Regulation of GDSL Lipase Gene Expression by the MPK3/MPK6 Cascade and Its Downstream WRKY Transcription Factors in *Arabidopsis* Immunity

Xiaofei Han,¹ Sen Li,¹ Miao Zhang,¹ Liuyi Yang,¹ Yidong Liu,² Juan Xu,^{1,†} and Shuqun Zhang^{2,†}

¹State Key Laboratory of Plant Physiology and Biochemistry, College of Life Sciences, Zhejiang University, Hangzhou, Zhejiang 310058, China; and ²Division of Biochemistry, Interdisciplinary Plant Group, Bond Life Sciences Center, University of Missouri, Columbia, MO 65211, U.S.A.

Accepted 5 December 2018.

Mitogen-activated protein kinase (MAPK) cascades serve as unified signaling modules in plant development and defense response. Previous reports demonstrated an essential role of *Arabidopsis* GLIP1, a member of the GDSL-like-motif lipase family, in both local and systemic resistance. *GLIP1* expression is highly induced by pathogen attack. However, the one or more signaling pathways involved are unknown. Here, we report that two pathogen-responsive MAPKs, MPK3 and MPK6, are implicated in regulating gene expression of *GLIP1* as well as *GLIP3* and *GLIP4*. After gain-of-function activation, MPK3 and MPK6 can strongly induce the expression of *GLIP1*, *GLIP3*, and *GLIP4*. Both *GLIP1* and *GLIP3* contribute to the plant resistance to *Botrytis cinerea*. WRKY33, a MPK3/MPK6 substrate, is essential for the MPK3/MPK6-dependent *GLIP1* induction. In addition, WRKY2 and WRKY34, two close homologs of WRKY33, have a minor effect in MPK3/MPK6-regulated *GLIP1* expression in *B. cinerea*-infected plants. Chromatin immunoprecipitation-quantitative polymerase chain reaction analysis demonstrated that the *GLIP1* gene is a direct target of WRKY33. In addition, we demonstrated that MPK3/MPK6-induced *GLIP1* expression is independent of ethylene and jasmonic acid, two important hormones in plant defense. Our results provide insights into the regulation of the *GLIP* family at the transcriptional level in plant immunity.

Plants have multifaceted defense systems that are either constitutive or inducible in response to pathogen invasion.

Sequence data from this paper are available in the *Arabidopsis* Genome Initiative or GenBank/EMBL databases under the following accession numbers: *MPK3* (At3g45640), *MPK6* (At2g43790), *EF1α* (At5g60390), *GLIP1* (At5g40990), *GLIP2* (At1g53940), *GLIP3* (At1g53990), *GLIP4* (At3g14225), *GLIP5* (At1g53920), *GLIP6* (At1g71120), *GLIP7* (At5g15720), *WRKY2* (At5g56270), *WRKY33* (At2g38470), *WRKY34* (At4g26440), *ETR1* (At1g66340), *EIN2* (At5g03280), *EIN3* (At3g20770), *CTR1* (At5g03730), and *COI1* (At2g39940), and *ACT8* (At1g49240).

[†]Corresponding authors: J. Xu; xujuan@zju.edu.cn and S. Zhang; Zhangsh@missouri.edu

Funding: This research was supported by grants from the Young Elite Scientist Sponsorship Program by CAST (2018QNRC001) and National Natural Science Foundation of China (31570297) to J. Xu and from the 111 Project (B14027) to S. Zhang.

*The e-Xtra logo stands for “electronic extra” and indicates that eight supplementary figures and one supplementary table are published online.

Inducible defense responses are initiated after plant sensing of pathogen or microbe-associated molecular patterns or pathogen-originated effectors, which stimulates a set of early defense events, including activation of mitogen-activated protein kinases (MAPKs), flux of calcium ion, burst of reactive oxygen species, and changes in plant hormone biosynthesis, such as ethylene biosynthesis. These early defense responses play critical roles in signaling multiple intermediate and late events, including cell-wall modification, defense gene activation, hypersensitive-response cell death, phytoalexin accumulation, and finally lead to the induction of plant resistance (Bacete et al. 2018; Boller and Felix 2009; Coll et al. 2011; Dixon 2001; Glazebrook 2005; Jwa and Hwang 2017; Kotchoni and Gachomo 2006; Meng and Zhang 2013). Additionally, these localized defense responses can induce uninfected parts of plants to elicit systemic acquired resistance (SAR), conferring elevated resistance against subsequent pathogen challenge (Sticher et al. 1997). There are many up-to-date reviews available that GDSL-motif lipase/esterase 1 (GLIP1) possesses antimicrobial activity in local resistance and is also involved in SAR (Kim et al. 2013, 2014; Kwon et al. 2009; Oh et al. 2005).

GLIPs, a subfamily of lipolytic enzymes, consist of a characteristic GDSL motif that differs from the common lipases (Akoh et al. 2004). Like other esterase and lipase families, GLIPs possess the same invariant residues (serine, aspartic acid, and histidine) that make up the conserved catalytic triad. However, the classical lipases feature sequence GxSxxG is replaced by GxSxxxxG, where the serine at the active sites is found to be relatively closer to the N-terminus rather than near the center (Brick et al. 1995). Increasing evidence implicates that GLIPs play critical roles in plant immunity, especially in rice and *Arabidopsis* (Gao et al. 2017; Lee et al. 2009; Oh et al. 2005). There are seven *GLIP* members in the *Arabidopsis* genome (Oh et al. 2005). Secretome analysis identified *Arabidopsis* GLIP1 as a salicylic acid (SA)-responsive secreted protein (Oh et al. 2005). The protein sequences show that GLIP1 has 68 to 78% identity to GLIP2, GLIP3, and GLIP4, while it is 32 to 52% identical to GLIP5, GLIP6, and GLIP7. It has been shown that GLIP2 negatively regulate auxin signaling to combat invading pathogens (Lee et al. 2009). At present, the functions of the other five GLIPs are poorly understood. *GLIP1* is highly induced after pathogen invasion. Nonetheless, the one or more signaling pathways involved are lacking.

MAPK cascades, conserved signaling modules during plant immunity, convert external stress signal from upstream receptors and sensors into a variety of cellular responses (Bigeard and Hirt 2018; Chen et al. 2001; Colcombet and Hirt 2008;

Dóczi et al. 2012; Pitzschke 2015; Taj et al. 2010; Xu and Zhang 2015a; Zhang et al. 2018). Each typical MAPK cascade generally contains three tiers of protein kinases, including a MAPK kinase kinase (known as MAPKKK or MEKK), its downstream MAPK kinase (known as MAPKK or MEK), and the terminal MAPK (Rodriguez et al. 2010). In *Arabidopsis thaliana*, there are four MAPKs that have been connected to immunity, including MPK3, MPK4, MPK6, and MPK11 (Bethke et al. 2012; Meng and Zhang 2013). Among them, MPK3 and MPK6, which are mostly functionally redundant, act downstream of two redundant MAPKKs, MKK4 and MKK5 (Asai et al. 2002; Meng and Zhang 2013; Pedley and Martin 2005; Wang et al. 2007). Another separate pathogen-responsive MAPK cascade consists of MPK4, two redundant MAPKKs, MKK1 and MKK2, along with the top tier MEKK1 (Gao et al. 2008; Ichimura et al. 2006; Kong et al. 2012; Meng and Zhang 2013; Qiu et al. 2008; Suarez-Rodriguez et al. 2007). At present, the kinases upstream of MPK11 are unclear.

Our recent studies demonstrate that MPK3 and MPK6 positively regulate defense responses, such as the induction ethylene biosynthesis (Guan et al. 2015; Han et al. 2010; Joo et al. 2008; Li et al. 2012; Liu and Zhang 2004), defense gene activation (Meng et al. 2013), camalexin production (Mao et al. 2011; Ren et al. 2008), indole glucosinolate (IGS) biosynthesis (Xu et al. 2016), stomatal immunity (Su et al. 2017), and photosynthesis inhibition (Su et al. 2018). The multifunctionality of MPK3 and MPK6 in plant disease resistance can be attributed to the multiple MAPK substrates. For instance, MPK3 and MPK6 are able to phosphorylate several WRKY transcription factors, including WRKY2, WRKY33, and WRKY34 (Guan et al. 2014; Mao et al. 2011; Ueda et al. 2017). WRKY33 was shown not only to positively regulate camalexin biosynthetic gene expression but it also promotes the expression of *ACS2* as well as *ACS6* downstream of MPK3 and MPK6 in plant immunity (Li et al. 2012; Mao et al. 2011). As the substrate of MPK3 and MPK6, both WRKY2 and WRKY34 transcription factors regulate pollen development and WRKY2 is also capable of upregulating *WOX-8* expression to polarize the zygote (Guan et al. 2014; Ueda et al. 2017).

It is demonstrated in this report that pathogen-induced expression of several members of the *GLIP* family, including *GLIP1*, *GLIP3*, and *GLIP4*, depends on the MPK3/MPK6 signaling pathway. In the conditional gain-of-function *GVG-NtMEK2^{DD}* (*DD*) and *Botrytis cinerea*-inoculated *Arabidopsis*, *GLIP1* expression is highly induced after MPK3 and MPK6 activation. We further found that both *GLIP1* and *GLIP3* play important roles in *Arabidopsis* resistance to *B. cinerea*. WRKY33, a MPK3/MPK6 substrate, is responsible for turning on the MPK3/MPK6-dependent *GLIP1* expression. Additionally, WRKY2 and WRKY34, two close homologs of WRKY33, are also involved in *GLIP1* induction in *B. cinerea*-infected plants. Chromatin immunoprecipitation-quantitative polymerase chain reaction (ChIP-qPCR) analysis demonstrated *GLIP1* gene is a direct target of WRKY33. Furthermore, it is found that the expression of *GLIP1* is independent of ethylene and jasmonic acid (JA) signaling. Our results provide insights into the regulation and the importance of the *GLIP* family in plant immunity.

