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The hnRNP-Q Protein LIF2 Participates in the Plant Immune Response

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Introduction

Living organisms are exposed to various abiotic and biotic stresses and continuously integrate diverse signals to limit the spread of microbial pathogens. RNA regulatory processes, which modulate transcriptional programs and post-transcriptional events in response to various cues, are an important component of the tolerance and adaptation strategies of organisms. Thus, RNA-binding proteins (RBPs), which are involved in various aspects of RNA processing (i.e., mRNA maturation, editing, splicing, and mRNA trafficking), are thought to be key regulators of stress responses in both animals and plants. However, this possibility has been scarcely studied.

The heterogeneous nuclear ribonucleoprotein (hnRNP) group of RBPs forms a large family of ancestral modular proteins with a high degree of functional diversification [1]. In humans, this group contains 37 members, only a few of which are involved in the stress response. For instance, hnRNP-K participates in the response to genotoxic stress [2]. The human NSI-associated protein 1/ synaptotagmin-binding cytoplasmic RNA-interacting protein (NSAP1/SYNCRIP), another cellular hnRNP protein, participates in the translational activation of both cellular and viral RNAs and functions in the Hepatitis C virus (HCV) life cycle by interacting with HCV RNA [3,4,5]. Knock-down of NSAP1/SYNCRIP significantly decreased the amount of HCV RNA in mammalian cells, suggesting that NSAP1/SYNCRIP is a negative regulator of viral defense responses. Recently, hnRNP-I was shown to interact with the long non-coding RNA RoR to modulate the expression of the tumor suppressor p53 in response to DNA damage and to participate in a surveillance network [6].

Plants contain more RBPs than do animals, but the number of plant hnRNPs is similar in both plants and animals. The functions of hnRNPs remain poorly described in plants [1,7]. Few studies have reported a role for plant hnRNPs in the stress response [8]. Until now, the glycine-rich RBP AtGRP7 was the only hnRNP known to play a role in the plant’s response to pathogens [9]. AtGRP7 is the substrate of the type III effector HopU1, which is injected by the bacterial pathogen Pseudomonas syringae into plant host cells. HopU1 mono-ADP ribosylates a conserved arginine
Table 1. Gene ontology (GO) analysis of the deregulated genes in the *lif2-1* mutant.

| Class          | Term                                      | Query item | Ref item | p-value    | FDR        | NF          |
|----------------|-------------------------------------------|------------|----------|------------|------------|-------------|
| Stimulus       | Response to stimulus GO:0050896           | 293        | 4057     | 8.60E-46   | 2.00E-42   | 2.78        |
|                | Response to chemical stimulus GO:0042221 | 172        | 2085     | 9.20E-36   | 7.10E-33   | 3.17        |
|                | Response to biotic stimulus GO:0009607   | 70         | 639      | 7.00E-23   | 2.30E-20   | 4.21        |
|                | Response to external stimulus GO:0009605 | 52         | 429      | 2.10E-19   | 4.90E-17   | 4.66        |
|                | Response to endogenous stimulus GO:0009719| 87         | 1068     | 2.60E-19   | 5.40E-17   | 3.13        |
|                | Response to abiotic stimulus GO:0009628  | 105        | 1471     | 1.00E-18   | 2.00E-16   | 2.75        |
| Stress         | Response to stress GO:0006950             | 193        | 2320     | 5.00E-40   | 5.80E-37   | 3.20        |
|                | Response to wounding GO:0009611           | 43         | 197      | 1.50E-26   | 7.10E-24   | 8.39        |
|                | Defense response GO:0006952               | 72         | 766      | 1.10E-19   | 2.90E-17   | 3.61        |
| Biotic stimulus| Response to fungus GO:0009620             | 25         | 158      | 8.40E-13   | 1.20E-10   | 6.09        |
|                | Response to bacterium GO:0009617          | 30         | 247      | 5.60E-12   | 7.20E-10   | 4.67        |
| Hormones       | Response to jasmonic acid stimulus GO:0009753 | 33        | 215      | 5.80E-16   | 1.00E-13   | 5.90        |
|                | Response to hormone stimulus GO:0009725  | 66         | 982      | 1.80E-11   | 2.00E-09   | 2.58        |
|                | Jasmonic acid mediated signaling pathway GO:0009867 | 12        | 49       | 3.80E-09   | 2.90E-07   | 9.42        |
|                | Response to abscisic acid stimulus GO:0009737 | 31        | 378      | 3.90E-08   | 2.50E-06   | 3.15        |
|                | Glicosinolate metabolic process GO:0019757 | 12        | 62       | 6.50E-08   | 3.80E-06   | 7.44        |
| Various        | Response to chitin GO:0010200             | 27         | 151      | 4.90E-15   | 8.10E-13   | 6.88        |
|                | Response to carbohydrate stimulus GO:0009743 | 31        | 240      | 4.90E-13   | 7.60E-11   | 4.97        |
|                | Lipid localization GO:0010876             | 11         | 24       | 7.10E-12   | 8.70E-10   | 17.63       |
|                | Response to water deprivation GO:0009414  | 28         | 229      | 2.30E-11   | 2.50E-09   | 4.30        |
|                | Immune response GO:0006955                | 36         | 367      | 2.60E-11   | 2.70E-09   | 3.77        |
|                | Response to osmotic stress GO:0006970     | 38         | 408      | 3.60E-11   | 3.30E-09   | 3.58        |
|                | Response to salt stress GO:0009651        | 33         | 366      | 1.40E-09   | 1.20E-07   | 3.47        |
|                | Response to oxidative stress GO:0006979   | 31         | 332      | 1.90E-09   | 1.50E-07   | 3.59        |

Among the 1008 deregulated genes in *lif2-1*, 982 are associated with a GO term and were analysed using the agriGO toolkit. GO terms with the best p-value (p<10^-18) were selected to illustrate *lif2* transcriptome specificities. Query item: number of deregulated genes in *lif2* in a given GO class. Reference item: total number of genes in a given GO class. The *A. thaliana* genome accounts for a total of 37767 items in the agriGO toolkit. The normed frequency (NF) = (Query item/Ref item)/(982/37767).
residue of AtGRP7, preventing binding to immunity-related RNA [10,11].

In a previous study, we identified LIF2, a novel hnRNP of the cruciferous plant Arabidopsis thaliana [12]. LIF2 is a nucleocytoplasmic RBP that contains three RNA Recognition Motifs (RRMs), the most frequent RNA-binding domain in the hnRNP family. LIF2 and its close homologs, AtLIL1 and AtLIL2, belong to the hnRNP-Q subfamily and LIF2 is structurally homologous to human NSAP1/SYNCRIP [12]. LIF2 interacts in vitro with LHP1, a Polycomb Repressive Complex1 (PRC1) subunit [13,14]. LIF2 is involved in the maintenance of plant cell identity and cell fate decision [12]. Indeed, loss-of-function of LIF2 affected various aspects of growth and development, such as flowering time and leaf size. More dramatically, lif2 mutations induced indeterminate growth of ovaries, resulting in the formation of ectopic inflorescences bearing severely affected flowers. Besides its developmental functions, LIF2 might be involved in the stress response. Indeed, a large set of stress-related genes (193) was found to be deregulated in lif2-1. The set of common genes deregulated in both lif2-1 and lhp1 mutants was even more enriched in stress-related genes than was that in lhp1-1 [12]. Furthermore, loss-of-function of LHP1 (also named TFL2 or TUD) altered the glucosinolate profile and reduced symptoms in response to infection by the obligate biotrophic fungus Plasmodiophora brassicae, which causes clubroot disease, a damaging disease in Brassicaceae [15,16].

