Another large functional group of DEGs was enriched in the regulation of cellular components. These categories were consistent with the response of the plant to environmental stress known to derive to strong metabolic readjustment, defense mechanism and turning off the photosynthesis machinery. Our data are in accordance with previous findings in response to biotic and abiotic stresses in Arabidopsis. Modern 'omic' technologies can generate countless lists of biological identifiers/descriptions. Visualization of the identified overlapping genes/proteins can provide new insights into phenotypes and better understanding of the biological significance. The co-expression data and PPI networks were incorporated and visualized to identify the regulation of the up- and down-regulated genes/proteins (Table 2). Our analysis of the interactive networks of proteins matches with what has been previously reported that biological networks may elucidate the roles of hub proteins in distinct pathway(s) involved in response to multiple environmental changes. STRING is a distinctive database in a way that it not only allows the visualization of gene/protein lists of an interactome network, but also links it directly to a comprehensive annotation of the gene/protein lists; thereby providing biological implications of these overlapping as well as non-overlapping gene/protein sets. The integration of gene expression data with
the interactome network can provide further biological information associated with the function of these genes in response to multiple environmental challenges.

The genes involved in co-expression analysis have been underlying in molecular network formation. The co-expressed genes might be validated by their regulation is such having similar cis-regulatory elements for a TF. Thus, co-regulation studies validate the relationship of correlated genes. Among these is RAP2.4, which is involved in ET-activated signaling pathway and was repressed by B. cinerea (Fig. 5). As most RAP2.4 TFs, RAP2.4d protein mediates the fine-control and adjusts the availability of three chloroplast peroxidases. In addition, RAP2.4 can bind to both the dehydration-responsive element (DRE) and the ET-responsive GCC-box. The T-DNA insertion line of RAP2.4 showed increased resistance to B. cinerea. The B. cinerea-induced RAP2.2 which also relies on ET signaling pathway, showed, on the other hand, decreased resistance to this fungal pathogen. This indicates that RAP2.4 appears to be important in response to abiotic and biotic stresses, particularly in the tolerance to drought and pathogenesis of B. cinerea. Although resistance mediated by SA has been reported to be mainly against biotrophic pathogens, other reports have shown the involvement of SA-mediated genes in response to necrotrophic pathogens including B. cinerea. Similar to our results, PAD4 gene was repressed but PR1 gene was induced after B. cinerea infection, regardless of the inoculated genotype (Fig. 6). In comparison to wild-type, rap2.4 mutant plants exhibited significantly decreased expression levels of PR1, PR2 and PAD4, indicating that the basal expression level of SA-responsive genes may depend on RAP2.4. In addition, JA/ET-mediated response contributes to plant defense against necrotrophic pathogens. The JA/ET responsive genes, PDF1.2, PR3 and PR4, were induced by B. cinerea in wild-type and rap2.4 plants. The VSP2 gene that is regulated by the MYC2 can be activated by JA and wound but suppressed by ET. Our results showed that the expression of VSP2 and MYC2 was enhanced in rap2.4 but repressed in wild type plants after B. cinerea infection. These findings are in agreement with a previous study that infection with B. cinerea induce Arabidopsis VSP2 and MYC2 at 14 hpi, followed by a significant decrease at 24 hpi. This might be attributed the increased levels of the stress hormone ET leading to down-regulation of VSP2 and MYC2 in the wild-type-infected plants. Interestingly, our qRT-PCR analysis showed that the reduced transcript levels of VSP2 (Fig. 6) seems to contradict the results obtained from the microarray data analysis (Table 2). This discordance could be due to the different plant growth conditions and pathogen infection procedures in this study and the datasets obtained from publicly available microarrays.

Previous studies have reported that the cyclopentenone OPDA regulates gene expression in concert with JA to fine-tune the expression of defense genes. Arabidopsis EXLA2 and OPR3 genes can modulate gene expression through COI1 or the electrophile effect of the cyclopentenones under biotic stress conditions. In addition, Arabidopsis and tomato mutants of OPR3 enhanced resistance to B. cinerea. Consistent with that, it was found that the transcript levels of OPR3 were dramatically reduced in rap2.4-infected plants, indicating a common regulation of gene expression in response to electrophilic oxylipins and B. cinerea. The detoxification-related genes, GST6 and GSTU19, that are known to be highly up-regulated by OPDA and PPs were also induced in the wild-type, but not in the rap2.4 mutant, after infection. This confirms that the regulation of these genes is affected by the down-regulation of RAP2.4 toward B. cinerea. We speculate that rap2.4 mutant accumulates JA as well as cyclopentenone oxylipins upon infection with B. cinerea. To test this hypothesis, quantification of JA, OPDA and phytotropostane Aβ (PPAβ) in rap2.4 mutant before and after infection is among our priorities. Future research to dissect the importance of RAP2.4 in the regulation of JA- and/or cyclopentenone-mediated gene responses and to map all implicated elements in stress signal transduction pathways should be a major focus.