RESULTS

Induction of *GLIP* expression after MPK3/MPK6 activation in the conditional gain-of-function *DD Arabidopsis* plants.

Expression profiling of the conditional gain-of-function *DD* plants (Ren et al. 2002; Yang et al. 2001) revealed that *GLIP1* and its homologs *GLIP3* and *GLIP4* are extremely induced

upon dexamethasone (DEX) treatment (Tsuda et al. 2013). Previous studies demonstrated that *GLIP1* is a pathogen- and SA-responsive secreted protein and is involved in *Arabidopsis* resistance to biotrophic and necrotrophic pathogens (Kim et al. 2013, 2014; Kwon et al. 2009; Oh et al. 2005). However, the signaling pathways underlying the induction of *GLIP1* gene expression remain unclear. As a result, we decided to further investigate the regulation of *GLIPs* by the MPK3/MPK6 signaling pathway. As the first step, we confirmed the induction of these *GLIPs* after MPK3/MPK6 activation, using quantitative reverse transcription (RT)-PCR. The levels of *GLIP1*, *GLIP3*, and *GLIP4* expression were greatly induced in *DD* plants in response to DEX treatment (Fig. 1). In contrast, *GLIP2*, *GLIP5*, *GLIP6*, and *GLIP7* expression could be reliably detected but at very low levels and not inducible. We calculated the expression levels of *GLIP* genes versus that of *EF1α* and were able to compare relative expression levels between distinct *GLIP* genes through this calculation (Fig. 1A). To better assess gene activation levels, we also calculated their induction fold over their basal levels. As shown in Figure 1B, transcripts of *GLIP1*, *GLIP3*, and *GLIP4* were induced by approximately 4,500-, 300-, and 100-fold, respectively. Consistently, no increases of *GLIP2*, *GLIP5*, *GLIP6*, and *GLIP7* expression were observed. These results demonstrate that the transcription of *GLIP1*, *GLIP3*, and *GLIP4* genes can be activated after activation of MPK3/MPK6 in *DD Arabidopsis*.

Induction of *GLIP1*, *GLIP3*, and *GLIP4* gene expression is dependent on the MPK3/MPK6 pathway.

To determine whether *GLIP1*, *GLIP3*, and *GLIP4* gene induction depends on MPK3 or MPK6 in *DD* plants, we analyzed the expression of *GLIP1*, *GLIP3*, and *GLIP4* in *DD*, *DD mpk3*, and *DD mpk6* plants. We found that absence of either *MPK3* or *MPK6* compromised the induction of *GLIP1*, *GLIP3*, and *GLIP4* expression in *DD* plants after DEX application (Fig. 2). Moreover, *GLIP4* expression in *DD mpk6* plants was higher at 24 h after DEX treatment than that in *DD* plants, possibly since delayed cell death occurred in the *DD mpk6* plants. These data support the idea that endogenous MPK3/MPK6 activation is responsible for *GLIP1*, *GLIP3*, and *GLIP4* gene induction in *DD* seedlings.

Botrytis cinerea, a necrotrophic pathogen, highly activates MPK3 and MPK6 in *Arabidopsis* (Han et al. 2010). It was previously reported that the expression of *Arabidopsis GLIP1* is induced by *Alternaria brassicicola* (Kwon et al. 2009; Oh et al. 2005), a necrotrophic pathogen that has the same lifestyle as *B. cinerea* (Glazebrook 2005). As a result, we examined whether *B. cinerea* can induce *GLIP1*, *GLIP3*, and *GLIP4* gene expression and whether this induction is MPK3/MPK6-dependent. As indicated in Figure 3A to C, the *GLIP1*, *GLIP3*, and *GLIP4* expression was obviously induced in *Arabidopsis* that was inoculated with *B. cinerea* spores. To obtain loss-of-function data to further understand the function of MPK3/MPK6 cascade in *B. cinerea*-induced *GLIP* gene activation, we first examined its expression in wild type (Col-0) and *mpk3* and *mpk6* single mutants. Loss of function of *MPK3* was associated with a mild decrease of *GLIP1* induction (Fig. 3A), whereas no significant reduction was detected in *mpk6* mutant. The subtle change in *mpk3* and *mpk6* single mutants could result from functional redundancy.

MPK3 and MPK6 have mostly redundant impacts on a variety of biological processes, for instance, embryogenesis (Wang et al. 2007; Zhang et al. 2017). Mutation of both *MPK3* and *MPK6* results in an embryo-lethal phenotype. We utilized a chemical genetic method to circumvent this problem and generate a conditional double mutant system for functional analyses (Bishop et al. 2000). A chemical-sensitized MPK6 variant,

MPK6^{YG}, was used to successfully rescue the *mpk3 mpk6* lethal phenotype, and the generating plants were designed as *MPK6SR* plants (genotype: *mpk3 mpk6 P_{MPK6}:MPK6^{YG}*) (Xu et al. 2014). In the absence of 4-amino-1-tert-butyl-3-(1'-naphthyl)pyrazolo [3,4-d]pyrimidine (NA-PP1) inhibitor, *MPK6SR* plants grow and develop normally, similar to *mpk3* single mutant. When we apply NA-PP1 to *MPK6SR* plants, MPK6^{YG} activity is inhibited in vivo, resulting a *mpk3 mpk6* double-activity null mutant. NA-PP1 is an analog of PP1 kinase inhibitor, which has a bulkier side chain. As a result, it cannot access the ATP binding site of a wild-type kinase (Bishop et al. 2000), ensuring the specificity of this approach. Likewise, we bred a rescued *mpk3 mpk6* double mutant employing a NA-PP1-sensitive MPK3 variant, MPK3^{TA} (genotype: *mpk3 mpk6 P_{MPK3}:MPK3^{TA}*, designed as *MPK3SR* plants) (Xu et al. 2016).

In *MPK6SR* plants without NA-PP1 treatment (equivalent to the *mpk3* single mutant), induction of *GLIP1* expression was partially compromised (Fig. 3D), consistent with what we observed in the *mpk3* single mutant (Fig. 3A). In contrast, in the presence of NA-PP1, *GLIP1* induction in *MPK6SR* plants was severely reduced after *B. cinerea* inoculation (Fig. 3D). In *MPK3SR* plants without NA-PP1, induction of *GLIP1* expression was not affected, similar to the *mpk6* single mutant, whereas *B. cinerea*-induced *GLIP1* gene activation was dramatically reduced in *MPK3SR* plants after NA-PP1 treatment. These data demonstrate the redundant function of MPK3 and MPK6 in induction of *GLIP1* expression after *B. cinerea* inoculation. At a late timepoint such as 24 h after pathogen inoculation, *GLIP1* induction in NA-PP1 pretreated *MPK3SR* and *MPK6SR* increased, possibly a result of loss of NA-PP1 inhibitor due to plant metabolism. Alternatively, this residual activation could be a result of one or more MPK3/MPK6-independent pathways. We also examined *GLIP3* and *GLIP4* gene expression in *MPK6SR* and *MPK3SR* plants inoculated

with *B. cinerea*. In Figure 3E and F, the induction levels of *GLIP3* and *GLIP4* expression were severely compromised in the loss-of-function plants, similar to the expression pattern of *GLIP1*. These results further support the role of MPK3 and MPK6 in the expression regulation of *GLIP1*, *GLIP3*, and *GLIP4* in plant immunity.

***GLIP1* and *GLIP3* contribute to the plant resistance to *B. cinerea*.**

The MPK3/MPK6-dependent high-level induction of *GLIP1*, *GLIP3*, and *GLIP4* genes prompted us to test the resistance of *glip1*, *glip3*, and *glip4* mutant plants against *B. cinerea*. We obtained a T-DNA insertion line for *GLIP3* (SALK_201623C, named as *glip3-1*) and two T-DNA insertion lines for *GLIP4* (WiscDsLox489-492B15 and SALK_048818, named *glip4-1* and *glip4-2*, respectively) (Supplementary Fig. S1). These mutant plants did not express *GLIP3* or *GLIP4* transcripts, respectively, as determined by RT-PCR analysis. The *glip1-1* and *glip1-2* mutants were the same as previously reported (Oh et al. 2005). Three days after inoculation with 10- μ l droplets of *B. cinerea* spore suspension, the disease phenotype in Col-0 and mutant plants was observed and the lesion sizes were measured. *B. cinerea* caused much bigger lesions in the *glip1-1* and *glip1-2* mutant plants than in Col-0 control plants (Fig. 4A and B). The *glip3-1* mutant plants also exhibited larger lesions compared with Col-0 but smaller than those on *glip1-1* and *glip1-2* mutants. In contrast, mutation of *GLIP4* did not alter the *B. cinerea* susceptibility. These results demonstrate the importance of *GLIP1* and *GLIP3* in plant resistance to *B. cinerea*.

WRKY33 is involved in the MPK3/MPK6-regulated *GLIP1* gene activation.