These data suggest that LIF2 and LHP1 have common functions in the stress response and prompted us to investigate the function of LIF2 in biotic stress responses. Here, we show that lif2 mutations conferred altered susceptibility to the necrotrophic fungal pathogens Sclerotinia sclerotiorum (S. sclerotiorum) and Botrytis cinerea (B. cinerea) and to the hemi-biotrophic bacterial pathogen P. syringae pv. tomato (Ptu), three biological agents with substantial impacts on the agronomical production of various plant species [17,18,19]. To better understand the altered pathogen susceptibility of the lif2 mutants, we investigated the expression of defense marker genes regulated by jasmonic acid (JA) and salicylic acid (SA), two critical signaling hormones in the activation of plant defense, as well as the production of stress-associated metabolites and hormones. Finally, we showed that the transcriptomic profiles of stress-response factors in lif2 are specific and we identified key defense regulators (such as the WRKY18 and WRKY33 transcription factors, which are known to be involved in B. cinerea susceptibility), whose deregulation may contribute to the observed pathogen response. Together, our data suggest that the LIF2 hnRNP-Q may suppress the plant immune response by an unknown SA-independent pathway. Given that the human homolog of LIF2, the NSAP1/SYNCRIP protein, is a trans-acting factor involved in both cellular processes and the viral life cycle [3,4,5], we propose that the conserved hnRNP-Q proteins may have an evolutionary conserved function in regulating the trade-off between growth and defense in eukaryotes.

Results
Analysis of the lif2-1 transcriptome reveals a potential stress-related function for LIF2

In a previous transcriptome profiling experiment, we showed that genes that were deregulated in the A. thaliana lif2-1 null mutant were greatly enriched in Gene Ontology (GO) terms involved in responses to stress stimuli [12]. Here, we further examined the deregulated gene set (1008 genes) using Singular Enrichment Analysis (SEA) implemented in the agriGO toolkit [20]. This analysis revealed 293 deregulated genes associated with the GO term “response to stimulus” and 193 with “response to stress” (Table 1). We noticed that the normed frequency (NF) of the “response to biotic stimulus” (GO:000960, NF 4.22, p-value 7 × 10⁻⁸⁵) was higher than that of the “response to abiotic stimulus” (GO:0009628, NF 2.75, p-value 2 × 10⁻¹⁶) (Table 1). Furthermore, several genes were associated with GO terms related to the JA-defense signaling pathway, the glucosinolate metabolic pathway, and responses to fungal and bacterial pathogens (Table 1). These data suggest that even if LIF2 is induced only weakly upon pathogen infection (Fig. S1), this gene might be involved in defense responses to various bioaggressors.

JA and SA pathways are altered in the lif2-1 mutant

Based on the lif2 transcriptome data [12], we further analyzed the expression of a key marker gene of the JA-mediated defense, PDF1.2 (a JA-responsive gene), and the expression of genes of the JA biosynthesis and signaling pathways, LOX3, AOS, AOC, and OPR3 (genes encoding enzymes of the JA biosynthesis pathway), JAR1 (encoding an enzyme that converts JA to the bioactive JA-Ile molecule), and COH1 (encoding an F-box subunit of the JA-receptor complex) [21] (Fig. 1A). All of these genes were downregulated in lif2-1, suggesting that the JA defense pathway is globally repressed in the lif2-1 mutant.

Since the JA-dependent defense network cross-communicates with the SA-dependent signaling pathway to fine-tune the plant’s defense response [22,23], we assessed the expression of ICS1/ SID2, which is involved in SA biosynthesis; ENHANCED DISEASE SUSCEPTIBILITY1 (EDS1) and PAD4, which are involved in the SA-signaling pathway; and NON-EXPRESSOR OF PR GENES 1 (NPR1) and PATHOGENESIS-RELATED 1 (PR1), which are important positive regulators of SA responses [24,25]. EDS1, PAD4, and ICS1 were upregulated in the lif2-1 mutant relative to wild-type plants (Fig. 1B). PR1 was also upregulated in lif2-1, whereas NPR1 expression was not significantly affected in lif2-1 (Fig. 1B). These data suggest that most genes involved in the SA-mediated defense pathway are constitutively upregulated in the lif2 mutants, in the absence of pathogen. The upregulation of genes of the SA signaling pathway and the downregulation of genes of the JA signaling pathway in lif2 illustrates the antagonistic action of these two pathways.

Prompted by the finding that genes involved in JA- and SA-defense-related networks are differentially expressed in lif2-1, we quantified the endogenous levels of these hormones in lif2-1 using HPLC-electrospray-MS/MS (Fig. 1C). Surprisingly, whereas ICS1 was upregulated in lif2-1, free SA levels were similar in the mature rosette leaves of lif2-1 and wild-type plants (Fig. 1C). Similarly, the JA levels were not significantly affected in the lif2-1 mutant (Fig. 1C). Furthermore, we found that the levels of auxin (IAA) and abscisic acid (ABA), two other phytohormones recently shown to be involved in the stress response [26,27], were not significantly altered in lif2-1 in the absence of pathogen challenge (Fig. 1C).

The lif2 mutants are less susceptible to the bacterial pathogen Pseudomonas syringae

As JA- and SA-defense-related gene expression were altered in the lif2 mutant (Fig. 1A-B), we investigated the response of lif2 to inoculation with the hemi-biotrophic bacterial pathogen P. syringae [28]. The bacterial growth of the virulent DC3000 strain was 10 times lower in lif2-1 leaves than in wild-type leaves at 24 hours post-inoculation (hpi) (Fig. 2A). Consistently, the disease symptoms were reduced on the leaves of two independent lines, lif2-1 and lif2-3, whereas severe symptoms were observed on the leaves of wild-type plants five days post-inoculation (dpi) (Fig. 2B). As expected, the lif2-2 line, a lif2-1 mutant line complemented with LIF2 under the control of its own regulatory regions, was as
susceptible as the wild-type plant (Fig. 2B). Similar results were obtained with the avirulent DC3000 \textit{avrRpm1} strain (Fig. 2C).

These data are consistent with the observation that SA-related genes are constitutively expressed in the \textit{lif2} mutant. In response to \textit{P. syringae} infection, the JA-related biosynthesis genes were upregulated in wild-type plants and to a lesser extent in \textit{lif2-1} (Fig. 3), with the exception of \textit{JAR1}, which was not induced. \textit{COI1}, which encodes a component of the JA-receptor complex interacting with the bacterial phytotoxin coronatine, was upregulated in both genotypes (Fig. 3A). \textit{PDF1.2} was downregulated in wild-type plants and not activated upon infection in \textit{lif2}. In contrast, genes involved in SA biosynthesis and signaling pathways were upregulated in both wild-type and \textit{lif2} plants upon DC3000 inoculation (Fig. 3B). \textit{PAD4} induction was weaker in \textit{lif2} than in wild-type plants, whereas \textit{PR1} expression was similar in the mutant and wild-type plants upon \textit{P. syringae} infection. Therefore,
despite a significant basal activation in the absence of pathogen, lif2 mutant plants were able to further activate SA-related defense genes in response to pathogen attack, and the response of the lif2 mutant to P. syringae infection differed from the wild type only in the level of defense gene expression. Together, these results indicate that LIF2 is not necessary for the activation of SA- and JA-related defense genes.