Our data provided a detailed picture of the highly dynamic interactive network involving several signaling proteins, in which inoculation with B. cinerea served as major control parameter to multiple stress responses in Arabidopsis. A recent genome-wide association study has revealed the combinatorial effect of P. rapae and drought on the regulation of genes in response to B. cinerea; thus by differentially regulating the level of resistance to B. cinerea. The meta-analysis of the current study revealed that upon individual or multiple stress(es), several common genes play a significant role in the defense mechanism of the plant. We conclude that RAP2.4 has the potential to serve in plant defense through the regulation of endogenous signal molecules and/or pathogen-derived effectors. Future investigations into the identification of pathogen-suppressed RAP2.4 gene expression and the relationship with membrane-associated microbe pattern (MAMP)-triggered defense will help explain the functions of RAP2.4 in innate immunity against B. cinerea. The long-term goal is to identify target genes, which can be helpful in breeding programs and agricultural biotechnology, and to generate crop varieties resistant to pathogen challenges, climatic changes and hormonal imbalances.

Methods

Plant growth and treatment conditions. We analyzed data from publicly available microarray datasets on Arabidopsis plants (ecotype Col-0) either infected or treated with the necrotrophic fungus pathogen B. cinerea, other biotic (P. syringae pv tomato DC3000 and avrRpm1, A. brassicicola and P. rapae), abiotic (oxidative stress and wounding) or hormonal (SA, MeJA, ACC or ABA) stresses. The microarray datasets were downloaded from NASCArrays at the BAR for Arabidopsis functional genomics database (http://bar.utoronto.ca/). The reference numbers for the corresponding stresses can be found in Table 3. Briefly, five-week-old Arabidopsis plants were inoculated by placing four 5-µl drops of 5 × 10^5 spore mL^-1 solution of B. cinerea on detached leaves. Responses to B. cinerea infection were assayed at 0 and 18 hpi of adult leaves. For bacterial infections, P. syringae pv. tomato DC3000 or avrRpm1, were infiltrated with 1 × 10^6 CFU mL^-1. Bioassays using drop-inoculation of 3-µl drops of 1 × 10^6 spores mL^-1 of A. brassicicola were carried out on detached leaves of 5-week-old plants. Infestations with P. rapae were performed by transferring 5 chewing larvae per plant. Responses to these biotic stresses were tested at 0 and 24 hpi.

For the oxidative stress or wounding treatment, 18-day-old seedlings were exposed to 10µM methyl viologen (paraquat) or punctured with pins, respectively, at 0 and 24 hours post treatment (hpt) as previously described. Arabidopsis seedlings grown in MS media were treated with 10µM SA, MeJA, ACC and ABA. Expressions
levels of hormonal treatments were determined at 3 hpt. Only shoot tissues were analyzed for all eleven stresses. No infection/no treatment control (designated as 0 hpi/hpt) was used from the obtained dataset NASCAarray-137.

Microarray procedure and normalization method. Affymetrix Expression Console (EC) software was used to treat the publicly available raw CEL files. This included probeset signal integration, background correction and quantile normalization. Transcriptome Analysis Console (TAC) software was used to analyze DEGs.

Affymetrix GeneChip ATH1 genome arrays representing 22,810 Arabidopsis genes were considered in each dataset from which, those with the absence (A) and medium (M) signal detection calls were removed and those which showed present (P) were only considered for further analysis. Depending on the treatment, the number of tested samples (n) for each replicate varies (Table 3). Three technical replicates were used for the analysis for each gene and their average was taken as the signal intensity. The FC for each gene was calculated by dividing the signal intensity (SI) of that particular gene in the treated data by the SI of that gene in the non-treated. The FC gene expression of ≥2 (up-regulated) or ≤0.5 (down-regulated) was set as default filter criteria for significant DEGs at P ≤ 0.05. Log2-transformed expression level data were used to generate scatter plots to detect the effect of each stress at 0 and the corresponding 3 hpt for hormones, 18 hpi for B. cinerea or 24 hpi/hpt for other biotic and abiotic stresses on plant gene expression. The gene identities and GO across microarray datasets were established using The Arabidopsis Information Resources (TAIR; www.arabidopsis.com). Although we compared gene sets across experiments and identified overlapping patterns of DEGs, the raw data was re-normalized under identical platforms regardless of spatiotemporal variability in the data.