Because of the importance of *GLIP1* in plant immunity (Kim et al. 2013, 2014; Kwon et al. 2009; Oh et al. 2005) and its

Fig. 1. Activation of GDSL LIPASE (*GLIP*) gene expression after MPK3/MPK6 activation in the gain-of-function *GVG-NtMEK2^{DD}* (*DD*) *Arabidopsis* seedlings. Conditional gain-of-function *DD Arabidopsis* seedlings (12 days old) were treated with 1 μ M dexamethasone (DEX). Samples were collected at indicated times. Real-time polymerase chain reaction was used to quantify the expressions of all seven *GLIP* genes. **A**, *GLIP* gene activation was expressed as the percentage of *EF1α* expression, allowing the comparison of expression levels among different *GLIP* genes. **B**, Induction of *GLIP* gene expression (fold of induction relative to the level before treatment) was calculated by the 2^{- $\Delta\Delta C_t$} method. In both calculations, the expression of *EF1α* was used as a reference. Error bars represent standard deviations ($n = 3$).

higher induction levels (Figs. 1, 2, and 3), we focused on this isoform for more detailed analysis. WRKY33, a MPK3/MPK6 substrate, functions as the transcriptional activator in promotion of camalexin biosynthetic genes expression and camalexin generation in *Arabidopsis* (Mao et al. 2011). To determine whether WRKY33 also participates in the modulation of *GLI1* gene expression downstream of the MPK3/MPK6 cascade, we performed real-time qPCR to quantify its expression in *DD* and *DD wrky33-1* plants after DEX treatment. As seen in Figure 5A, the activation of *GLI1* gene expression by *DD* transgene was greatly reduced in the background of *wrky33-1* mutant. Examination of *DD wrky33-2* plants gave a similar result (Supplementary Fig. S2A). Based on the residual level of *GLI1* gene activation in *DD wrky33* plants, we can also conclude that, in addition to WRKY33, one or more other transcription factors are also involved and can partially replace WRKY33 in its absence.

We then tested whether pathogen-induced *GLI1* expression requires WRKY33 by quantifying the expression of *GLI1* in *B. cinerea*-induced Col-0 and *wrky33-1* plants. As expected,

Fig. 2. Activation of *GLI1*, *GLI3*, and *GLI4* gene expression in gain-of-function *GVG-NtMEK2^{DD}* (*DD*) seedlings is dependent on MPK3 and MPK6. The 12-day-old *DD*, *DD mpk3*, and *DD mpk6* seedlings were treated with 1 μ M dexamethasone (DEX). Samples were collected at indicated times. **A**, Real-time polymerase chain reaction was used to quantify the expressions of *GLI1*, **B**, *GLI3*, and **C**, *GLI4* genes. The *GLI* genes transcript levels were calculated as a percentage of the *EF1α* transcript. Error bars represent standard deviations ($n = 3$). Two-way analysis of variance was performed to compare the levels of *GLI* gene expressions in different genotypes after DEX treatment. Different lowercase letters above the brackets represent statistically significant differences ($P < 0.05$).

the induction of *GLI1* mRNA by *B. cinerea* infection was reduced in the *wrky33-1* mutant in comparison with the Col-0 control (Fig. 5B). Examination of *wrky33-2* mutant gave a similar result. The remaining *GLI1* induction detected in the *B. cinerea*-induced *wrky33* mutant once more points to the involvement of one or more other transcription factors.

***Botrytis cinerea*-induced *GLI1* expression is partially dependent on WRKY2 and WRKY34.**

Our previous research demonstrated that WRKY34 and WRKY2, two highly homologous transcription factors with WRKY33, function in pollen development downstream of MPK3/MPK6 (Guan et al. 2014). MPK3/MPK6-dependent phosphorylation of WRKY34 is crucial to its role in the development of plant pollen. Both WRKY34 and WRKY2 have broad expression patterns, despite the fact that WRKY34 expression is much higher in pollen (Guan et al. 2014), suggesting their one or more roles in other cells and tissues. To determine whether WRKY2 and WRKY34 are responsible for the residual *GLI1* induction in *wrky33* mutant, we crossed *wrky2 wrky34* double mutants into *wrky33* background and identified a *wrky2 wrky33 wrky34* triple mutant. We then monitored the expression of *GLI1* in Col-0, *wrky33* single-, *wrky2 wrky34* double-, and *wrky2 wrky33 wrky34* triple-mutant plants at different times after infection by *B. cinerea*. There was a slight reduction of *B. cinerea*-triggered *GLI1* induction in *wrky2 wrky34* double-mutant plants in comparison with Col-0 (Fig. 6). We also found that *GLI1* expression level in *wrky2 wrky33 wrky34* triple mutant was lower than that in the *wrky33* single mutant and the *wrky2 wrky34* double mutant after *B. cinerea* infection, confirming the involvement of WRKY2 and WRKY34 in *B. cinerea*-induced *GLI1* expression. This result can explain the residual induction of *GLI1* in *wrky33* mutant plants inoculated with *B. cinerea* (Fig. 5B). We also examined the resistance phenotypes of Col-0, *wrky33*, *wrky2 wrky34*, and *wrky2 wrky33 wrky34* mutant plants in response to *B. cinerea* (Supplementary Fig. S3). In comparison with Col-0 control plants, *wrky33* single mutant plants developed more extensive necrosis. In contrast, *wrky2 wrky34* double mutant plants showed wild type-like resistance to *B. cinerea*. Although *wrky2 wrky33 wrky34* triple mutant plants were more susceptible to *B. cinerea* than Col-0 plants, no obvious difference between the triple mutants and *wrky33* single mutants was observed. These results indicated that WRKY33 play a more important role in resistance to *B. cinerea* and the regulation of *GLI1*, whereas its two close homologs, WRKY2 and WRKY34, play a minor role in regulating *GLI1* expression in response to *B. cinerea* infection.

WRKY33 binds to the W-boxes in the promoter of *GLI1* gene in vivo.

Next, we further investigated the detail function of WRKY33 in regulating the expression of the *GLI1* gene. Sequence analysis identified three W-box elements in the 2.2-kb promoter region of the *GLI1* gene (Fig. 7B). We then performed ChIP-qPCR to examine whether *GLI1* is a direct target of the WRKY33 transcription factor in plant defense. WRKY33 protein has five MAPK phosphorylation sites in the N-terminus, including serine-54, serine-59, serine-65, serine-72, and serine-85 (Mao et al. 2011). For this experiment, we generated transgenic plants expressing WRKY33^{SD}, a phosphomimicking form of WRKY33 with all five serine (S) residues mutated to aspartic acid (D) (Fig. 7A), in *wrky33* mutant background. The WRKY33^{SD} transgene was driven by the 35S promoter and was attached with a 4myc epitope tag at the N termini. As indicated in Figure 7C, in 35S:4myc-WRKY33^{SD}/*wrky33* plants, the antimyc antibody was able to highly enrich the W-box-containing *GLI1* promoter region

via coimmunoprecipitation with the 4myc-WRKY33^{SD} protein. In contrast, in control reactions, immunoglobulin G (IgG) antibody failed to enrich the *GLIP1* gene promoter. As another control, both antimyc and IgG antibody failed to coimmunoprecipitate *GLIP1* gene promoter sequence from the non-transgenic Col-0 plants. We also performed ChIP-qPCR in 35S::4myc-WRKY33^{WT}/*wrky33* plants. As shown in Supplementary Figure S4, the wild-type form of WRKY33 exhibits similar binding activity to the *GLIP1* promoter as does WRKY33^{SD}, which is consistent with our previous conclusion that phosphorylation of WRKY33 by MPK3/MPK6 does not alter its DNA-binding ability to the W-box *cis*-element (Mao et al.

2011). These in-vivo data indicated the direct binding of WRKY33 to the *GLIP1* promoter.

Ethylene- and JA-independent regulation of *GLIP1* expression in the MPK3/MPK6 cascade.

It was reported that pathogen-induced *GLIP1* expression involves ethylene pathways (Kim et al. 2014; Oh et al. 2005). Plants that are inoculated with *B. cinerea* spores or DD plants that are treated with DEX exhibit enhanced levels of ethylene (Li et al. 2012; Liu and Zhang 2004). To detect whether the induction of *GLIP1* gene expression observed in Figures 1, 2A, 3A, and 3D relies on ethylene sensing or signaling, we tested *GLIP1*

Fig. 3. Activation of *Botrytis cinerea*-induced *GLIP1*, *GLIP3*, and *GLIP4* genes is dependent on functional MPK3 and MPK6. **A to C**, Wild-type (Col-0), *mpk3*, and *mpk6* seedlings (12 days old) were inoculated with *B. cinerea* (Bc) spores, and **D to F**, 12-day-old wild-type (Col-0) and chemical-genetically rescued *mpk3 mpk6* double mutant (*MPK6SR* genotype: *mpk3 mpk6 P_{MPK6}:MPK6^{YG}*; *MPK3SR* genotype: *mpk3 mpk6 P_{MPK3}:MPK3^{TA}*) seedlings were inoculated with *B. cinerea* spores after pretreatment with 4-amino-1-tert-butyl-3-(1'-naphthyl)pyrazolo [3,4-d]pyrimidine (NA-PP1) (5 μM final concentration) or dimethyl sulfoxide (DMSO), the solvent of NA-PP1 stock solution, for 30 min. Samples were collected at indicated times. Real-time polymerase chain reaction was used to quantify the expressions of *GLIP1* (A and D), *GLIP3* (B and E), and *GLIP4* (C and F) genes. *GLIP* gene transcript levels were calculated as a percentage of the *EF1α* transcript. Error bars represent standard deviations (n = 3). Two-way analysis of variance was performed to compare the expression levels of *GLIP* genes in different genotypes after *B. cinerea* inoculation. Different lowercase letters above the brackets represent statistically significant differences (P < 0.05).

expression in *DD*, *DD etr1-1*, *DD ein2*, and *DD ein3* plants treated with DEX. Although we detected a small decrease in *GLIP1* expression in *DD ein2* plants at 12 h after DEX treatment, *GLIP1* expression was not reduced at other timepoints. Furthermore, there was no decrease in *GLIP1* induction in *DD etr1-1* and *DD ein3* plants (Fig. 8A), suggesting that activation of the MPK3/MPK6 pathway activates the expression of the *GLIP1* gene independent of ethylene sensing or signaling.