Response of the lif2 sid2 double mutant to the biotrophic P. syringae pathogen

Since neither the high level of PR1 expression in the lif2 mutant nor the reduced susceptibility of lif2 to P. syringae infection were correlated with an increase in free SA content, we hypothesized that LIF2 may act downstream of SA production. To test this hypothesis, we crossed the lif2 mutant with the salicylic acid induction deficient2 (sid2/ics1) mutant. The rosettes of sid2 lif2-1 double mutant plants were smaller than those of the two parental lines, sid2-2 and lif2-1, in both short-day (SD) and long-day (LD) conditions (Fig. 4A and S2). Interestingly, in SD conditions, similarly to lif2-1 plants, the double mutant was early flowering (Fig. S2) and produced indeterminate ovaries (IDO) with an ectopic inflorescence, suggesting that the lif2-1 mutation is epistatic to sid2-2.

We then investigated the response of the lif2-1 sid2-2 double mutant to pathogens. Bacterial growth of the DC3000 strain was reduced at 24 hpi (by about 10-fold) in the lif2-1 sid2-2 rosette leaves compared with those of the wild type (Fig. 4B), similarly to lif2-1. These results suggest that the reduced susceptibility of lif2 is independent of SA biosynthesis. Interestingly, the level of PR1 expression in lif2-1 sid2-2 was similar to that of the wild type (Fig. 4C), suggesting that the decreased susceptibility of lif2 may involve an SA-independent defense pathway.

Response of the lif2 mutant to a necrotrophic fungal pathogen

Since responses to pathogens are usually largely dependent on the pathogen lifestyle, we then assessed the defense response of lif2 to the necrotrophic fungal pathogen S. sclerotiorum (strain S55). We used two A. thaliana accessions as controls, Rubezhnoe-1 (Rbz-1) and Shahdara (Sha), which were previously shown to be resistant and susceptible to S. sclerotiorum, respectively [29]. After inoculation, the symptoms were stronger in lif2 than in wild-type plants (Fig. 5A and B), revealing that lif2 is susceptible to S. sclerotiorum. This observation is consistent with the constitutive downregulation of JA-related genes in lif2 reported in this study, and the finding that the JA pathway is essential for resistance to this pathogen [29,30,31].

We then inoculated these plants with a second necrotrophic fungal pathogen, B. cinerea. Unexpectedly, we observed that lif2 was less susceptible to two virulent strains of B. cinerea, the wild-type virulent B0510 and Bd90 strains, the latter of which was less virulent (Fig. 6A-C). These data suggest that the immune response of lif2 is independent of the pathogen's lifestyle.

To decipher the molecular basis for the resistance of lif2 to B. cinerea, we assessed the expression of a number of regulators known to be involved in disease resistance to B. cinerea (Table 2). For instance, mutations in the MIB46 transcription factor gene [32], ATGXR513 [33], or the basic helix-loop-helix MYC2 transcription

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Figure 2. The lif2 mutants are less susceptible to P. syringae infection. (A) Bacterial growth of the virulent DC3000 strain in wild-type (WT) and lif2-1 rosette leaves at 24 hours post-inoculation (hpi). (B) Rosette leaves imaged 5 days post-inoculation (dpi) with the virulent DC3000 strain. The lif2-1 and lif2-3 plants had similar responses, whereas the complemented lif2-1 mutant (lif2-c) behaved similarly to WT plants. Four independent experiments were performed with similar results. (C) Bacterial growth of the avirulent DC3000 avrRpm1 strain in rosette leaves at 24 hpi. For bacterial growth experiments, each data point represents the mean value from at least thirty leaves. Similar results were obtained in two independent experiments. The bars represent standard deviation. (Student’s t-test, * p<0.05).

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factor [34] led to increased resistance to *B. cinerea*. In our transcriptome data, *ATGXRS13* was upregulated in *lif2*, while *MYB46* was not affected, suggesting that these two genes are probably not involved. However, we found that *MYC2* was downregulated in *lif2* (Fig. 6D), which is in agreement with the decreased susceptibility of *lif2* to *B. cinerea*. We also evaluated the expression profiles of a few other candidate genes whose downregulation led to an increased susceptibility to *B. cinerea* (i.e., *PAD2*, *CYP71B15/PAD3*, *BIK1*, *WRK35*, *WRK40*, and *WRK33*) [35,36,37,38,39,40]. Interestingly, only the expression of *WRK33* was significantly different in the two mutant alleles compared with wild-type or *lif2-c* plants.

The loss-of-function of *CATALASE 2* (*CAT2*) triggers pathogen defense responses and resistance [41]. Furthermore, the inhibition of NADPH oxidase and of other flavoprotein enzymes involved in oxidative stress limits *B. cinerea* infection [42]. We found that *CAT2* was downregulated in *lif2*, which is in agreement with the reduced susceptibility of this mutant to *B. cinerea*. Thus, *CAT2* and the *MYC2*, *WRK35*, and *WRK33* transcription factors are interesting candidate proteins that participate in the *lif2* response to *B. cinerea*.

Glucosinolate content is altered in the *lif2* mutant

Due to the observed enrichment in the GO term “glycosinolate metabolic process” (GO:0019757) in the *lif2-1* transcriptome (Table 1), and to the established role of glucosinolates (GLSs) and their breakdown products in plant defense [43], we extracted and quantified twenty-one of these secondary metabolites (*β*-thioglucoside-N-sulfated oximes), belonging to three main GLS families (aliphatic, indolic, and benzoate GLSs) in mutant and wild-type seedlings (Table S1). The GLSs were analyzed by negative electrospray ionization liquid chromatography coupled with mass spectrometry (ESI-HPLC-MS). Globally, the GLS content was lower in the *lif2* mutant than in wild-type plantlets, with decreases observed in fourteen GLSs belonging to all three main GLS families [Fig. 7A]. The levels of indol-3-ylmethyl glucosinolate (*I3M*), 4-methylsulfinylbutyl glucosinolate (*4MSOB*), 4-methylthiobutyl glucosinolate (*4MTB*), and 5-methylthiopentyl glucosinolate (*5MTP*) were significantly decreased in the *lif2* mutant. Interestingly, the indolic GLS family was less affected by the *lif2* mutation, with only a decrease in indol-3-ylmethyl glucosinolate (*glucobrassicin, I3M*) being observed. As a control, we quantified the GLS content in the *lhp1* mutant (Fig. 7B), which is known to have an altered glucosinolate profile [15]. In *lhp1*, significantly increased levels were observed for six GLSs (i.e., *8MSOO, 3MTP, 4MSOB, 5MSOP, 6MSOH,* and *7MSOH*), whereas only one GLS, the indolic *4MOI3M* GLS, had decreased levels relative to the wild type. Similar results were obtained using *lhp1* seeds [15]. Next, we quantified the levels of raphanusamic acid (RA), an important breakdown product of GLS that forms during the biotic stress response [44]. PEN2 myrosinase catalyzes the formation of RA from the I3M or 4MOI3M substrates. We observed a significant reduction in RA in both *lif2* (0.91 ng/mg, n = 7–8, p < 0.01) and *lhp1* (0.89 ng/mg, n = 7–8, p < 0.001) compared with wild-type plants (1.39 ng/mg).

Therefore, *lif2* has a GLS profile that is distinct from that of wild-type and *lhp1* seedlings. Thus, the control of GLS metabolism...
seems to be dependent on LIF2 and partially independent of the LHP1/LIF2 interaction [12]. Alterations to the GLS pathways may also contribute to the responses of the lif2 mutant to pathogens.