The heatmap was generated using Morpheus (https://software.broadinstitute.org/morpheus/) to visualize large data matrices. To analyze the generated gene expression datasets, the top 50 BUGs and BDGs were plotted along with their corresponding DEGs of the other tested datasets. Log2 values of FC were scaled as −4 to +4; and denoted by color intensity from red (down-regulated genes) to green (up-regulated genes). Black was denoted for the absence of gene expression in the specific dataset. To identify the common DEGs across the datasets, Venn diagrams were generated using Venny 2.1 software (http://bioinfogp.cnb.csic.es/tools/venny/index.html).

Arabidopsis PPI network. The PPI dataset was downloaded from A. thaliana Protein Interaction Network (AtPIN). The interactome data includes the experimentally identified PPIs and computationally predicted interactions. The PPI network was created and visualized using Cytoscape Version 3.7.0. Node-edge attributes for the genes/proteins of interest were downloaded from STRING database (http://string-db.org/) which contains the known and predicted PPIs of the query. The network was modified based on these genes/proteins using a Cytoscape plugin Style (formerly known as Vizmapper). In the network, nodes represent the proteins/edges; whereas lines represent the interactions/connections. The attributes of the nodes were altered for the visualization of the network in respect to the genes/proteins of interest. Another plugin, BINGO v3.0.3, was used to determine the GO categories, which were statistically overrepresented in the selected set of proteins.

Fungal culture, plant inoculation and T-DNA insertion lines. For disease assays, B. cinerea strain rap2-2rap1-2rap2-4rap1-4 bo5-10 was grown on V8 agar media (36% V8 juice, 0.2% CaCO₃ and 2% Agar). The fungal cultures were sub-cultured by transferring a piece of agar containing the mycelium to a fresh plate of V8 agar and incubated at 25° C. In order to study the response of B. cinerea, we followed the procedure of disease assays coming from the microarray reference obtained from the BAR. Four rosette leaves from five-week-old soil grown Arabidopsis plants were drop-inoculated by placing a 3 µL each specific left and right primer (Table S1, Supporting Information). T-DNA lines were confirmed via qRT-PCR using primers provided in Table S1 (Supporting Information). Fungal biomass was assessed by accumulation of used as an endogenous reference for normalization. Expression levels were calculated by the comparative cycle variance (ANOVA) and Duncan’s multiple range test were performed to determine the statistical significance.

Statistical analysis. For qRT-PCR assay, three technical replicates of each sample were used with a minimum of three biological replicates. Results were expressed as means ± standard deviation (SD) of the number of experiments. A Student’s t-test for the values was performed at P < 0.05.

Data of B. cinerea growth and lesion size in inoculated plants represent the mean ± SD (n = 20). Analysis of variance (ANOVA) and Duncan’s multiple range test were performed to determine the statistical significance. Mean values followed by a different letter are significantly different from the corresponding control (P < 0.05). All experiments were carried out independently in triplicate with similar results.
References

1. Coolen, S., Van Pelt, J. A., Van Wees, S. C. & Pieterse, C. M. J. Mining the natural genetic variation in Arabidopsis thaliana for adaptation to sequential biotic and abiotic stresses. Planta 249, 1087–1105 (2019).

2. Suzuki, N., Rivero, R. M., Shulaev, V., Blumward, E. & Mittler, R. Abiotic and biotic stress combinations. New Phytol. 20, 32–43 (2014).

3. Atkinson, N. & Urwin, P. E. The interaction of plant biotic and abiotic stresses, From genes to the field. J. Exp. Bot. 63, 3523–3544 (2012).

4. Shaar-Moshe, L., Blumwald, E. & Peleg, Z. Unique physiological and transcriptional shifts under combinations of salinity, drought, and heat. Plant Physiol. 174, 421–437 (2017).