We further investigated the expression of the *GLIP1* gene in Col-0, *etr1-1*, *ein2*, *ein3*, and *ctr1* plants after *B. cinerea* challenge. No decrease in *GLIP1* activation was observed in *etr1-1*, *ein2*, or *ein3* mutant after infection by *B. cinerea* (Fig. 8B). Instead, *GLIP1* induction in these mutants was significantly higher. All of these indicated that pathogen-induced expression of *GLIP1* is not ethylene-dependent but ethylene-sensing or -signaling is required to limit the levels of *GLIP1* gene activation. Alternatively, the lack of ethylene sensing or signaling blocks a subset of defense responses, which allows deployment of more resources in activation of ethylene-independent defense responses such as *GLIP1* activation. We also compared the basal levels of *GLIP1* transcript in Col-0, *etr1-1*, *ein2*, *ein3*, and *ctr1* plants (Supplementary Fig. S5). Although the differences were significant, the fold changes are minimal, around twofold. In the constitutive ethylene-response mutant *ctr1*, the minimal increase in *GLIP1* transcript level indicates that constitutive activation of ethylene signaling in *ctr1* is not sufficient to activate *GLIP1* expression. Consistent with this, 1 and 10 μ M 1-aminocyclopropane-1-carboxylic acid (ACC) treatment resulted in only two- and 3.5-fold induction of *GLIP1* expression in

Col-0, respectively (Supplementary Fig. S6), suggesting a subtle role of ethylene in *GLIP1* expression induction. Taken together, these findings show that *GLIP1* expression induced by MPK3/MPK6 is independent of ethylene.

MPK6 was reported to be an essential regulator in *Arabidopsis* JA signaling pathway (Takahashi et al. 2007), although earlier research in our lab indicated that the activation of SIPK and WIPK, tobacco homologs of *Arabidopsis* MPK6 and MPK3, respectively, do not elevate JA level in tobacco (Kim et al. 2003). To investigate whether JA signaling pathway functions in the enhanced expression of *GLIP1* after MPK3/MPK6 activation, we examined *GLIP1* expression in *DD* and *DD coi1* plants. *COI1*, an F-box protein, is a coreceptor of JA and essential to JA-regulated responses (Gfeller et al. 2010). As indicated in Figure 8C, the *GLIP1* expression was not significantly affected in *DD coi1* plants at 0, 6, and 12 h, although its level was slightly reduced at 24 h in *DD coi1* in comparison with *DD*. We also analyzed the expression of *GLIP1* in Col-0 and *coi1* mutant plants after *B. cinerea* inoculation. Loss of *COI1* was not sufficient to block *GLIP1* expression (Fig. 8D). To the contrary, *GLIP1* expression was higher in *coi1* at 24 h after *B. cinerea* invasion. Furthermore, the level of *GLIP1* transcript was not induced in Col-0 after methyl jasmonate (MeJA) treatment. Thus, we can conclude that JA signaling is not related to the MPK3- and MPK6-dependent induction of *GLIP1*.

DISCUSSION

The function of *GLIP1* in *Arabidopsis* immunity has been characterized recently (Kim et al. 2013, 2014; Kwon et al.

Fig. 4. Resistance phenotype in *glip1*, *glip3*, and *glip4* mutant plants inoculated with *Botrytis cinerea*. The 4-week-old wild-type (Col-0), *glip1-1*, *glip1-2*, *glip3-1*, *glip4-1*, and *glip4-2* plants grown under short-day conditions were drop-inoculated with 10 μ l of *B. cinerea* spore suspension (5×10^5 spores/ml). Disease symptoms were scored 3 days after inoculation. **A**, Necrotic lesion phenotypes. **B**, Measurement of lesion size. Error bars represent standard deviations (*n* = 10). Asterisks represent significant differences from Col-0 plants (Student's *t* test, asterisks [***] indicate *P* < 0.001).

2009; Oh et al. 2005). High induction of *GLIP1* expression is found during the plant immune process. However, the molecular mechanism underlying the induction of *GLIP1* gene expression is unclear. In this report, the positive regulation of *GLIP1* expression by MPK3 and MPK6, two important pathogen-responsive MAPKs, is investigated (Figs. 1, 2, and 3). It is also revealed that the implication of WRKY33, a known MPK3/MPK6 substrate (Mao et al. 2011), in *B. cinerea* triggered *GLIP1* induction (Fig. 5). ChIP-qPCR analysis demonstrated that the *GLIP1* gene is a direct target of WRKY33 (Fig. 7). In addition, WRKY2 and WRKY34, two WRKY33 homologs downstream of MPK3 and MPK6 during pollen development (Guan et al. 2014), are involved in the residual activation of *GLIP1* expression in the *wrky33* mutant (Fig. 6), suggesting that these two WRKYs can either substitute partially the role of WRKY33 in its loss or function redundantly with WRKY33, as illustrated in our working model (Supplementary Fig. S7).

Redundant function of MPK3 and MPK6 in *B. cinerea*-induced *GLIP* expression.

Gain-of-function *DD*-induced *GLIP1*, *GLIP3*, and *GLIP4* gene expression was compromised by a single mutation of *MPK3* or *MPK6*, whereas mutation of both *MPK3* and *MPK6* is required to compromise *B. cinerea*-triggered *GLIP1*, *GLIP3*, and *GLIP4* gene activation (Figs. 2 and 3). We previously observed a similar phenomenon in MPK3/MPK6 cascade-mediated *ACS2* and *ACS6* gene activation and camalexin

induction after inoculation by *B. cinerea* (Li et al. 2012; Ren et al. 2008). There are several possible explanations for these seemingly paradoxical findings. First, MPK3/MPK6-regulated *GLIP1*, *GLIP3*, and *GLIP4* expression will be influenced by two pathways, the MPK3/MPK6-mediated phosphorylation of key transcriptional activators such as WRKY33 and dephosphorylation of these transcription factors by one or more unknown phosphatases. Both MPK3 and MPK6 are required to conquer the activity of their phosphatases and effectively keep the phosphorylation of downstream transcription factors to subsequently drive *GLIP1*, *GLIP3*, and *GLIP4* expression in *DD* plants. When either *MPK3* or *MPK6* is mutant, the phosphorylation strength is unable to counteract the dephosphorylation action and *GLIP1*, *GLIP3*, and *GLIP4* expression is severely inhibited. In contrast, *B. cinerea* infection not only activates the MPK3/MPK6 pathway (Li et al. 2012), leading to WRKY33 phosphorylation, but may also inactivate its dephosphorylation process. As a result, the absence of either MAPK may not be able to suppress *GLIP1*, *GLIP3*, and *GLIP4* expression. An alternative explanation is that *B. cinerea* induction can activate various signaling pathways in plants. In addition to the MAPK cascade, the activation of other pathways can probably make up for the weakened MAPK cascade in both *mpk3* and *mpk6* single mutants, which makes it essential to employ a *mpk3 mpk6* double-mutant system to observe the loss-of-function phenotype after *B. cinerea* spore-inoculation.

WRKY transcription factors function downstream of MPK3 and MPK6 in modulation of *GLIP1* gene expression.

Accumulating evidence revealed the crucial function of MPK3 and MPK6 in the regulation of a range of pathogen-triggered defense responses (Guan et al. 2015; Lassowskat et al. 2014; Lee et al. 2015; Meng and Zhang 2013; Meng et al. 2013;

Fig. 5. WRKY33 is involved in the MPK3/MPK6-regulated *GLIP1* gene activation. **A**, *GVG-NtMEK2^{DD}* (*DD*) and *DD wrky33-1* seedlings (12 days old) were treated with 1 μ M dexamethasone (DEX), and **B**, 12-day-old wild-type (*Col-0*) and *wrky33-1* seedlings were treated with *Botrytis cinerea* (*Bc*) spores. Samples were collected at indicated times. Real-time polymerase chain reaction was used to quantify the expression of *GLIP1* gene. *GLIP1* transcript level was calculated as a percentage of the *EF1α* transcript. Error bars represent standard deviations ($n = 3$). Two-way analysis of variance was performed to compare the level of *GLIP1* gene expression in different genotypes after DEX or *B. cinerea* treatment. Different lowercase letters above the brackets represent statistically significant differences ($P < 0.05$).

Fig. 6. WRKY2 and WRKY34 play a slight role in the MPK3/MPK6-regulated *GLIP1* expression in response to *Botrytis cinerea*. Wild-type (*Col-0*), *wrky33*, *wrky2 wrky34*, and *wrky2 wrky33 wrky34* seedlings (12 days old) were treated with *B. cinerea* (*Bc*) spores. Samples were collected at indicated times. Real-time polymerase chain reaction was used to quantify the expression of the *GLIP1* gene. *GLIP1* transcript level was calculated as a percentage of the *EF1α* transcript. Error bars represent standard deviations ($n = 3$). Two-way analysis of variance was performed to compare the level of *GLIP1* gene expression in different genotypes after *B. cinerea* inoculation. Different lowercase letters above the brackets represent statistically significant differences ($P < 0.05$).