**Molecular signature of the lif2 mutant**

In an attempt to identify the key genes involved in the lif2 response to biotic stress, we further analyzed the expression of transcription factors (TFs), due to their prominent role in regulating gene expression. Among the 66 TFs deregulated in the lif2-1 transcriptome [12], we noticed that the APETALA2/ethylene-responsive element-binding protein (AP2/EREBP), WRKY, MYB, and basic helix-loop-helix (bHLH) transcription factor families were overrepresented (Table S2) (Fig. 8A). We also noticed a strong bias towards downregulation of genes of these families (Fig. 8). For example, 11 of the 13 deregulated genes of the AP2-EREBP family were downregulated in lif2 (Fig. 8). Out of the four subfamilies of AP2/EREBP (i.e., AP2, RAV (related to ABI3/VP1), dehydration-responsive element-binding protein (DREB), and ERF) [45,46], only the two largest ones, the DREB and ERF subfamilies, were deregulated in the lif2-1 mutant. The plant-specific WRKY transcription factors are key regulators of stress and plant immune responses [47], whereas the NAC TFs are involved in both development and the abiotic and/or biotic stress responses. Two stress-responsive NACs (SNACs) that were recently described [48,49] were deregulated in the lif2-1 transcriptome. In the MYB and bHLH families, MIF2, which encodes a key defense transcription factor involved in JA responses, was deregulated (Fig. 6D) as well as the JA-ASSOCIATED MIF2 LIKE1 gene (JAM1).

Furthermore, we noticed that 6 genes associated with the GO term “negative regulators of defense response” (GO:0031348) were downregulated in the lif2-1 transcriptome. These genes might participate in the lif2 stress response (Fig. 8B). Among these genes, JAR1 represses the SA-dependent signaling pathway; PEN3, which is an ABC transporter, restricts pathogen proliferation in the hosts; and BAP1 is a negative regulator of programmed cell death and is involved in membrane trafficking in response to external conditions [50]. These data suggest that LIF2 affects a variety of defense-related pathways.

The 38 TFs that were both deregulated in the lif2-1 rosette transcriptome (Table S2) and belong to the AP2/EREBP, WRKY, NAC, MYB, or bHLH family, as well as the 6 negative regulators (Fig. 8B) represent a sort of molecular signature of the lif2 mutant phenotype. We thus wondered how these 44 genes were deregulated in wild-type genetic backgrounds in response to biotic stress. To address this question, we selected 62 transcriptome profiles from plants treated with pathogens compared with those that were not, in the Genevestigator database [51] and extracted the expression of these 44 genes. We then performed a hierarchical
clustering analysis using of the MultiExperiment Viewer application [52], and identified three main gene clusters, which are characterized by specific co-expression profiles of \textit{MYC} and \textit{MYB} (Fig. 8C). The small Cluster I (containing \textit{MYC4/MYB28/MYB48}) contained genes that are upregulated in \textit{lif2-1} and downregulated in response to most pathogen treatments. The large Cluster II (containing \textit{MYC2/MYB15}) contained genes downregulated in \textit{lif2-1} and up- or downregulated in response to pathogen treatments, with no obvious link to the biology of the pathogens. Finally, Cluster III (containing \textit{MYC7E/MYB34}) mainly contained genes repressed in \textit{lif2-1} and in response to most biotic stresses, except in response to \textit{P. syringae}, and thus, which are probably associated with the JA pathway due to the presence of JAR1. In conclusion, our clustering analysis revealed that (i) the expression profiles of the 44 selected genes involved in the response to pathogens were diverse, (ii) the \textit{MYC} and \textit{MYB} genes were co-expressed in different combinations, and (iii) \textit{lif2} exhibited a unique expression pattern, with opposite profiles to most of the 62 analyzed transcriptome profiles, which might be in line with its specific response to pathogens.

**Discussion**

Plants have evolved both constitutive and induced defense mechanisms to counteract pathogen attacks. Most of the induced mechanisms are based on the production of a complex repertoire of plant metabolic compounds and signaling hormones, which activate appropriate defense pathways, based on the nature of the pathogens [53,54]. Salicylic acid, a phenolic phytohormone, and the jasmonic acid phytohormone, a polyunsaturated fatty acid derived from \(\alpha\)-linolenic acid, are two key molecules in plant defense mechanisms [55]. SA, which also possesses medicinal properties [24] and plays a central role in animal immunity [56], and its derivatives, have crucial roles in defense against biotrophic pathogens [57]. They also regulate various aspects of abiotic stress responses, plant growth, and development. The JA and SA defense signaling pathways have been shown to cross-communicate and to be mostly antagonistic, providing plants with a regulatory potential to fine-tune their defense reaction depending on the type of pathogen encountered [22,23]. SA and JA are mainly involved in the response to biotrophic and necrotrophic pathogens, respectively. However, complex crosstalk exists between the hormone signaling pathways involved in plant responses to pathogens [26]. Secondary metabolites, such as glucosinolates, are also involved in the biotic stress response in plants [43].

Only a few RBPs have emerged as regulators of the biotic stress response in plants [8,38,59,60]. For instance, AtRBP-DR1, a putative component of the resistance protein RPS2 complex, positively regulates defense responses mediated by SA in \textit{A. thaliana} [61]. The conserved MOS2 protein, a putative RBP with both G-patch and KWO domains, is required for resistance against \textit{P. syringae pv. maculicola} ES4326 and \textit{Peronospora parasitica} Emoy2 [62], whereas overexpression of the \textit{Gossypium hirsutum} GhZF1 protein, which contains a CCCH-type zinc finger present in some RBPs, has been shown to enhance both disease resistance to the biotrophic oomycete \textit{Hyaloperonospora arabidopsidis} [9]. Except for the hnRNP AtGRP7 [9], the RBP-mediated defense mechanisms remain poorly understood.

**Figure 5. LIF2 is involved in the plant defense response to \textit{S. sclerotiorum}.** Leaves of four-week-old plants were inoculated with \textit{S. sclerotiorum} strain S55. The \textit{lif2} alleles and the complemented \textit{lif2-c} line in the Col-0 background were analyzed. \textit{A. thaliana} Col-0, Rubezhnoe-1 (Rbz-1) (more resistant than Col-0), and Shahdara (Sha) (more susceptible than Col-0) accessions were used as controls. (A) Symptoms at 7 dpi. (B) The disease score was evaluated for each line at 7 dpi. Means and standard deviations were based on at least twenty plants per line. A significant difference in susceptibility relative to that of the Col-0 accession is indicated with an asterisk (Kruskal and Wallis’s test, * \(p<0.05\)).

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Here, we studied the role of A. thaliana RBP LIF2 in the plant’s response to biotic stress. We demonstrated that LIF2 loss-of-function results in pathogen resistance, in a manner that is independent of the pathogen’s lifestyle or infection strategy. Indeed, the lif2 mutant was less susceptible to both the hemi-biotrophic bacterial P. syringae pv. tomato pathogen and the necrotrophic ascomycete B. cinerea, two pathogens with substantial impacts on agronomical production due to their wide range of host specificities [17,18]. However, lif2 was more susceptible to the necrotrophic ascomycete S. sclerotiorum, which attacks more than 400 plant species around the world.