5. AbuQamar, S. F. et al. Expression profiling and mutant analysis reveals complex regulatory networks involved in Arabidopsis response to Botrytis infection. Plant J. 48, 28–44 (2006).

6. Segarra, G., Santipere, G., Elena, G. & Trillas, I. Enhanced Botrytis cinerea resistance of Arabidopsis plants grown in compost may be explained by increased expression of defense-related genes, as revealed by microarray analysis. PLoS One 8, e56075 (2013).

7. Balan, B., Marra, F. P., Caruso, T. & Martinielli, E. Transcriptional responses to biotic stresses in Malus x domestica, a meta-analysis study. Sci. Rep. 8, 1970 (2018).

8. Kilian, J. et al. 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 (2007).

9. Schen, P. M. et al. Coordinated plant defense responses in Arabidopsis revealed by microarray analysis. Proc. Natl. Acad. Sci. USA 97, 11655–11660 (2000).

10. Sham, A. et al. Transcriptome analysis reveals genes commonly induced by Botrytis cinerea infection, cold, drought and oxidative stresses in Arabidopsis. PLoS One 9, e113718 (2014).

11. Sham, A. et al. Identification of Arabidopsis candidate genes in response to biotic and abiotic stresses using comparative microarrays. PLoS One 10, e0125666 (2015).

12. Coolen, S. et al. Transcriptome dynamics of Arabidopsis during sequential biotic and abiotic stresses. Plant J. 86, 249–267 (2016).

13. Suzuki, N. Hormone signalling pathways under stress combinations. Plant Signal. Behav. 11, e1247139 (2016).

14. AbuQamar, S. F., Moustafa, K. & Tran, L. S. Mechanisms and strategies of plant defense against Botrytis cinerea. Crit. Rev. Biotechnol. 37, 362–274 (2017).

15. Verma, V., Ravindran, P. & Kumar, P. P. Plant hormone-mediated regulation of stress responses. BMC Plant Biol. 16, 86 (2016).

16. AbuQamar, S. F., Ajb, S., Sham, A., Enan, M. R. & Iatrini, R. A mutation in the expansin-like A2 gene enhances resistance to necrotrophic fungal and hypersensitivity in Arabidopsis thaliana. Mol. Plant Pathol. 14, 813–827 (2013).

17. AbuQamar, S. F., Moustafa, K. & Tran, L. S. Omics’ and plant responses to Botrytis cinerea. Front. Plant Sci. 7, 1658 (2016).

18. Rehrig, E. M., Appel, H. M., Jones, A. D. & Schultz, J. C. Roles for jasmonate- and ethylene-induced transcription factors in the ability of Arabidopsis to respond differentially to damage caused by two insect herbivores. Front. Plant Sci. 5, 407 (2014).

19. AbuQamar, S. F., Chai, M.-F., Luo, H., Song, F. & Mengiste, T. Tomato protein kinase 1b mediates signaling of plant responses to necrotrophic fungal infection and insect herbivory. Plant Cell 20, 1964–1983 (2008).

20. Dean, R. et al. The top 10 fungal pathogens in molecular plant pathology. Mol. Plant Pathol. 13, 414–430 (2012).

21. Laluk, K. & Mengiste, T. Necrotroph attacks on plants, wanton destruction or covert extortion? Arabidopsis Book 8, e0136 (2010).

22. Mengiste, T., Laluk, K. & AbuQamar, S. F. Mechanisms of induced resistance against B. cinerea. In Post-Harvest Pathology, Plant Pathology in the 21st century, Vol 2, Chap 2, eds Prusky, D. & Gullino, M. L. (Dordrecht, Springer Science + Business Media, B. V), 13–30 (2010).

23. Penninckx, I. A., Thomma, B. P., Buchala, A., Métrax, J. P. & Broekaert, W. F. Concomitant activation of jasmonate and ethylene response pathways is required for induction of a plant defensin gene in Arabidopsis. Plant Cell 10, 2103–2113 (1998).

24. AbuQamar, S. F., Luo, H., Laluk, K., NickelBarl, M. & Mengiste, T. Cross-talk between biotic and abiotic stress responses is mediated by the tomato AIM1 transcription factor. Plant J. 58, 347–360 (2009).

25. Jiang, Y. & Yu, D. WRKY57 regulates JA and ABA responses transcriptionally to compromise Botrytis cinerea resistance in Arabidopsis thaliana. Plant Physiol. 171, 2771–2782 (2016).