Rasmussen et al. 2012; Rodriguez et al. 2010; Sheikh et al. 2016; Su et al. 2017; Tsuda et al. 2009, 2013; Xu et al. 2016). The multifunctionality of MPK3 and MPK6 can be attributed to their capacity to phosphorylate distinct substrates. MPK3- and MPK6-mediated phosphorylation of several ACS isoforms can stabilize these ACS proteins and induce ethylene production (Han et al. 2010; Joo et al. 2008; Liu and Zhang 2004; Xu and Zhang 2015b). WRKY33 phosphorylated by MPK3/MPK6 upregulates the camalexin synthetic gene expression and drives the induction of camalexin production by responding to invading pathogen. WRKY33 is also implicated in the transcriptional modulation of *ACS2* and *ACS6*, two ACS isoforms, which results in abundant ethylene generation by fungi (Li et al. 2012; Mao et al. 2011). Besides WRKYs, MPK3 and MPK6 also phosphorylate other transcription factors, for instance, ERF6 (Meng et al. 2013). Phosphorylated ERF6 activates the transcription of defense genes such as *PDF1.1* and *PDF1.2a* and contributes to plant resistance to fungal pathogen (Meng et al. 2013). Recently, we found the MPK3/MPK6-ERF6 pathway plays critical roles in the dynamic metabolism of IGSs by regulating several key regulators, such as *MYB51* and *MYB122*, and key biosynthesis genes, including *CYP81F2*, *IGMT1*, and *IGMT2* (Xu et al. 2016).

Defense-related *GLI1* expression is dependent on WRKY33 downstream of MPK3 and MPK6 (Fig. 5). WRKY33, as a transcription factor, specifically binds to the C/TTGACT/C DNA motif (W-box) in the promoters of its target genes (Ciolkowski et al. 2008; Pandey and Somssich 2009). ChIP-qPCR results indicated the direct binding of WRKY33 to *GLI1* promoter, which contributes to MPK3- and MPK6-dependent *GLI1* expression after *B. cinerea* inoculation (Fig. 7). However, a recent ChIP-Seq analysis of flg22-treated plants failed to identify *GLI1* as a target of WKY33 (Birkenbihl et al. 2017). This could be due to the sensitivity difference between ChIP-qPCR and ChIP-Seq. We found that the flg22-induced *GLI1* expression was very low in comparison with the pathogen incubation (Supplementary Fig. S8), which possibly is a result of transient activation of MPK3 and MPK6 by responding to flg22. Both *B. cinerea* and flg22 can trigger activation of MPK3 and MPK6, but the activation kinetics are different between the two treatments. Plant sensing of *B. cinerea* infection triggers a long-lasting MPK3 and MPK6 activation, which leads to high-level camalexin accumulation (Mao et al. 2011; Ren et al. 2008). Conversely, plants treated with flg22

only show a transient activation of MPK3 and MPK6 (Liu and Zhang 2004; Xu et al. 2014), which is not sufficient in inducing camalexin biosynthesis (Millet et al. 2010; Schenke et al. 2011).

Plant defense-related hormones in the activation of *GLI1* gene expression.

Plant hormones, including salicylic acid (SA), ethylene, and jasmonic acid (JA), play important roles in mediating plant defense response against pathogens (Pieterse et al. 2012). In general, SA signaling is implicated in plant defense against the biotrophic pathogens, while JA and ethylene are involved in the necrotrophic pathogens (Glazebrook 2005). There is a competitive intercommunication or crosstalk between the signaling pathways of SA and JA, such as the SA antagonistic effects on JA-responsive genes (Caarls et al. 2015, 2017; Shigenaga et al. 2017; Verma et al. 2016). On the other hand, JA and ethylene function synergistically in plant immunity (Broekgaarden et al. 2015).

Arabidopsis MPK3 and MPK6 are involved in both the modulation of ethylene synthesis and JA signaling in plant immunity (Guan et al. 2015; Han et al. 2010; Li et al. 2012; Liu and Zhang 2004; Takahashi et al. 2007). Plants challenged by *B. cinerea* generate high levels of ethylene, which is dependent on the MKP3/MPK6 cascade (Han et al. 2010; Li et al. 2012; Liu and Zhang 2004). Here, we found MPK3- and MPK6-mediated *GLI1* expression is not dependent on ethylene signaling when plants are infected with *B. cinerea* (Fig. 8B). This seems to conflict with results reported in previous publications (Kim et al. 2013; Kwon et al. 2009; Oh et al. 2005), but the opposite conclusion we reached may suggest that the role of ethylene signaling components in induction of *GLI1* is subtle and influenced by experimental conditions. Otherwise, the ethylene signaling may play differential roles in regulating *GLI1* expression in response to different biotic stimulus. MPK3- and MPK6-mediated *GLI1* expression is also independent in JA signaling against the pathogen *B. cinerea* (Fig. 8D), which is consistent with previous results (Kwon et al. 2009; Oh et al. 2005). Previous reports showed that the participation of *GLI1* in *Arabidopsis* SAR as a SA-responsive secreted protein (Kim et al. 2014; Kwon et al. 2009; Oh et al. 2005). Whether MPK3- and MPK6-mediated *GLI1* expression involves SA signaling remains to be further investigated.

Fig. 7. WRKY33 transcription factor binds to the promoter of the *GLI1* gene in vivo. **A**, Mitogen-activated protein kinase phosphorylation sites in the N terminus of WRKY33 and the phospho-mimicking WRKY33 protein with all five serine (S) mutated to aspartic acid (D) (WRKY33^{SD}). **B**, The number and relative position of the W-boxes in the *GLI1* promoter. Line arrows indicate the position of primers used for quantitative polymerase chain reaction (qPCR) after chromatin immunoprecipitation (ChIP). **C**, WRKY33 binds to the promoter of the *GLI1* gene in vivo. ChIP-qPCR analysis was performed using 12-day-old Col-0 and 35S:WRKY33^{SD}/wrky33 seedlings inoculated with *B. cinerea* spores for 12 h. Tagged 4myc-WRKY33^{SD} protein-chromatin complex was immunoprecipitated with an antimyc antibody. A control reaction was processed side-by-side, using mouse immunoglobulin G (IgG). ChIP- and input-DNA samples were quantified by real-time qPCR, using primers specific to the W-box-containing region (B). The ChIP results are presented as a percentage of input DNA. Error bars indicate standard deviations ($n = 3$). Asterisks represent significant differences in DNA abundance of antimyc and IgG antibody in the same seedlings (Student's t test, asterisks [**] indicate $P < 0.01$).

MATERIALS AND METHODS

Plant materials.

Columbia (Col-0) ecotype is applied as the background of all *Arabidopsis thaliana* mutants and transgenic lines in this work. Steroid-inducible promoter-driven tobacco *GVG-NtMEK2^{DD}* transgenic *Arabidopsis* plants (*DD*) (Ren et al. 2002), *glip1-1* and *glip1-2* (Oh et al. 2005), *mpk3-1* (Wang et al. 2007), *mpk6-2* (Liu and Zhang 2004), *wrky33-1* and *wrky33-2* (Zheng et al. 2006), *etr1-1* (Chang et al. 1993), *ein2-1* (Roman et al. 1995), *ein3-1* (Chao et al. 1997), *ctr1-1* (Kieber et al. 1993), and *coi1-1* (Xie et al. 1998) mutants were described previously. *DD mpk3* (Wang et al. 2007), *DD mpk6* (Liu and Zhang 2004), *35S:4myc-WRKY33^{WT}/wrky33* (Mao et al. 2011), *wrky2 wrky34* (Guan et al. 2014), as well as chemical genetically rescued *mpk3 mpk6* double mutants (*MPK6SR* and *MPK3SR*) (Xu et al. 2014, 2016) were previously described. The T-DNA insertion alleles *glip3-1* (SALK_201623C), *glip4-1* (WiscDsLox489-492B15), and *glip4-2* (SALK_048818) were obtained from the *Arabidopsis* Biological Resource Center.

For mutant genotyping, the LBb1.3 (5'-ATTTTGCCGAT TTCGGAAC-3') and P745 (5'-AACGTCCGCAATGTGTTAT TAAGTTGTC-3') border primers were used to detect the SALK lines (*glip3-1* and *glip4-2*) and the Wisconsin line (*glip4-1*), respectively. The gene-specific primers used were as follows: *GLIP3-LP*, 5'-AGAACAACAACTTGACCATGC-3' and *GLIP3-RP*, 5'-CATGAAGGCGTATCTAAAACC-3' for the *glip3-1* mutant; *GLIP4-LP*, 5'-TGATTATTGA CGCTGTCTTCGT-3' and *GLIP4-RP*, 5'-ACCGTAACGAG AAGGATTGTTG-3' for *glip4-1* and *glip4-2* mutants.

Plant growth conditions and treatment.

Arabidopsis seedlings were grown and treated as described before (Mao et al. 2011). *Arabidopsis* seeds were maintained, surface-sterilized, and vernalized for about 3 to 5 days at 4°C. All the seeds were sown in petri dishes containing liquid half-strength Murashige and Skoog (MS) media and were incubated under continuous light at 22°C in a growth chamber as described before (Li et al. 2012). Seedlings were transferred 5 days later to 20-ml gas chromatography (GC) vials containing 6 ml of the same liquid media (10 seedlings for each vial) and were retained under similar growth circumstances. The 12-day-old seedlings that were grown in GC vials were used for treatment. The seedlings were collected at various times after utilization of DEX (final concentration of 1 µM), inoculation of *Botrytis cinerea* spores (4.0 × 10⁵ spores in each vial), ACC (1 or 10 µM), or MeJA (50 or 100 µM). Processes for maintenance of *B. cinerea* as well as preparation of spores were as earlier indicated (Han et al. 2010; Li et al. 2012; Ren et al. 2008).

Generation of transgenic plants.

