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Research paper

Phytochrome B regulates jasmonic acid-mediated defense response against *Botrytis cinerea* in *Arabidopsis*

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ABSTRACT

The phytochrome B mediated light signaling integrates with various phytohormone signalings to control plant immune response. However, it is still unclear whether phyB-mediated light signaling has an effect on the biosynthesis of jasmonate during plant defense response against *Botrytis cinerea*. In this study, we demonstrated that phyB-mediated light signaling has a role in this process. Initially, we confirmed that *phyb* plants were obviously less resistant to *B. cinerea* while *phyB* overexpressing plants showed significantly enhanced resistance. We also found that the expression of numerous JA biosynthesis genes was promoted upon treatment with red or white light when compared to that of darkness, and that this promotion is dependent on phyB. Consistent with the gene expression results, *phyb* plants accumulated reduced pool of JA-Ile, indicating that phyB-mediated light signaling indeed increased JA biosynthesis. Further genetic analysis showed that light-mediated JAZ9 degradation and phyB-enhanced resistance were dependent on the receptor COI1, and that *pif1/3/4/5* (*pifq*) can largely rescue the severe symptom of *phyb*. Taken together, our study demonstrates that phyB may participate in plant defense against *B. cinerea* through the modulation of the biosynthesis of JA.

1. Introduction

In the process of interaction with various pathogens, plants have evolved effective immune system to protect themselves. The precisely regulated defense responses are vital for plants to successfully defense against various pathogens. Based on their lifestyles, plant pathogens can be mainly clarified into two types, namely biotrophic pathogens and necrotrophic pathogens. *Botrytis cinerea*, as a typical necrotrophic pathogen, kills plants mainly through cell wall-degrading enzymes and toxins and obtains nutrients from the remains (Mengiste, 2012). It causes serious devastating diseases in both horticultural and agronomic crops and has been widely used as model fungus to study the interaction between plants and pathogens.

The phytohormone JA is exquisitely tuned by the ever-changing environmental conditions and is important for defense responses to multiple pathogens and herbivorous insects (Browse, 2009; Bhosale et al., 2013; Havko et al., 2016). JA biosynthesis occurs at chloroplast, peroxisome, and cytoplasm, and is catalyzed by a series of enzymes, including 13-lipoxygenase (LOX), allene oxide synthase (AOS), allene oxide cyclase (AOC), OPDA reductase 3 (OPR3), and jasmonoyl-isoleucine synthetase (JAR1) (Wang et al., 2019). One of the end products, JA-Ile, a bioactive form of JA, is perceived by the F-box protein CORONATINE INSENSITIVE1 (COI1), which subsequently facilitates the ubiquitination and degradation of JAZ proteins via the SCF^{COI1}–26S proteasome pathway (Xie et al., 1998; Xu et al., 2002; Thines et al., 2007; Yan et al., 2009). Disruption of JA biosynthesis and signaling pathways greatly impact plant defense response against *B. cinerea*. For example, several JA biosynthesis and signaling associated mutants, such as *aos*, *opr3*, *jar1*, and *coi1*,

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show compromised resistance to *B. cinerea* (Méndez-Bravo et al., 2011; Rowe et al., 2010; Scalschi et al., 2015; Chehab et al., 2011). Conversely, *jaz* decuple (*jazD*) is highly resistant to *B. cinerea* (Guo et al., 2018).

In *Arabidopsis thaliana*, phytochromes are red (R) and far-red (FR) light sensing photoreceptors. Among the five members of phytochromes, phyA and phyB are the most important phys. There are two forms of phytochromes: the inactive Pr and the active Pfr. The Pr and Pfr form are separately located in cytoplasm and nucleus, and can interconvert to each other (Kircher et al., 2002; Sakamoto and Nagatani, 1996; Yamaguchi et al., 1999). Upon red light irradiation, the inactive Pr form of phyB converts to active Pfr which translocates into nucleus, where it interacts with a group of basic helix-loop-helix transcription factors called phytochrome-interacting factors (PIFs), to modulate plant growth and adaptations, including seed germination, morphogenesis, shade avoidance, and disease resistance (Kami et al., 2010; Chen and Chory, 2011). There is emerging evidence that phytochrome-mediated light signaling is involved in defenses against pathogens and herbivorous insects (Ballaré, 2014; Ballaré and Austin, 2019). For example, in tobacco, phyB positively influences defense responses to cucumber mottle virus by modulating the expression of salicylic acid (SA)-, JA-, and ET-mediated defense genes (Li et al., 2015). Similarly, the tomato phyB also positively regulates plant defenses against the herbivorous insect *Spodoptera eridania* (Izaguirre et al., 2006). In rice, phytochromes mediate the developmentally controlled resistance to the blast fungus (*Magnaporthe grisea*) by regulating the expression of both JA and SA signaling-associated genes (Xie et al., 2011). Both phytochrome A (phyA) and phyB in *A. thaliana* contribute to defense responses against the incompatible bacterial strain *Pseudomonas syringae* pv. *tomato* (*Pto*) DC3000 through interactions with the pathogen/SA-mediated signal transduction pathway (Genoud et al., 2002).