26. Sham, A. et al. Microarray analysis of Arabidopsis WRKY53 mutants in response to the necrotrophic fungus Botrytis cinerea. PLoS One 12, e0172343 (2017).

27. Zheng, Z., AbuQamar, S., Mengiste, T. & Chen, Z. Arabidopsis WRKY53 transcription factor is required for resistance to necrotrophic fungal pathogens. Plant J. 48, 592–605 (2006).

28. Liu, S. The role of Arabidopsis WRKY53 in modulating host immunity towards the necrotroph Botrytis cinerea (Doctoral dissertation). University of Cologne and Max Planck Institute for Plant Breeding in Cologne, Germany (2014).

29. Bow, M. J. & Sutton, A. J. Quality control in systematic reviews and meta-analyses. Eur. J. Vasc. Endovasc. Surg. 40, 669–677 (2010).

30. Toufighi, K., Brady, S. M., Austin, R., Ly, E. & Provart, N. J. The Botany Array Resource, e-northerns, expression analysis, and promoter analyses. Plant J. 43, 153–163 (2005).

31. Asselbergh, B., De VreeSchauwer, D. & Hofte, M. Global switches and fine-tuning-ARA modules plant pathogen defense. Mol. Plant Microbe Interact. 21, 709–719 (2008).

32. Windram, O. et al. Arabidopsis defense against Botrytis cinerea, Chronology and regulation deciphered by high-resolution temporal transcriptomic analysis. Plant Cell 24, 3530–3557 (2012).

33. Szklarczyk, D. et al. The STRING database in 2017. Quality-controlled protein–protein association networks, made broadly accessible. Nucleic Acids Res. 45, D362–D366 (2016).

34. Birkenbihl, R. P., Diezel, C. & Somssich, I. E. Arabidopsis WRKY33 is a key transcriptional regulator of hormonal and metabolic responses toward Botrytis cinerea infection. Plant Physiol. 159, 260–285 (2012).

35. Stintzi, A., Weber, H., Reymond, P., Browse, J. & Farmer, E. E. Plant defense in the absence of jasmonic acid, The role of cyclopentenones. Proc. Natl. Acad. Sci. USA 98, 12837–12842 (2001).

36. Taki, N.

37. AbuQamar, S. F. et al. 12-oxo-phytodienoic acid triggers expression of a distinct set of genes and plays a role in wound-induced gene expression in Arabidopsis. Plant Physiol. 139, 1268–1283 (2005).

38. Lorenzo, O., Chico, J. M., Sanchez-Serrano, J. J. & Solano, R. JASMONE-SENSITIVITE1 encodes a MYC transcription factor essential to discriminate between different jasmonate-regulated defense responses in Arabidopsis. Plant Cell 16, 1938–1950 (2004).

39. Mueller, S. et al. General detoxification and stress responses are mediated by oxidized lipids through TGA transcription factors in Arabidopsis. Plant Physiol. 20, 768–785 (2010).

40. Stotz, H. U., Mueller, S., Zoeller, M., Mueller, M. J. & Berger, S. TGA transcription factors and jasmonate-independent COI1 signalling specific plant responses to reactive oxylipins. J. Exp. Bot. 64, 963–973 (2013).

41. Lee, S., Rojas, C. M., Ishiga, Y., Pandey, S. & Mysore, K. S. Arabidopsis heterotrimERIC G proteins play a critical role in host and nonhost resistance against Pseudomonas syringae pathogens. PLoS One 8, e82445 (2013).

42. Meena, K. K. et al. Abiotic stress responses and microbe-mediated mitigation in plants, The omics strategies. Front. Plant Sci. 8, 172 (2017).
69. Crespo-Salvador, O., Escamilla-Aguilar, M., Lopez-Cruz, J., Lopez-Rodas, G. & Gonzalez-Bosch, C. Determination of histone

68. SAS Institute. The SAS System for Windows. Cary, NC, Release 9.0 SAS Institute Inc (2002).

67. Lee, S.

66. Scalschi, L.

65. Irizarry, R. A.

64. Brandão, M. M., Dantas, L. L. & Silva-Filho, M. C. AtPIN, Arabidopsis thaliana protein interaction network.

63. Goda, H.

62. Chehab, E. W.

61. AbuQamar, S. Expansins: Cell wall remodeling proteins with a potential function in plant defense.

60. Sweeney, T. E., Haynes, W. A., Vallania, F., Ioannidis, J. P. & Khatri, P. Methods to increase reproducibility in differential gene expression via meta-analysis. *Nucleic Acids Res.*, 45, e1–e1 (2017).