The three pathogens used in this study induce different plant defense pathways and their associated defense molecules: the JA and ethylene pathways, and possibly the SA pathway, are activated in response to S. sclerotiorum infection [29,30]; the SA pathway is key in the plant’s response to P. syringae infection; and JA and camalexin are involved in the response to B. cinerea infection [64]. Our analysis of the lif2 mutant revealed that SA-related genes (ICS1/SID2, PR1) were constitutively activated. The upregulation of PR1 was in agreement with the decreased susceptibility of lif2 to P. syringae, and this response was also observed in other mutants with reduced susceptibility to P. syringae, such as cpr1 and cpr5 [65]. However, the constitutive activation of the SA biosynthesis pathway did not lead to an overaccumulation of free SA in the lif2 mutant. SA undergoes various chemical modifications, which affect its activity, catabolism, transport, and storage [66]. The accumulation of free SA is fine-tuned by the transcriptional regulation of genes involved in SA biosynthesis, but also by the modulation of enzymes that modify SA [24]. Our data suggest that genes of the SA pathway may undergo post-transcriptional regulation or that a homeostasis mechanism may control free SA accumulation and maintain it at wild-type levels in lif2. Furthermore, the SA production pathway is not fully elucidated in A. thaliana, since no isochorismate pyruvate lyase,

Figure 6. LIF2 is involved in the plant’s susceptibility to B. cinerea. (A-C) Six-week-old plants were infected with a mycelium plug of the virulent B. cinerea B0510 (A-B) and BD90 (C) strains. (A) Symptoms at 3 dpi with the B0510 strain. (B-C) Lesion diameters were measured at 1 to 3 dpi. Stars indicate a significant difference from wild-type leaves on the corresponding day (Mann and Whitney’s test, with a p-value of <0.05). (D) Expression of marker genes involved in the defense response to B. cinerea. doi:10.1371/journal.pone.0099343.g006
which is required to convert isochorismate into SA in bacteria, has been identified to date. A second SA biosynthesis pathway, which is regulated by phenylalanine ammonia lyase (PAL), has been proposed to exist in plants [57,67]. In lif2, the discrepancy between the constitutive upregulation of the SA/ICS1-dependent pathway and the lack of SA accumulation may also suggest some crosstalk between the ICS1 and PAL pathways to fine-tune SA production. Finally, the lif2 mutant, a double mutant was less susceptible than wild-type plants to P. syringae, despite exhibiting a PR1 expression level similar to that of wild-type plants. Therefore, the reduced susceptibility of the lif2 mutant to P. syringae was independent of SA in non-challenged plants, suggesting that LIF2 participates in plant defense via a novel defense pathway that is independent of the SA-signaling defense pathway.

Furthermore, we showed that the GLS profile of the lif2 mutant was altered. These changes might indirectly participate in the increased resistance phenotype of lif2. Indeed, GLSs function in the plant’s defense response to herbivores and fungal pathogens [44,68,69,70]. Ward et al. (2010) suggested that GLSs do not play a direct role in the response to bacteria, but possibly have an intermediary role by influencing some defense pathways. Indeed, after infection of A. thaliana with P. syringae DC3000, a significant reduction in I3M and 4MOI3M was observed [71]. In the lif2 mutant, a low level of I3M was observed, but no change was observed in 4MOI3M in the absence of bacterial challenge. The global decrease in GLS and RA contents observed in lif2 might thus contribute to the control of the basal defense pathways. Interestingly, JA is a regulator of GLS gene expression and of GLS accumulation via multiple pathways [72,73].

Several TFs have recently been shown to be involved in GLS gene regulation, including MYB28, MYB29, and MYB76, which are key regulators of the aliphatic-GLSs, and MYB34 and MYB51, which are regulators of the indole-GLSs. Furthermore, MYC2, MYC3, and MYC4 can form protein complexes with all known GS-related MYBs to regulate GLS biosynthesis [74]. In the lif2 mutant, MYB28 is downregulated, whereas MYB34 is upregulated, and the levels of members of the two GLS families were decreased. However, MYC2 is downregulated in the lif2 mutant. Furthermore, several TFs related to defense responses are deregulated in lif2. WRKY33 is an essential transcription factor in the defense against B. cinerea that acts by controlling the expression of genes involved in redox homeostasis, SA signaling, and camalexin biosynthesis and thus affecting the SA-JA balance [40]. The activation of WRKY33 observed in lif2 may have downstream effects, which may play a role in the resistance of lif2 to B. cinerea. Therefore, it is likely that multiple components of different defense pathways contribute to the primed state of lif2 in the absence of pathogen, and to its reduced pathogen susceptibility. Interestingly, LIF2 interacts with the chromatin-associated protein LHP1, a subunit of the Polycomb Repressive Complex, which interacts with numerous genomic sites and regulates their expression [12,14]. Some defense-related genes are present among the LHP1 targets. Whether LIF2 acts coordinately with LHP1 at these defense-related loci to control them constitutes an interesting research question, as the role of chromatin proteins in plant immunity is poorly documented [75,76,77,78,79]. For instance, PIE1, a member of the SWR1 subfamily, was shown to negatively regulate plant defense [80], whereas loss-of function of the SIRTUIN2 histone deacetylase, a homolog of the yeast Silent information repression 2 (Sir2) protein [81] and of SDG8 histone methyltransferase [82] alter plant-pathogen responses. It was proposed that chromatin modifications may participate in defense priming in plants [83]. Thus, our study highlights an emerging role for the LIF2 chromatin-associated protein in biotic stress responses, with a putative suppressor function in plant immunity (Fig. 9).

Finally, LIF2 is also involved in the maintenance of growth and cell determination during floral development [12]. Therefore, our study illustrates the trade-off existing between plant development and plant defense against various enemies in a changing environment [84,85]. LIF2 may thus regulate the balance between development and plant immunity by limiting the cost associated with the plant defense response (Fig. 9). It was suggested that priming of SA-related defense responses greatly enhances disease resistance and plant fitness, but diminishes fitness in the absence of pathogens [85]. Furthermore, since both developmental and plant defense processes require adaptation to environmental conditions, sharing common elements with dual functions may allow for better fine-tuning [86]. Since the human hnRNP-Q homolog of LIF2, SNAP1/SYNGR1, also acts as a cellular RBP and as a suppressor of human immunity against virus infection, our data highlight a conserved role for hnRNP-Q in eukaryote immunity. The dual function of hnRNP-Q proteins in development and defense is likely to involve key conserved molecular events in eukaryotic cells.

### Table 2. Genes involved in susceptibility to B. cinerea.

| Name | AGI | Mutant phenotype | Reference |
|------|-----|------------------|-----------|
| GRX13 | AT1G03850 | Decreased susceptibility | La Camera et al. 2011 |
| MYC2 | AT1G32640 | Decreased susceptibility | Lorenzo et al. 2004 |
| MYB46 | AT5G12870 | Decreased susceptibility | Ramírez et al. 2011 |
| BIR1 | AT2G39660 | Increased susceptibility | Veronese et al. 2006 |
| COI1 | AT2G39940 | Increased susceptibility | Thomma et al. 1999 |
| JAR1 | AT2G46370 | Increased susceptibility | Thomma et al. 1999 |
| PAD2 | AT4G23100 | Increased susceptibility | Ferrari et al. 2003 |
| PAD3 | AT3G26830 | Increased susceptibility | Ferrari et al. 2003 |
| WRKY18 | AT4G31800 | Increased susceptibility* | Xu et al. 2006 |
| WRKY40 | AT1G80840 | Increased susceptibility* | Xu et al. 2006 |
| WRKY33 | AT2G38470 | Increased susceptibility | Zheng et al. 2006 |
| WRKY70 | AT3G56400 | Increased susceptibility | AbuQamar et al. 2006 |

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Figure 7. Glucosinolate (GLS) contents in \textit{lif2-1} and \textit{lhp1-1} young seedlings. The GLSs were quantified in \textit{lif2-1} (A) and \textit{lhp1-1} (B) using ESI-HPLC-MS. The full names of the GLSs are listed in Table S1. doi:10.1371/journal.pone.0099343.g007
Figure 8. Stress-response genes deregulated in the lif2 transcriptome. (A) The distribution of deregulated TFs in lif2. (B) Deregulated genes belonging to the GO term “Negative regulators of defence response”. The log ratio and the p-value were extracted from our CATMA transcriptome data. (C) Hierarchical clustering analysis performed using the MultiExperiment Viewer application, the Pearson correlation as current metric, and complete linkage clustering as the linkage method. The expression profiles of 38 deregulated TFs and 6 negative regulators in different biotic conditions were used in this analysis. Three gene clusters (I to III) were identified.