Interestingly, recent research has produced evidence that the JA-triggered defense responses can be suppressed by the inactivation of phytochrome B (phyB) during the shade avoidance response via a mechanism involving the increased stability of JAZ repressors, enhanced degradation of MYCs, and reduced plant sensitivity to JA (Moreno et al., 2009; Robson et al., 2010; Cerrudo et al., 2012, 2017; de Wit et al., 2013; Chico et al., 2014; Leone et al., 2014; Cargnel et al., 2014), suggesting that phyB-mediated light signaling plays an important role in regulating JA signaling pathways. Most recently, it has been reported that far-red light supplementation which inactivates phyB and facilitates shade avoidance response, affects the JA-Ile content of plants to keep a balance between growth and resistance via ST2a-involved sulfation (Fernández-Milmanda et al., 2020), suggesting that phyB-mediated light signaling has a function in JA metabolism. Moreover, light has been found to promote jasmonate biosynthesis to regulate hypocotyl elongation in a COI1-JAZ-MYCs-dependent manner (Yi et al., 2020).

In this study, we demonstrated that phyB-mediated light signaling enhances plant defense responses by positively regulating the concentration of JA. We also demonstrated that the phyB mediated defense is closely associated with JA signaling. Thus, our results provide compelling evidence that the phyB functions coordinately with JA pathway to control plant defense response against *B. cinerea*.

2. Materials and methods

2.1. Materials and growth conditions

The wild type and mutant plants used in this study are in Col-0 background. The mutants *phya* (SALK_014575C), *phyb* (SALK_069700C), and *pif1/3/4/5* (*pifq*, CS66049) were purchased from Arabidopsis Biological Resource Center. The seeds of *coi1-16* were provided by Zhixiang Chen (Purdue University). The overexpression plants *phyB-OX* (Jiang et al., 2016) and *JAZ9OE2* (Yang et al., 2012) have been described. The *phya/phyb*, *JAZ9OE2/coi1-16*, *coi1-16/phyB-OX*, *JAZ9OE2/phyB-OX*, and *phyb/pifq* were generated by genetic crossing. Seeds were sterilized in 10% bleach, and then sown on 1/2 MS medium (pH 5.8) containing 0.5% agar and 1% sucrose. After 3 days at 4 °C, seeds were grown under 16 h light/8 h dark (LDs) or 12 h light/12 h dark at 22 °C. Approximately 60 μmol m^{-2} s^{-1} of red light and 100 μmol m^{-2} s^{-1} of white light were used for light treatment. Primers used for PCR genotyping and sequencing are listed in Supplementary Table S1.

2.2. Botrytis cinerea infection

Botrytis cinerea (B05.10) strain was cultivated and propagated on Potato Dextrose Agar. We resuspended and collected the spores in Sabouraud Maltose Broth. The concentration of spore suspension was calculated by using hemocytometer. Plants and detached leaves were spray- and drop-inoculated respectively.

2.3. Quantitative real-time PCR analysis

We extracted the total RNA from Arabidopsis seedlings using the TRIzol reagent (Invitrogen), and then synthesized the cDNA from 1ug of total RNA using PrimeScript™ RT reagent Kit (Takara). 1 μl cDNA was subjected to qPCR on a Roche LightCycler 480, using the SYBR Premix Ex Taq (Takara). *ACTIN2* used as the reference gene. The primers used for RT-qPCR are provided in Supplementary Table S1.

2.4. Measurement of OPDA, JA and JA-Ile contents

Plants were grown on 1/2 MS medium under LD conditions for 8 days. After pre-treated with darkness for 12 h, seedlings were treated with darkness, red or white light for another 24 h separately. Samples were harvested at indicated time. OPDA, JA and JA-Ile were extracted and analyzed on UPLC-MS/MS system (LCMS-8040, Shimadzu) as described previously (Qi et al., 2018). Quantification of OPDA was according to its standard curve. $^{13}C_6$-JA and $^{13}C_6$-JA-Ile used as the internal standards for JA and JA-Ile respectively.