To generate the phospho-mimicking WRKY33 variant (WRKY33^{SD}), the wild-type WRKY33 complementary (c) DNA was amplified via PCR, using primers WRKY33-F1, 5'-ATGGCTGCTTCTTTCTTACAAATGG-3' and WRKY33-B1, 5'-TCAGGGCATAAACGAATCGAAAAATGAGG-3' and was transferred into the vector pBluescript II KS. Site-directed mutagenesis was performed to get the mutations in the phosphorylation sites of WRKY33. The mutations were verified via sequencing. Briefly, the five target phosphorylation sites of WRKY33, serine-54, serine-59, serine-65, serine-72, and serine-85, were designated as 1, 2, 3, 4, and 5, respectively. Primers that were utilized for mutagenesis were presented as below: 12DDF1, 5'-CTC CTTCTTCAATCTCTATCGATCCTTCTCTTGTCGATCCTT CCACCTGTTTC-3'; 34DDF1, 5'-ATCCTTCCACTTGTTTCGA TCCCTCTCTTTTCTCGATGACCCCTGCTTTTGTCTCC-3'; 5SDF1, 5'-CTCTGCTAACGTTCTAGCTGATCCAACACAGG

AGC-3', as well as their complementary primers. The mutated nucleotides are in bold and underlined. We generated the WRKY33^{SD} with all five serine residues mutated to aspartic acid residues, according to three sequential mutagenesis steps.

For the purposes of establishing the *35S:4myc-WRKY33^{SD}* construct, we amplified the WRKY33^{SD} cDNA fragment using the WRKY33-F1/WRKY33-B1 primer pair and subcloned this fragment into an altered vector pBluescript II KS with the N-terminal 4myc tag. This 4myc-tagged WRKY33^{SD} cDNA was shifted into modified vector pBI121 by using the *SpeI/XhoI* sites. In addition, transformation of *Agrobacterium tumefaciens* GV3101 that carried the construct was implemented into *Arabidopsis* using a floral-dipping approach (Clough and Bent 1998). Immunoblot analyses were used to select independent lines expressing 4myc-tagged WRKY33^{SD}. Successfully transformed plants comprising a single copy of T-DNA insertion were identified on the basis of 3:1 segregation of kanamycin resistance in the T2 generation. Homozygous transgenic lines in the T3 generation were applied for further experiments.

Fig. 8. MPK3/MPK6-induced *GLIP1* expression is independent of ethylene and jasmonic acid. **A**, The 12-day-old *GVG-NtMEK2^{DD}* (*DD*), *DD etr1-1*, *DD ein2*, and *DD ein3* seedlings were treated with 1 µM dexamethasone (DEX), and **B**, 12-day-old wild-type (*Col-0*), *etr1-1*, *ein2*, *ein3*, and *ctr1-1* seedlings were inoculated with *Botrytis cinerea* (*Bc*) spores. **C**, *DD* and *DD coi1* seedlings (12 days old) were treated with 1 µM DEX, and **D**, 12-day-old wild-type (*Col-0*) and *coi1* seedlings were inoculated with *B. cinerea* spores. Samples were collected at indicated times. Real-time polymerase chain reaction was used to quantify the expression of the *GLIP1* gene. The *GLIP1* transcript level was calculated as a percentage of the *EF1α* transcript. Error bars represent standard deviations ($n = 3$). Two-way analysis of variance was performed to compare the level of *GLIP1* gene expression in different genotypes after DEX or *B. cinerea* treatment. Different lowercase letters above the brackets represent statistically significant differences ($P < 0.05$).

RNA extraction and real-time PCR analysis.

The extraction of total plant RNA was performed using the TRIzol reagent (Invitrogen). After treatment with DNase (Invitrogen), total RNA (1 µg) was deployed to synthesize first-strand cDNA. RT-PCR and real-time PCR were performed to characterize the T-DNA insertion mutants and gene expression, respectively. A real-time PCR machine (Eppendorf) was used to conduct a real-time PCR analysis as presented by Ren et al. (2008). Normalization of samples was performed using the reference gene *EF1α*. The expression levels of genes were evaluated utilizing two different approaches. The first approach was the commonly used $2^{-\Delta\Delta C_t}$ cycle threshold method offering fold of gene induction that is relative to basal level before treatment (0 h timepoint). The other approach shows the transcript level that is relative to the *EF1α* gene in the same sample, which is deemed a superior method to compare the expression levels among diverse genes. The primers that were applied in real-time PCR and RT-PCR are presented in Supplementary Table S1.

B. cinerea resistance assay.

For the *B. cinerea* resistance assay, seeds were sown in soil and were maintained in a growth chamber at 22°C and about 65% relative humidity under a 10-h light and 14-h dark cycle for 4 weeks. Detailed procedures of *B. cinerea* maintenance and spore preparation were as described (Ferrari et al. 2003; Meng et al. 2013). Mature rosette leaves detached from 4-week-old plants were placed in petri dishes containing 0.8% agar, with the petiole embedded in the medium. Each leaf was inoculated with a 10-µl drop of spore suspension (5×10^5 spores/ml). Leaves were photographed and lesion size was measured at 3 days after inoculation.

ChIP-qPCR analysis.

ChIP-qPCR processing and assay was implemented as presented before (Xu et al. 2016). The 12-day-old seedlings inoculated with *B. cinerea* spores for 12 h were harvested for this experiment. First, these samples were subjected to cross-linking and processing as described by Kaufmann et al. (2010). One gram of the resulting tissue was used to isolate the chromatin, and the chromatin was then sheared with an enzymatic shearing kit (Active Motif). Subsequently, immunoprecipitation was performed by incubation of chromatin samples with 2 µg of antimyc antibody (Millipore) or mouse IgG (negative control) at 4°C for about 1 h. The protein-chromatin immunocomplexes were captured by utilization of Protein G-Dynal magnetic beads (Invitrogen). Purification of the immunoprecipitated DNA was performed after Proteinase K digestion with a ChIP DNA clean and concentrator kit (Zymo Research). The immunoprecipitated DNA and input DNA were then subjected to qPCR with the primers that were specific for the promoter region of *GLI1*. These forward and backward primer pairs that were utilized for the purpose of ChIP-qPCR included 5'-CGAGTGAACAACGAAGCA-3' and 5'-TCGGAAAATGCCGTCTAGT-3', both of which are near the W-boxes in the *GLI1* promoter. The ChIP-qPCR outcomes are expressed as the percentage of input DNA.

Statistical analyses.

All the experiments mentioned in this research were performed independently at least three times with similar outcomes, and one representative dataset is shown. GraphPad Prism 6.0 was used to perform statistical analyses. The differences are statistically significant ($P < 0.05$), very significant ($P < 0.01$), or extremely significant ($P < 0.001$) as indicated by single, double, or triple asterisks, respectively (Student's *t* test). Tukey's post hoc test was used to perform a two-way analysis

of variance when time-course data of different mutants were compared (Brady et al. 2015). Statistically significant differences ($P < 0.05$) are shown in figures by different letters above the brackets.

LITERATURE CITED

- Akoh, C. C., Lee, G.-C., Liaw, Y.-C., Huang, T.-H., and Shaw, J.-F. 2004. GDSL family of serine esterases/lipases. *Prog. Lipid Res.* 43:534-552.
- Asai, T., Tena, G., Plotnikova, J., Willmann, M. R., Chiu, W.-L., Gomez-Gomez, L., Boller, T., Ausubel, F. M., and Sheen, J. 2002. MAP kinase signalling cascade in *Arabidopsis* innate immunity. *Nature* 415:977-983.
- Bacete, L., Mérida, H., Miedes, E., and Molina, A. 2018. Plant cell wall-mediated immunity: Cell wall changes trigger disease resistance responses. *Plant J.* 93:614-636.
- Bethke, G., Pecher, P., Eschen-Lippold, L., Tsuda, K., Katagiri, F., Glazebrook, J., Scheel, D., and Lee, J. 2012. Activation of the *Arabidopsis thaliana* mitogen-activated protein kinase MPK11 by the flagellin-derived elicitor peptide, flg22. *Mol. Plant-Microbe Interact.* 25: 471-480.
- Bigeard, J., and Hirt, H. 2018. Nuclear signaling of plant MAPKs. *Front. Plant Sci.* 9:469.
- Birkenbihl, R. P., Kracher, B., and Somssich, I. E. 2017. Induced genome-wide binding of three *Arabidopsis* WRKY transcription factors during early MAMP-triggered immunity. *Plant Cell* 29:20-38.
- Bishop, A. C., Ubersax, J. A., Petsch, D. T., Matheos, D. P., Gray, N. S., Blethrow, J., Shimizu, E., Tsien, J. Z., Schultz, P. G., Rose, M. D., Wood, J. L., Morgan, D. O., and Shokat, K. M. 2000. A chemical switch for inhibitor-sensitive alleles of any protein kinase. *Nature* 407:395-401.
- Boller, T., and Felix, G. 2009. A renaissance of elicitors: Perception of microbe-associated molecular patterns and danger signals by pattern-recognition receptors. *Annu. Rev. Plant Biol.* 60:379-406.
- Brady, S. M., Burrow, M., Busch, W., Carlborg, Ö., Denby, K. J., Glazebrook, J., Hamilton, E. S., Harmer, S. L., Haswell, E. S., Maloof, J. N., Springer, N. M., and Kliebenstein, D. J. 2015. Reassess the *t* test: Interact with all your data via ANOVA. *Plant Cell* 27:2088-2094.
- Brick, D. J., Brumlik, M. J., Buckley, J. T., Cao, J.-X., Davies, P. C., Misra, S., Tranbarger, T. J., and Upton, C. 1995. A new family of lipolytic plant enzymes with members in rice, *Arabidopsis* and maize. *FEBS Lett.* 377: 475-480.
- Broekgaarden, C., Caarls, L., Vos, I. A., Pieterse, C. M. J., and Van Wees, S. C. M. 2015. Ethylene: Traffic controller on hormonal crossroads to defense. *Plant Physiol.* 169:2371-2379.
- Caarls, L., Pieterse, C. M., and Van Wees, S. C. 2015. How salicylic acid takes transcriptional control over jasmonic acid signaling. *Front. Plant Sci.* 6:170.
- Caarls, L., Van der Does, D., Hickman, R., Jansen, W., Verk, M. C., Proietti, S., Lorenzo, O., Solano, R., Pieterse, C. M., and Van Wees, S. C. 2017. Assessing the role of ETHYLENE RESPONSE FACTOR transcriptional repressors in salicylic acid-mediated suppression of jasmonic acid-responsive genes. *Plant Cell Physiol.* 58:266-278.
- Chang, C., Kwok, S. F., Bleecker, A. B., and Meyerowitz, E. M. 1993. *Arabidopsis* ethylene-response gene ETR1: Similarity of product to two-component regulators. *Science* 262:539-544.
- Chao, Q., Rothenberg, M., Solano, R., Roman, G., Terzaghi, W., and Ecker, J. R. 1997. Activation of the ethylene gas response pathway in *Arabidopsis* by the nuclear protein ETHYLENE-INSENSITIVE3 and related proteins. *Cell* 89:1133-1144.
- Chen, Z., Gibson, T. B., Robinson, F., Silvestro, L., Pearson, G., Xu, B., Wright, A., Vanderbilt, C., and Cobb, M. H. 2001. MAP kinases. *Chem. Rev.* 101:2449-2476.
- Ciolkowski, I., Wanke, D., Birkenbihl, R. P., and Somssich, I. E. 2008. Studies on DNA-binding selectivity of WRKY transcription factors lend structural clues into WRKY-domain function. *Plant Mol. Biol.* 68:81-92.
- Clough, S. J., and Bent, A. F. 1998. Floral dip: A simplified method for *Agrobacterium*-mediated transformation of *Arabidopsis thaliana*. *Plant J.* 16:735-743.
- Colcombet, J., and Hirt, H. 2008. *Arabidopsis* MAPKs: A complex signalling network involved in multiple biological processes. *Biochem. J.* 413:217-226.
- Coll, N. S., Eppele, P., and Dangl, J. L. 2011. Programmed cell death in the plant immune system. *Cell Death Differ.* 18:1247-1256.
- Dixon, R. A. 2001. Natural products and plant disease resistance. *Nature* 411:843-847.
- Dóczi, R., Okrés, L., Romero, A. E., Paccanaro, A., and Bögre, L. 2012. Exploring the evolutionary path of plant MAPK networks. *Trends Plant Sci.* 17:518-525.