42. Seo, J. S. et al. Identification of a novel jasmonate-responsive element in the AtJMT promoter and its binding protein for AtJMT repression. *PLoS One* 8, e55482 (2013).

48. Rudnik, R., Bulcha, J. T., Reischneider, E., Ellersiek, U. & Baier, M. Specificity versus redundancy in the RAP2.4 transcription factor family of Arabidopsis thaliana. Transcriptional regulation of genes for chloroplast peroxidases. *BMC Plant Biol.* 17, 144 (2017).

43. Wang, Y. et al. Arabidopsis RAP2.2 plays an important role in plant resistance to Botrytis cinerea and ethylene responses. *New Phytol.* 195, 450–460 (2012).

49. Li, N., Han, X., Feng, D., Yuan, D. & Huang, L.-J. Signaling crosstalk between salicylic acid and ethylene/jasmonate in plant defense. Do we understand what they are whispering? *Int. J. Mol. Sci.* 20, 671 (2019).

50. Leon-Reyes, A. et al. Salicylate-mediated suppression of jasmonate-responsive gene expression in Arabidopsis is targeted downstream of the jasmonate biosynthesis pathway. *Plant Biol.* 232, 1423–1432 (2010).

51. Zhao, Z. et al. Transcriptome analysis of gene expression patterns potentially associated with premature senescence in Nicotiana tabacum L. *Mol. Cell* 23, 2856 (2018).

52. Wang, Y., Thilmony, R. & Gu, Y. Q. NetVenn, an integrated network analysis web platform for gene lists. *Nucleic Acids Res.* 42, W161–166 (2014).

53. Vandereyken, K., Leene, J. V., Coninck, B. D. & Cammue, B. P. A. Ubiquitin strategy, taking a closer look at *Arabidopsis* co-regulation. *Plant Gene* 1, 29–34 (2015).

54. Zhao, Y. et al. Arabidopsis RAP2.2 plays an important role in plant resistance to Botrytis cinerea and ethylene responses. *New Phytol.* 195, 450–460 (2012).

55. Li, N., Han, X., Feng, D., Yuan, D. & Huang, L.-J. Signaling crosstalk between salicylic acid and ethylene/jasmonate in plant defense. Do we understand what they are whispering? *Int. J. Mol. Sci.* 20, 671 (2019).

56. Leon-Reyes, A. et al. Salicylate-mediated suppression of jasmonate-responsive gene expression in Arabidopsis is targeted downstream of the jasmonate biosynthesis pathway. *Plant Biol.* 232, 1423–1432 (2010).

57. AbuQamar, S. Expansins: Cell wall remodeling proteins with a potential function in plant defense. *Plant Biochem. Physiol.* 2, 1 (2014).

58. Zhang, R., Qi, H., Sun, Y., Xiao, S. & Lim, B. L. Transgenic *Arabidopsis thaliana* containing increased levels of ATP and sucrose is more susceptible to *Pseudomonas syringae* syringae. *Nucleic Acids Res.* 45, e1–e1 (2017).

59. Hemsley, P. A. et al. The Arabidopsis mediator complex subunits MED16, MED14, and MED2 regulate mediator and RNA polymerase II recruitment to CBP-responsive cold regulated genes. *Plant Cell* 26, 465–484 (2014).

Acknowledgements
This project was funded by Khalifa Center for Biotechnology and Genetic Engineering-UAEU (Grant #: 31R081) to S.AQ.

Author contributions
K.T. and S.AQ. designed the research. K.T. and S.AQ. supervised the study. A.S., H.A. and K.W. performed experiments. A.S. performed the PPI network. K.T. and S.AQ. analyzed the data. H.A., K.W. and R.I. assisted with experiments and/or data evaluation. K.T. and S.AQ. wrote the manuscript. All authors critically revised the manuscript and approved the final version.

Competing interests
The authors declare no competing interests.

Additional information
Supplementary information is available for this paper at https://doi.org/10.1038/s41598-019-53694-1.

Correspondence and requests for materials should be addressed to K.T. or S.AQ.

Reprints and permissions information is available at www.nature.com/reprints.