2.5. Western blotting

Six-day-old seedlings grown on 1/2 MS medium under LD conditions were placed in darkness for 12 h before treatment. Then, seedlings were treated with darkness, red light and white light for 24 h separately. Samples were harvested at indicated time, total proteins were extracted using extraction buffer containing 100 mM Tris—HCl, pH 7.5, 100 mM NaCl, 5 mM EDTA, 1% SDS, 1 × complete protease inhibitor cocktail (Roche) and 50 μM MG132. Protein concentration was determined by the Bio-Rad protein assay. The anti-HA (Santa Cruz) and anti-Actin (Affinity) were used to detect HA-JAZ9 and Actin, respectively. The immunoblot bands were quantified by ImageJ.

2.6. MeJA treatment

The seven-day-old WT and *phyb* plants grown on soil were sprayed with the indicated concentrations of MeJA each day at ZT0 and ZT12 for 20 days under 12 h light/12 h dark at 22 °C.

3. Results

3.1. phyB enhances plant defense against Botrytis cinerea

Previous studies discovered that low red/far-red ratios, perceived by phyB, decrease the resistance of *A. thaliana* to the necrotrophic fungal pathogen *B. cinerea* (Cerrudo et al., 2012, 2017; de Wit et al., 2013). To further characterize phyB regarding defenses response against *B. cinerea*, the *phyb* mutant and *phyB*-overexpressing (*phyB-OX*) lines were inoculated with this pathogen. Compared with the wild type (WT) plants, the *phyb* mutant plants developed more severe necrotic symptoms, whereas the *phyB-OX* plants had less extensive disease symptoms and most of their leaves remained green (Fig. 1A). Moreover, when isolated leaves were drop-inoculated with *B. cinerea* spores, the lesion on the *phyb* mutant leaves was 2-times larger than that on the WT leaves. However, the *phyB-OX* leaves had less severe lesions than the WT leaves (Fig. 1B). To confirm these disease symptoms, we quantified the fungal biomass in inoculated plants by examining the accumulation of *B. cinerea β-tubulin* transcripts. Compared with the WT plants, the *phyb* plants accumulated more *β-tubulin* mRNA, while *phyB-OX* had less (Fig. 1C). Thus, consistent with previously reported results (Cerrudo et al., 2012, 2017; de Wit et al., 2013), our data indicated that *phyB* positively regulates plant defense against *B. cinerea* in *Arabidopsis*.