- Ferrari, S., Plotnikova, J. M., De Lorenzo, G., and Ausubel, F. M. 2003. *Arabidopsis* local resistance to *Botrytis cinerea* involves salicylic acid and camalexin and requires *EDS4* and *PAD2*, but not *SID2*, *EDS5* or *PAD4*. *Plant J.* 35:193-205.
- Gao, M., Liu, J., Bi, D., Zhang, Z., Cheng, F., Chen, S., and Zhang, Y. 2008. MEKK1, MKK1/MKK2 and MPK4 function together in a mitogen-activated protein kinase cascade to regulate innate immunity in plants. *Cell Res.* 18:1190-1198.
- Gao, M., Yin, X., Yang, W., Lam, S. M., Tong, X., Liu, J., Wang, X., Li, Q., Shui, G., and He, Z. 2017. GDSL lipases modulate immunity through lipid homeostasis in rice. *PLoS Pathog.* 13:e1006724.
- Gfeller, A., Liechti, R., and Farmer, E. E. 2010. *Arabidopsis* jasmonate signaling pathway. *Sci. Signal.* 3:cm4.
- Glazebrook, J. 2005. Contrasting mechanisms of defense against biotrophic and necrotrophic pathogens. *Annu. Rev. Phytopathol.* 43:205-227.
- Guan, R., Su, J., Meng, X., Li, S., Liu, Y., Xu, J., and Zhang, S. 2015. Multilayered regulation of ethylene induction plays a positive role in *Arabidopsis* resistance against *Pseudomonas syringae*. *Plant Physiol.* 169:299-312.
- Guan, Y., Meng, X., Khanna, R., LaMontagne, E., Liu, Y., and Zhang, S. 2014. Phosphorylation of a WRKY transcription factor by MAPKs is required for pollen development and function in *Arabidopsis*. *PLoS Genet.* 10:e1004384.
- Han, L., Li, G.-J., Yang, K.-Y., Mao, G., Wang, R., Liu, Y., and Zhang, S. 2010. Mitogen-activated protein kinase 3 and 6 regulate *Botrytis cinerea*-induced ethylene production in *Arabidopsis*. *Plant J.* 64:114-127.
- Ichimura, K., Casais, C., Peck, S. C., Shinozaki, K., and Shirasu, K. 2006. MEKK1 is required for MPK4 activation and regulates tissue-specific and temperature-dependent cell death in *Arabidopsis*. *J. Biol. Chem.* 281:36969-36976.
- Joo, S., Liu, Y., Lueth, A., and Zhang, S. 2008. MAPK phosphorylation-induced stabilization of ACS6 protein is mediated by the non-catalytic C-terminal domain, which also contains the cis-determinant for rapid degradation by the 26S proteasome pathway. *Plant J.* 54:129-140.
- Jwa, N.-S., and Hwang, B. K. 2017. Convergent evolution of pathogen effectors toward reactive oxygen species signaling networks in plants. *Front. Plant Sci.* 8:1687.
- Kaufmann, K., Muiño, J. M., Østerås, M., Farinelli, L., Krajewski, P., and Angenent, G. C. 2010. Chromatin immunoprecipitation (ChIP) of plant transcription factors followed by sequencing (ChIP-SEQ) or hybridization to whole genome arrays (ChIP-CHIP). *Nat. Protoc.* 5:457-472.
- Kieber, J. J., Rothenberg, M., Roman, G., Feldmann, K. A., and Ecker, J. R. 1993. CTR1, a negative regulator of the ethylene response pathway in *Arabidopsis*, encodes a member of the raf family of protein kinases. *Cell* 72:427-441.
- Kim, C. Y., Liu, Y., Thorne, E. T., Yang, H., Fukushige, H., Gassmann, W., Hildebrand, D., Sharp, R. E., and Zhang, S. 2003. Activation of a stress-responsive mitogen-activated protein kinase cascade induces the biosynthesis of ethylene in plants. *Plant Cell* 15:2707-2718.
- Kim, H. G., Kwon, S. J., Jang, Y. J., Chung, J. H., Nam, M. H., and Park, O. K. 2014. GDSL lipase 1 regulates ethylene signaling and ethylene-associated systemic immunity in *Arabidopsis*. *FEBS Lett.* 588:1652-1658.
- Kim, H. G., Kwon, S. J., Jang, Y. J., Nam, M. H., Chung, J. H., Na, Y. C., Guo, H., and Park, O. K. 2013. GDSL LIPASE1 modulates plant immunity through feedback regulation of ethylene signaling. *Plant Physiol.* 163:1776-1791.
- Kong, Q., Qu, N., Gao, M., Zhang, Z., Ding, X., Yang, F., Li, Y., Dong, O. X., Chen, S., Li, X., and Zhang, Y. 2012. The MEKK1-MKK1/MKK2-MPK4 kinase cascade negatively regulates immunity mediated by a mitogen-activated protein kinase kinase in *Arabidopsis*. *Plant Cell* 24:2225-2236.
- Kotchoni, S. O., and Gachomo, E. W. 2006. The reactive oxygen species network pathways: an essential prerequisite for perception of pathogen attack and the acquired disease resistance in plants. *J. Biosci.* 31:389-404.
- Kwon, S. J., Jin, H. C., Lee, S., Nam, M. H., Chung, J. H., Kwon, S. I., Ryu, C. M., and Park, O. K. 2009. GDSL lipase-like 1 regulates systemic resistance associated with ethylene signaling in *Arabidopsis*. *Plant J.* 58:235-245.
- Lassowskat, I., Böttcher, C., Eschen-Lippold, L., Scheel, D., and Lee, J. 2014. Sustained mitogen-activated protein kinase activation reprograms defense metabolism and phosphoprotein profile in *Arabidopsis thaliana*. *Front. Plant Sci.* 5:554.
- Lee, D. S., Kim, B. K., Kwon, S. J., Jin, H. C., and Park, O. K. 2009. *Arabidopsis* GDSL lipase 2 plays a role in pathogen defense via negative regulation of auxin signaling. *Biochem. Biophys. Res. Commun.* 379:1038-1042.
- Lee, J., Eschen-Lippold, L., Lassowskat, I., Böttcher, C., and Scheel, D. 2015. Cellular reprogramming through mitogen-activated protein kinases. *Front. Plant Sci.* 6:940.
- Li, G., Meng, X., Wang, R., Mao, G., Han, L., Liu, Y., and Zhang, S. 2012. Dual-level regulation of ACC synthase activity by MPK3/MPK6 cascade and its downstream WRKY transcription factor during ethylene induction in *Arabidopsis*. *PLoS Genet.* 8:e1002767.
- Liu, Y., and Zhang, S. 2004. Phosphorylation of 1-aminocyclopropane-1-carboxylic acid synthase by MPK6, a stress-responsive mitogen-activated protein kinase, induces ethylene biosynthesis in *Arabidopsis*. *Plant Cell* 16:3386-3399.
- Mao, G., Meng, X., Liu, Y., Zheng, Z., Chen, Z., and Zhang, S. 2011. Phosphorylation of a WRKY transcription factor by two pathogen-responsive MAPKs drives phytoalexin biosynthesis in *Arabidopsis*. *Plant Cell* 23:1639-1653.
- Meng, X., Xu, J., He, Y., Yang, K.-Y., Mordorski, B., Liu, Y., and Zhang, S. 2013. Phosphorylation of an ERF transcription factor by *Arabidopsis* MPK3/MPK6 regulates plant defense gene induction and fungal resistance. *Plant Cell* 25:1126-1142.
- Meng, X., and Zhang, S. 2013. MAPK cascades in plant disease resistance signaling. *Annu. Rev. Phytopathol.* 51:245-266.
- Millet, Y. A., Danna, C. H., Clay, N. K., Songnuan, W., Simon, M. D., Werck-Reichhart, D., and Ausubel, F. M. 2010. Innate immune responses activated in *Arabidopsis* roots by microbe-associated molecular patterns. *Plant Cell* 22:973-990.
- Oh, I. S., Park, A. R., Bae, M. S., Kwon, S. J., Kim, Y. S., Lee, J. E., Kang, N. Y., Lee, S., Cheong, H., and Park, O. K. 2005. Secretome analysis reveals an *Arabidopsis* lipase involved in defense against *Alternaria brassicicola*. *Plant Cell* 17:2832-2847.
- Pandey, S. P., and Somssich, I. E. 2009. The role of WRKY transcription factors in plant immunity. *Plant Physiol.* 150:1648-1655.
- Pedley, K. F., and Martin, G. B. 2005. Role of mitogen-activated protein kinases in plant immunity. *Curr. Opin. Plant Biol.* 8:541-547.
- Pieterse, C. M., Van der Does, D., Zamioudis, C., Leon-Reyes, A., and Van Wees, S. C. 2012. Hormonal modulation of plant immunity. *Annu. Rev. Cell Dev. Biol.* 28:489-521.
- Pitzschke, A. 2015. Modes of MAPK substrate recognition and control. *Trends Plant Sci.* 20:49-55.
- Qiu, J.-L., Zhou, L., Yun, B.-W., Nielsen, H. B., Fiil, B. K., Petersen, K., Mackinlay, J., Loake, G. J., Mundy, J., and Morris, P. C. 2008. *Arabidopsis* mitogen-activated protein kinase kinases MKK1 and MKK2 have overlapping functions in defense signaling mediated by MEKK1, MPK4, and MKS1. *Plant Physiol.* 148:212-222.
- Rasmussen, M. W., Roux, M., Petersen, M., and Mundy, J. 2012. MAP kinase cascades in *Arabidopsis* innate immunity. *Front. Plant Sci.* 3:169.
- Ren, D., Liu, Y., Yang, K.-Y., Han, L., Mao, G., Glazebrook, J., and Zhang, S. 2008. A fungal-responsive MAPK cascade regulates phytoalexin biosynthesis in *Arabidopsis*. *Proc. Natl. Acad. Sci. U.S.A.* 105:5638-5643.
- Ren, D., Yang, H., and Zhang, S. 2002. Cell death mediated by MAPK is associated with hydrogen peroxide production in *Arabidopsis*. *J. Biol. Chem.* 277:559-565.
- Rodriguez, M. C., Petersen, M., and Mundy, J. 2010. Mitogen-activated protein kinase signaling in plants. *Annu. Rev. Plant Biol.* 61:621-649.
- Roman, G., Lubarsky, B., Kieber, J. J., Rothenberg, M., and Ecker, J. R. 1995. Genetic analysis of ethylene signal transduction in *Arabidopsis thaliana*: Five novel mutant loci integrated into a stress response pathway. *Genetics* 139:1393-1409.
- Schenke, D., Böttcher, C., and Scheel, D. 2011. Crosstalk between abiotic ultraviolet-B stress and biotic (flg22) stress signalling in *Arabidopsis* prevents flavonol accumulation in favor of pathogen defence compound production. *Plant Cell Environ.* 34:1849-1864.
- Sheikh, A. H., Eschen-Lippold, L., Pecher, P., Hoehenwarter, W., Sinha, A. K., Scheel, D., and Lee, J. 2016. Regulation of WRKY46 transcription factor function by mitogen-activated protein kinases in *Arabidopsis thaliana*. *Front. Plant Sci.* 7:61.
- Shigenaga, A. M., Berens, M. L., Tsuda, K., and Argueso, C. T. 2017. Towards engineering of hormonal crosstalk in plant immunity. *Curr. Opin. Plant Biol.* 38:164-172.
- Sticher, L., Mauch-Mani, B., and Métraux, J.-P. 1997. Systemic acquired resistance. *Annu. Rev. Phytopathol.* 35:235-270.
- Su, J., Yang, L., Zhu, Q., Wu, H., He, Y., Liu, Y., Xu, J., Jiang, D., and Zhang, S. 2018. Active photosynthetic inhibition mediated by MPK3/MPK6 is critical to effector-triggered immunity. *PLoS Biol.* 16:e2004122.
- Su, J., Zhang, M., Zhang, L., Sun, T., Liu, Y., Lukowitz, W., Xu, J., and Zhang, S. 2017. Regulation of stomatal immunity by interdependent functions of a pathogen-responsive MPK3/MPK6 cascade and abscisic acid. *Plant Cell* 29:526-542.