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Identifying the associated RNA partners of LIF2 RBP may elucidate the underlying mechanism.

Materials and Methods

Plant materials

Arabidopsis thaliana lines used in this study were in the Columbia (Col-0) accession. The homozygous lif2-1 and lif2-3 mutants were described in [12]. The lif2-ε line is a lif2-1 mutant complemented with the LIF2 genomic region under the control of a 3-kb promoter region. The sid2-2 [87] mutant was crossed with the lif2-1 allele. Double mutants were selected by PCR using specific primers (Table S3, [12]).

For P. syringae, B. cinerea, and S. sclerotiorum pathogen response analyses, plants were grown on soil in growth chambers, under controlled short-day conditions (8 hours light/16 hours dark), at 20˚C and 70% hygrometry.

Primers

All primers are described in Table S3.

Bacterial inoculation

The virulent strain P. syringae pv. tomato DC3000 (Pst DC3000) [88] and the avirulent strain P. syringae pv. tomato DC3000, carrying the avirulent gene avrRpm1 (Pst DC3000 avrRpm1) [28], were grown at 28˚C on King’s B solid medium plates containing the appropriate antibiotics. Briefly, a fresh bacterial suspension was scraped off the growth plate, and resuspended in 10 mM NaCl. The bacterial suspension was infiltrated into the abaxial side of 24 to 30 rosette leaves of seven-week-old plants (6 to 10 plants per genotype) using a 1-ml syringe without a needle. Control inoculations (Mock) were performed using 10 mM MgCl2. Bacterial growth was assessed 24 hours after infiltration. Whole leaves were homogenized in 500 µl of 10 mM MgCl2. After serial dilutions, homogenates were plated on King’s B medium and incubated at 28˚C for 2 days before colonies were counted. The surface area of each leaf was measured using ImageJ software in order to calculate cfu/cm². Day 0 titers ranged from log10 = 3.6–3.9. Leaves were photographed 5 days post-infiltration (dpi). All experiments were repeated at least twice with similar results.

Fungal pathogen inoculation procedure

Four-week-old plants were inoculated with discs carrying S. sclerotiorum (S55 strain) mycelia, as previously described [29]. Plant inoculations were performed in a Helios 1200 Phytotron under a light period of 9 h at 22˚C and 80% relative humidity during the first 3 days and 66% thereafter. At least 20 plants of each genotype were inoculated. The disease score of each line was evaluated seven days post-inoculation. Two independent experiments were performed with similar results.

Leaves of seven-week-old plants were inoculated with mycelial plugs of the wild-type strain B. cinerea B0510 and Bd90 (diameter of 3 mm), as previously described [89]. Infected leaves were placed in a Petri dish under high humidity and incubated at 21˚C. Lesion surfaces were measured daily for three days using ImageJ software.

Quantification of hormonal contents

Rosettes of seven-week-old plants were frozen immediately after harvest and ground in liquid nitrogen. Four pools of 6 rosettes were collected. Frozen material (100 mg) was extracted with 3 ml of acetone/water/acetic acid [80/19/1, v/v/v] containing the following stable isotope labelled internal standards: 10 ng [4-2H] ABA (NRG-CNRC Plant Biotechnology Institute, Saskatoon, Canada), 50 ng [4-2H] salicylic acid (Olchemim, Olomouc, Czech Republic), 1 ng [5-2H] jasmonic acid (CDN Isotopes CIL Chuzeau, Sainte Foy la Grande, France), and 10 ng [6-13C] indole-3-acetic acid (Cambridge Isotope Laboratory, Andover, MA). The extract was vigorously shaken for 30 s, sonicated for 1 min at 25 kHz, shaken for 10 min at room temperature, and then centrifuged (8230 g, 4˚C, 15 min). The supernatants were collected and the pellets were extracted again with 1 ml of the same extraction solution, then vigorously shaken (1 min) and sonicated (1 min, 25 kHz). Following centrifugation, the two supernatants were pooled and dried. The dry extract was dissolved in 140 µl acetonitrile/water (50/50, v/v), filtered, and submitted to analysis by HPLC-electrospray ionisation-MS/MS (HPLC-ESI-MS/MS). The compounds were introduced into the ESI source using a Waters 2695 separation module (Alliance; Waters, Milford, MA, USA) equipped with a Waters 2487 dual UV detector. Separation was achieved on a reverse-phase column (Uptisphere C18 ODB, 150 x 2.1 mm, Interchim, Montluçon, France), using a flow rate of 0.15 ml min⁻¹ and a binary gradient as follows: (A) acetic acid 0.1% (v/v) and (B) acetonitrile. The solvent gradient was programmed as follows: 0–5 min, 20% A; 5–15 min, 50% A; 15–30 min, 100% B; and 30–42 min, 20%. The analyses were performed on a Waters Quattro LC Triple Quadrupole Mass Spectrometer (Waters) operating in a Multiple Reaction Monitoring (MRM) scanning mode. The instrumental parameters were set as follows: capillary, 2.70 kV (negative mode); extractor, 3 V; and source block and desolvation gas temperatures, 120˚C and 350˚C, respectively. Nitrogen was used for the nebulization and desolvation (77 L h⁻¹ and 365 L h⁻¹, respectively), and argon was used as the collision gas at 2.83 x 10⁻⁶ mbar. For a 5-µL injection volume of sample prepared and reconstituted in 140 µl of 50/50 acetonitrile/H2O (v/v), the limit of detection (LOD) and limit of quantification (LOQ) were extrapolated for each hormone from a calibration curve and sample using the Quantify module of MassLynx (version 4.1 software). The parameters used for MRM quantification and the LOD and LOQ are listed in Tables S4 and S5, respectively. The amount of JA was expressed as a ratio of peak areas (209>62/214>62) per fresh weight, due to imurities contained in the D5-JA standard.

Glucosinolate extraction and quantification

Glucosinolate analyses were performed on 15-day-old in vitro seedlings grown under long-day conditions. The plant material was rapidly collected, frozen, and lyophilized. Lyophilized material (30–50 mg) was ground in liquid nitrogen. Then, 0.2 mg of sinigrin hydrate standard (Sigma Aldrich Ref. 85440) was added as an internal tracer for recovery and analytical
purposes. The samples were extracted using 2.5 ml of extraction solution (75% acetonitrile/25% water solution), vigorously homogenized for 5 min in a Polytron homogenizer (Fischer Scientific), and centrifuged (8000 g, 20°C, 15 min). The supernatants were collected and the pellets were extracted with 1 ml of the extraction solution and sonicated (15 min, 25 Hz). Following centrifugation, the supernatant was pooled with the first supernatant. The extracts were then evaporated to dryness using a Thermo Savant SpeedVac overnight, at ambient temperature. A water/acetonitrile solution (95/5) was added to the dried extracts, which were filtered and diluted to the third with water/acetonitrile (95/5), prior to negative electrospray LC-MS analysis on an Alliance 2695 system coupled to Quattro LC (Waters). Glucosinolates were identified by retention time, mass, isotopic pattern, and fragment ions [90,91]. The concentrations of the metabolites were quantified using the sinigrin response, as previously described [92]. Three independent extraction analyses were carried out per biological experiment and two to three biological replicates were performed. Biochemical information about the different classes of GLSs and their biosynthesis pathways can be found in various databases (e.g., http://www.genome.jp/kegg-bin/show_pathway?ko00966+C08417 or the AraCyc website).

Gene expression analysis
Total RNA was isolated from various tissues, using the RNeasy Plant Mini Kit (QIAGEN), and treated with RNase-free DNaseI (Invitrogen). Reverse transcription (RT) reactions were performed with Superscript II reverse transcriptase (Invitrogen), according to the manufacturer’s instructions. Quantitative real-time PCR (qPCR) was performed on Eppendorf Mastercycler® ep realpex (Eppendorf) using MESA FAST qPCR MasterMix Plus for SYBR® Assay (Eurogentec), as per the manufacturer’s instructions.