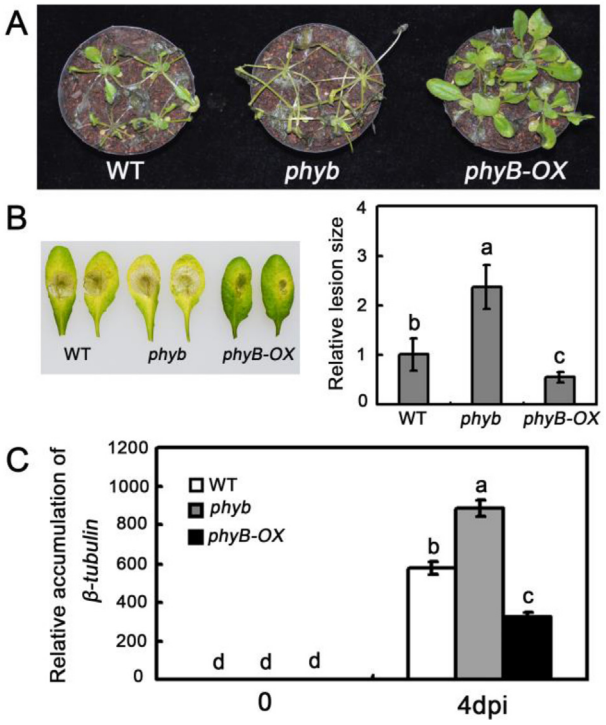

Fig. 1. Loss of phyB function compromises the resistance of *Arabidopsis* against *Botrytis cinerea*. (A) Disease phenotypes of spray-inoculated plants. The *phyb* and *phyB-OX* plants exhibit enhanced susceptibility and resistance to *B. cinerea* respectively. The 28-day-old WT, *phyb* and *phyB-OX* plants grown under 12 h light/12 h dark conditions were sprayed with *B. cinerea* spores (5×10^5 spores/ml). Representative plants were photographed at 6 dpi. The experiments were repeated three times with similar results. (B) The detached leaves of WT, *phyb* and *phyB-OX* were inoculated with 8 μl drop of a suspension of *B. cinerea* spores (5×10^5 spores/ml). Disease phenotypes of drop-inoculated leaves were photographed at 3 dpi. Relative lesion size was measured at 3 dpi. Values are mean SD over 20 leaves. (C) Accumulation of pathogen in plants sprayed with *B. cinerea*. RNA was isolated from spray-inoculated plants at 0 and 4 dpi, and the relative accumulation of *β-tubulin* mRNA was used to analyze the biomass of *B. cinerea*. Error bars indicate the SD of three independent experiments. In (B) and (C) the different letters above columns indicate significant differences (one-way ANOVA; $P < 0.05$).

3.2. phyB-mediated light signaling promotes the expression of JA biosynthesis genes

Considering JAZ proteins are stabilized under low red/far-red light conditions (Chico et al., 2014; Leone et al., 2014), we proposed that phyB-mediated light signaling may modulate JAZ stability by controlling JA biosynthesis. To test this possibility, we first examined the expression of JA-synthesis genes following an exposure to darkness, red or white light irradiation. Compared with the effects of darkness, the light treatments dramatically up-regulated the expression of JA synthesis and metabolism genes, including *AOS*, *AOC1*, *AOC2*, *AOC3*, *OPR3*, and *JAR1* (Fig. 2A). However, the white light-induced expression levels of *AOS*, *AOC1*, *AOC2*, and *OPR3* were considerably lower in the *phyb* and *phya/phyb* mutant plants than in the WT plants (Fig. 2B). Thus, these results demonstrated that phyB plays an important role in light promoted expression of JA biosynthesis genes.

3.3. phyB-mediated light signaling enhances JA biosynthesis and JAZ degradation

To verify whether phyB-promoted expression of JA biosynthesis genes could elevate JA biosynthesis, we analyzed the content of OPDA, JA, and JA-Ile in wild type, *phyb*, and *phya/phyb*. Consistent with the gene expression results, we observed that the white light treatment increased the contents of OPDA and JA-Ile (Fig. 3A and C). However, the synthesis of these compounds was greatly inhibited in the *phyb* and *phya/phyb* plants, and exogenous supplement of MeJA at the concentration of 50 or 100 μmol/L can decrease the relative lesion size of *phyb* leaves to the wild type level (Fig. 3A and C and Fig. S1). These results imply that phyB-mediated light signaling can positively modulate JA-Ile pool. To investigate whether the increased JA-Ile pool under light conditions contributes to JAZ degradation, we examined JAZ9 stability in response to light treatments. The red and white light irradiations promoted

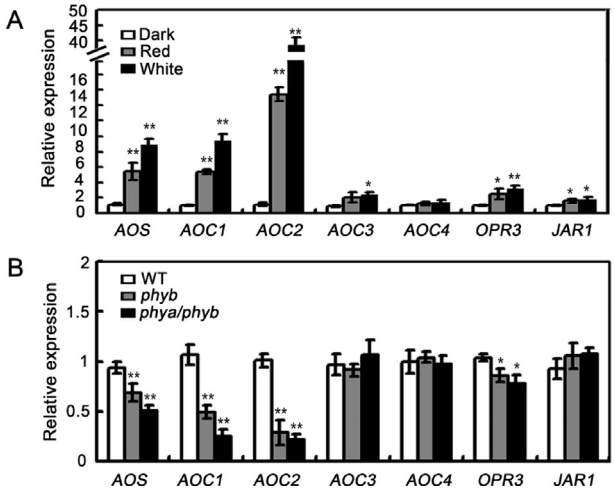

Fig. 2. Light promotes the expression of JA biosynthesis gene in a phyB-dependent manner. (A) The JA biosynthesis and metabolism genes expression in 8-day-old WT plants, pre-treated in darkness for 12 h and then treated with darkness, red and white light for 24 h. (B) The JA biosynthesis and metabolism genes expression in 8-day-old WT, *phyb* and *phya/phyb* plants, pre-treated in darkness for 12 h and then treated with white light for 24 h. For (A) and (B), error bars indicate the SD of three independent experiments. Asterisks indicate Student's *t*-test significant differences (*$P < 0.05$, **$P < 0.01$).