- Suarez-Rodriguez, M. C., Adams-Phillips, L., Liu, Y., Wang, H., Su, S. H., Jester, P. J., Zhang, S., Bent, A. F., and Krysan, P. J. 2007. MEKK1 is required for flg22-induced MPK4 activation in *Arabidopsis* plants. *Plant Physiol.* 143:661-669.
- Taj, G., Agarwal, P., Grant, M., and Kumar, A. 2010. MAPK machinery in plants: Recognition and response to different stresses through multiple signal transduction pathways. *Plant Signal. Behav.* 5:1370-1378.
- Takahashi, F., Yoshida, R., Ichimura, K., Mizoguchi, T., Seo, S., Yonezawa, M., Maruyama, K., Yamaguchi-Shinozaki, K., and Shinozaki, K. 2007. The mitogen-activated protein kinase cascade MKK3-MPK6 is an important part of the jasmonate signal transduction pathway in *Arabidopsis*. *Plant Cell* 19:805-818.
- Tsuda, K., Mine, A., Bethke, G., Igarashi, D., Botanga, C. J., Tsuda, Y., Glazebrook, J., Sato, M., and Katagiri, F. 2013. Dual regulation of gene expression mediated by extended MAPK activation and salicylic acid contributes to robust innate immunity in *Arabidopsis thaliana*. *PLoS Genet.* 9:e1004015.
- Tsuda, K., Sato, M., Stoddard, T., Glazebrook, J., and Katagiri, F. 2009. Network properties of robust immunity in plants. *PLoS Genet.* 5:e1000772.
- Ueda, M., Aichinger, E., Gong, W., Groot, E., Verstraeten, I., Vu, L. D., De Smet, I., Higashiyama, T., Umeda, M., and Laux, T. 2017. Transcriptional integration of paternal and maternal factors in the *Arabidopsis* zygote. *Genes Dev.* 31:617-627.
- Verma, V., Ravindran, P., and Kumar, P. P. 2016. Plant hormone-mediated regulation of stress responses. *BMC Plant Biol.* 16:86.
- Wang, H., Ngwenyama, N., Liu, Y., Walker, J. C., and Zhang, S. 2007. Stomatal development and patterning are regulated by environmentally responsive mitogen-activated protein kinases in *Arabidopsis*. *Plant Cell* 19:63-73.
- Xie, D.-X., Feys, B. F., James, S., Nieto-Rostro, M., and Turner, J. G. 1998. COI1: An *Arabidopsis* gene required for jasmonate-regulated defense and fertility. *Science* 280:1091-1094.
- Xu, J., Meng, J., Meng, X., Zhao, Y., Liu, J., Sun, T., Liu, Y., Wang, Q., and Zhang, S. 2016. Pathogen-responsive MPK3 and MPK6 reprogram the biosynthesis of indole glucosinolates and their derivatives in *Arabidopsis* immunity. *Plant Cell* 28:1144-1162.
- Xu, J., Xie, J., Yan, C., Zou, X., Ren, D., and Zhang, S. 2014. A chemical genetic approach demonstrates that MPK3/MPK6 activation and NADPH oxidase-mediated oxidative burst are two independent signaling events in plant immunity. *Plant J.* 77:222-234.
- Xu, J., and Zhang, S. 2015a. Mitogen-activated protein kinase cascades in signaling plant growth and development. *Trends Plant Sci.* 20:56-64.
- Xu, J., and Zhang, S. 2015b. Ethylene biosynthesis and regulation in plants. Pages 1-25 in: *Ethylene in Plants*. C. K. Wen, ed. Springer, Dordrecht, The Netherlands.
- Yang, K.-Y., Liu, Y., and Zhang, S. 2001. Activation of a mitogen-activated protein kinase pathway is involved in disease resistance in tobacco. *Proc. Natl. Acad. Sci. U.S.A.* 98:741-746.
- Zhang, M., Su, J., Zhang, Y., Xu, J., and Zhang, S. 2018. Conveying endogenous and exogenous signals: MAPK cascades in plant growth and defense. *Curr. Opin. Plant Biol.* 45 (Pt A):1-10.
- Zhang, M., Wu, H., Su, J., Wang, H., Zhu, Q., Liu, Y., Xu, J., Lukowitz, W., and Zhang, S. 2017. Maternal control of embryogenesis by MPK6 and its upstream MKK4/MKK5 in *Arabidopsis*. *Plant J.* 92: 1005-1019.
- Zheng, Z., Qamar, S. A., Chen, Z., and Mengiste, T. 2006. *Arabidopsis* WRKY33 transcription factor is required for resistance to necrotrophic fungal pathogens. *Plant J.* 48:592-605.