Supporting Information
Figure S1 LIF2 expression in response to P. syringae DC3000 and DC3000 avrRpm1 inoculations. lpi, hour post-infection.

Figure S2 Phenotypes of the lif2-1 sid2-2 double mutant. (A) Rosettes of 46-day-old plants grown in long-day (LD) conditions. (B) Rosette leaves in LD conditions. (C) Rosettes of 53-day-old plants grown in short-day (SD) conditions. (D) Rosette leaves in SD conditions. (E) The lif2-1 sid2-2 mutant is early flowering. Number of rosette leaves produced by plants grown in LD and SD conditions.

Table S1 The nomenclature of glucosinolate compounds.

Table S2 Selection of transcription factors differentially expressed in the lif2 transcriptome.

Table S3 Gene-specific oligonucleotides used in this study.

Table S4 Parameters for the LC-ESI-MS/MS analysis in negative mode.

Table S5 The limit of detection (LOD) and limit of quantification (LOQ).

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Author Contributions
Conceived and designed the experiments: DR MF VG. Performed the experiments: CLR SDP SBM FP CB MF VG. Contributed to the writing of the manuscript: VG.

References
1. Busch A, Hertel KJ (2012) Evolution of SR protein and hnRNP splicing regulatory factors. RNA 3: 1–12.
2. Moumen A, Magill C, Dry KL, Jackson SP (2013) ATM-dependent phosphorylation of heterogeneous nuclear ribonucleoprotein K promotes p33 transcriptional activation in response to DNA damage. Cell Cycle 12: 690–704.
3. Choi KS, Mizutani A, Lai MM (2004) SYNCRIP, a member of the heterogeneous nuclear ribonucleoprotein family, is involved in mouse hepatitis virus RNA synthesis. J Virol 78: 13153–13162.
4. Liu HM, Aizaki H, Choi KS, Machida K, Ou JJ, et al. (2009) SYNCRIP, a member of the heterogeneous nuclear ribonucleoprotein family, is involved in mouse hepatitis virus RNA replication. Virology 386: 249–256.
5. Park SM, Paek KY, Hong KY, Jang CJ, Cho S, et al. (2011) Translation-competent 48S complex formation on HIV IRES requires the RNA-binding protein NSAP1. Nucleic Acids Res 39: 7791–7802.
6. Zhang A, Zhou N, Huang J, Liu Q, Fukuda K, et al. (2013) The human long non-coding RNA-RoR is a p53 repressor in response to DNA damage. Cell Res 23: 340–350.
7. Loekovicz ZJ (2009) Role of plant RNA-binding proteins in development, stress response and genome organization. Trends Plant Sci 14: 229–236.
8. Ambroseo A, Costa A, Leone A, Grillo S (2012) Beyond transcription: RNA-binding proteins as emerging regulators of plant response to environmental constraints. Plant Science 182: 12–18.
9. Lee DH, Kim DS, Hwang BK (2012) The pepper RNA-binding protein CaRBP1 functions in hypersensitive cell death and defense signaling in the cytoplasm. Plant Journal 72: 235–248.
10. Fu ZQ, Guo M, Jeong BR, Tian F, Elshou TE, et al. (2007) A type III effector ADPribosylates RNA-binding proteins and quells plant immunity. Nature 447: 284–288.
11. Jeong BR, Lin Y, Joe A, Guo M, Korneli C, et al. (2011) Structure function analysis of an ADPribosyltransferase type III effector and its RNA-binding target in plant immunity. The Journal of Biological Chemistry 286: 43272–43281.
12. Latrasse D, Germann S, Houha-Heîrin N, Dubois E, Bui-Prodhomme D, et al. (2011) Control of flowering and cell fate by LIF2, an RNA binding partner of the polycomb complex component LHP1. PLoS One 6: e16592.
13. Gaudin V, Libault M, Pouteau S, Juul T, Zhao G, et al. (2011) Control of flowering and cell fate by LIF2, an RNA binding partner of the polycomb complex component LHP1. PLoS One 6: e16592.
14. Zhang X, Germann S, Bius BJ, Khorasanizadeh S, Gaudin V, et al. (2007) The Arabidopsis LHP1 protein colocalizes with histone H3 Lys27 trimethylation. Nat Struct Mol Biol 14: 896–871.
15. Kim JH, Durrett TP, Last RL, Jander G (2004) Characterization of the Arabidopsis T3G Glucosinolate Mutation, an Allele of TERMINAL FLOW ER.2. Plant Mol Biol 54: 671–692.
16. Ludwig-Muller J, Pieper K, Ruppel M, Cohen JD, Epstein E, et al. (1999) Indole glucosinolate and auxin biosynthesis in Arabidopsis thaliana (L.) Heynh. glucosinolate mutants and the development of clubroot disease. Planta 208: 409–419.
17. Mansfield J, Genin S, Maqsood S, Citovsky V, Spielmann M, et al. (2012) Top 10 plant pathogenic bacteria in molecular plant pathology. Mol Plant Pathology 13: 614–629.

18. Dean R, Van Ken JA, Pretorius ZA, Hammond-Kosack KF, Di Pietro A, et al. (2012) The Top 10 fungal pathogens in molecular plant pathology. Mol Plant Pathology 13: 414–430.

19. Mengiste T (2012) Plant immunity to necrotrophs. Annu Rev Phytopathol 50: 207–294.

20. Du Z, Zhou X, Ling Y, Zhang Z, Su Z (2010) agrigFO: a GO analysis toolkit for the agricultural community. Nuclear Acids Res 38: W64–70.

21. Wasternack C, Hause B (2013) Jasmonates: biosynthesis, perception, signal transduction and action in plant stress response, growth and development. Annals Botany 111: 1021–1058.

22. Thaler JS, Humphrey PT, Whitekin NK (2012) Evolution of jasmonate and salicylate signal crosstalk. Trends Plant Sci 17: 260–270.

23. Amico CJ, Colon C, Banks T, Ramonell KM (2012) Insights into the role of jasmonate-mediated defenses against necrotrophic and biotrophic fungal pathogens. Front Biol 7: 48–56.

24. Rivas-San Vicente M, Plasencia J (2011) Salicylic acid beyond defense: its role in plant growth and development. Journal Experimental Botany 62: 3231–3238.

25. Shah J (2003) The salicylic acid loop in plant defense.Curr Opin Plant Biol 6: 365–371.

26. Robert-Seilaniantz A, Grant M, Jones JD (2011) Hormone crosstalk in plant disease and defense: more than just jasmonate-salicylate antagonism. Annu Rev Phytopathol 49: 317–343.

27. Bari R, Jones JD (2009) Role of plant hormones in plant defence responses. Plant J 59: 977–989.

28. Robert-Seilaniantz A, Grant M, Jones JD (2011) Hormone crosstalk in plant

disease and defense.

29. Perchepied L, Balague C, Riou C, Claudel-Renard C, Riviere N, et al. (2010) The role of salicylic acid in Arabidopsis resistance to bacterial pathogens. Front Plant Sci 1: 257–268.

30. AbuQamar S, Chen X, Dhawan R, Bluhm B, Salmeron J, et al. (2006) Arabidopsis local

31. Ferrari S, Plotnikova JM, De Lorenzo G, Ausubel FM (2003) Arabidopsis local

32. Ramirez V, Agorio A, Coego A, Garcia-Andrade J, Hernandez MJ, et al. (2011) The role of the G protein beta subunit heterotrimer in plant defense. Front Plant Sci 2: 175–183.

33. Veronese P, Nakagami H, Bluhm B, Abuqamar S, Chen X, et al. (2006) The Arabidopsis EDS4 and PAD2, but not SID2, EDS5 or PAD4. Plant Journal 35: 193–205.

34. Lorenzo O, Chico JM, Sanchez-Serrano JJ, Solano R (2004) JASMONATE-DEPENDENT and COI1-INDEPENDENT defense responses against Sclerotinia sclerotiorumpathogens controlling disease resistance to Sclerotinia sclerotiorum in Arabidopsis thaliana. Mol Plant Microbe Interact 23: 846–860.

35. Abuqamar S, Chen X, Dhawan R, Bluhm B, Salmeron J, et al. (2006) Arabidopsis resistance to necrotrophic and biotrophic pathogens. Plant Cell 18: 257–273.

36. Ferrari S, Plotnikova JM, De Lorenzo G, Ausubel FM (2003) Arabidopsis local

37. Bari R, Jones JD (2009) Role of plant hormones in plant defence responses. Plant J 59: 977–989.

38. Robert-Seilaniantz A, Grant M, Jones JD (2011) Hormone crosstalk in plant disease and defense.

39. AbuQamar S, Chen X, Dhawan R, Bluhm B, Salmeron J, et al. (2006) Arabidopsis local

40. Ferrari S, Plotnikova JM, De Lorenzo G, Ausubel FM (2003) Arabidopsis local

41. Bari R, Jones JD (2009) Role of plant hormones in plant defence responses. Plant J 59: 977–989.

42. Govrin EM, Levine A (2000) The hypersensitive response facilitates plant

defense responses against Pseudomonas syringae pv. tomato. Plant Journal 48: 592–605.

43. Sonderby IE, Geu-Flores F, Halkier BA (2010) Biosynthesis of glucosinolates—A general inhibitors of programmed cell death. Plant Physiol. 159: 266–285.

44. Govrin EM, Levine A (2000) The hypersensitive response facilitates plant

defense responses against Pseudomonas syringae pv. tomato. Plant Journal 48: 592–605.

45. Govrin EM, Levine A (2000) The hypersensitive response facilitates plant

defense responses against Pseudomonas syringae pv. tomato. Plant Journal 48: 592–605.

46. Govrin EM, Levine A (2000) The hypersensitive response facilitates plant

defense responses against Pseudomonas syringae pv. tomato. Plant Journal 48: 592–605.

47. Ishihama N, Yoshioka H (2012) Post-translational regulation of WRKY transcription factors in plant immunity. Curr Opin Plant Biol 15: 431–437.

48. Chen L, Song Y, Li S, Zhang L, Zou C, et al. (2012) The role of WRKY transcription factors in plant abiotic stresses. Biochim Biochim Acta 1819: 120–129.

49. Nakashima K, Takahasi H, Misu J, Shinozaki K, Yamaguchi-Shinozaki K (2012) NAC transcription factors in plant abiotic stress responses. Biochim Biophys Acta 1819: 97–106.

50. Yang H, Yang S, Liu Y, Huo J (2007) The Arabidopsis AP1 and AP2 genes are general inhibitors of programmed cell death. Plant Physiol. 145: 135–146.

51. Hruz T, Laule O, Szabo G, Wessendorf F, Bleuler S, et al. (2008) Genevestigator v3: a reference expression database for the meta-analysis of transcriptional data. Advances Bioinformatics 2008: 420747.

52. Robert-Seilaniantz A, Grant M, Jones JD (2011) Hormone crosstalk in plant disease and defense: more than just jasmonate-salicylate antagonism. Annu Rev Phytopathol 49: 317–343.

53. Kliebenstein DJ, Figuth A, Mitchell-Olds T (2002) Genetic architecture of flower color regulation in Arabidopsis thaliana. Plant Physiol 126: 849–860.
76. Palma K, Thorgrimsen S, Malinovsky FG, Filø BK, Nielsen HB, et al. (2010) Autoimmunity in Arabidopsis acd11 is mediated by epigenetic regulation of an immune receptor. PLoS Pathog 6: e1001137.

77. Alvarez-Venegas R, Abdallat AA, Guo M, Alfano JR, Arrazola Z. (2007) Epigenetic control of a transcription factor at the cross section of two antagonistic pathways. Epigenetics 2: 106–113.

78. DeFraia CT, Zhang X, Moa Z. (2010) Elongator subunit 2 is an accelerator of immune responses in Arabidopsis thaliana. Plant Journal 64: 511–523.

79. Ma KW, Flores C, Ma W. (2011) Chromatin configuration as a battlefield in plant-bacteria interactions. Plant Physiol. 157: 535–543.

80. March-Diaz R, Garcia-Dominguez M, Lozano-Juste J, Leon J, Florencio FJ, et al. (2008) Histone H2A.Z and homologues of components of the SWR1 complex are required to control immunity in Arabidopsis. Plant Journal 53: 475–487.

81. Wang C, Gao F, Wu J, Dai J, Wei G, et al. (2010) Arabidopsis putative deacetylase AtSRT2 regulates basal defense by suppressing PAD4, EDS5 and SID2 expression. Plant Cell Physiol. 51: 1291–1299.

82. Berr A, McCallum EJ, Ahloua A, Heintz D, Heitz T, et al. (2010) Arabidopsis Histone Methyltransferase SET DOMAIN GROUP8 Mediates Induction of the Jasmonate/Ethylene Pathway Genes in Plant Defense Response to Nectrotrophic Fungi. Plant Physiol. 154: 1403–1414.

83. Conrath U (2011) Molecular aspects of defence priming. Trends Plant Sci 16: 524–531.

84. Winter CM, Austin RS, Blanvillain-Baufume S, Reback MA, Moureaux M, et al. (2011) LEAFY target genes reveal floral regulatory logic, cis motifs, and a link to biotic stimulus response. Dev. Cell 20: 430–443.

85. Todesco M, Balasubramanian S, Hu TT, Traw MB, Horton M, et al. (2010) Natural allelic variation underlying a major fitness trade-off in Arabidopsis thaliana. Nature 465: 632–636.

86. Alcazar R, Reymond M, Schmidt G, de Meaux J (2011) Genetic and evolutionary perspectives on the interplay between plant immunity and development.Curr Opin Plant Biol 14: 378–384.

87. Wildermuth MC, Dewdney J, Wu G, Ausubel FM (2001) Isochorismate synthase is required to synthesize salicylic acid for plant defence. Nature 414: 562–565.

88. Katagiri F, Thilmony R, He SY (2002) The Arabidopsis thaliana-Pseudomonas syringae interaction. Arabidopsis Book 1: e0039.

89. Morcx S, Kuma C, Choquer M, Assie S, Blondet E, et al. (2013) Disruption of Bech6, Bech6 or Bech6 chitin synthase genes in Botrytis cinerea and the essential role of class VI chitin synthase (Bch6). Fungal Genet Biol 52: 1–8.

90. Clark DB (2010) Glucosinolates, structures and analysis in food. Anal. Methods 2: 310–325.

91. Lee KC, Cheuk MW, Chan W, Lee AW, Zhao ZZ, et al. (2006) Determination of glucosinolates in traditional Chinese herbs by high-performance liquid chromatography and electrospray ionization mass spectrometry. Anal Bioanal Chem 386: 2225–2232.

92. Denance N, Ranocha P, Oriol N, Barlet X, Riviere MP, et al. (2012) Arabidopsis wat1 (walls are thin1)-mediated resistance to the bacterial vascular pathogen, Ralstonia solanacearum, is accompanied by cross-regulation of salicylic acid and tryptophan metabolism. Plant Journal 73: 225–239.