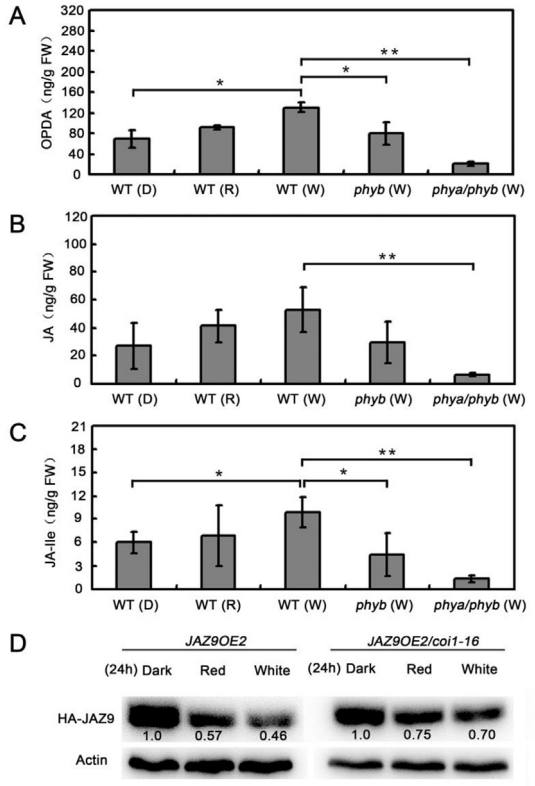

Fig. 3. phyB regulates JA-Ile pool and JA signaling. (A), (B) and (C) Measurement of OPDA, JA and JA-Ile contents in WT and indicated mutant plants in darkness (D), red (R) and white (W) light separately. (D) Western-blot detection of HA-JAZ9 degradation. HA-JAZ9/Actin ratios were calculated and shown. Similar results were obtained from three biological repeats. In (A), (B) and (C), error bars indicate the SD of three independent experiments. Asterisks indicate Student's *t*-test significance (*$P < 0.05$, **$P < 0.01$).

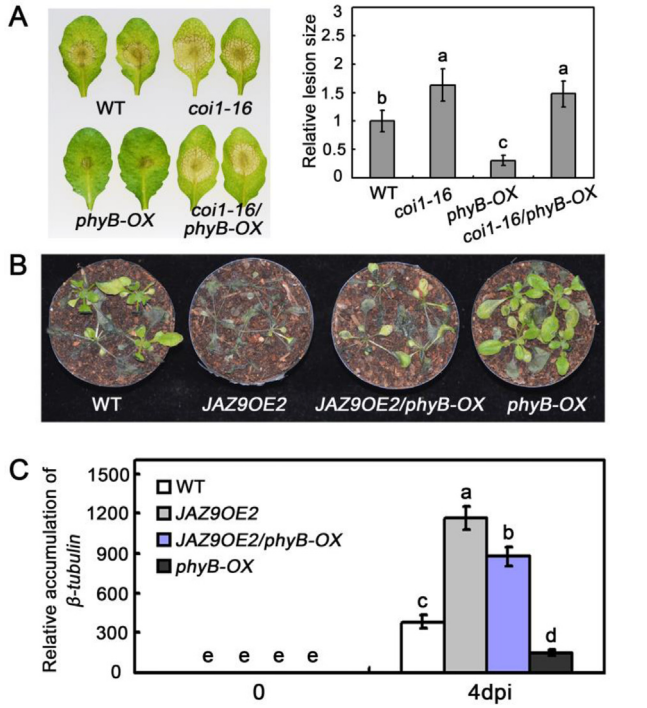

Fig. 4. The functional relationship between phyB and JA signaling in defense response. (A) The phenotypes of detached leaves of WT, *coi1-16*, *phyB-OX*, and *coi1-16*/*phyB-OX* inoculated with 8 μl spore suspension (5×10^5 spores/ml). At 3 dpi, disease phenotypes were taken and relative lesion size was measured. Error bars indicate SD of more than 15 leaves. (B) The disease phenotypes of WT, *JAZ9OE2*, *JAZ9OE2*/*phyB-OX* and *phyB-OX* plants. Two-week-old plants grown under 12 h light/12 h dark conditions were inoculated with *B. cinerea* (1×10^5 spores/ml), and photographs were taken at 4 dpi. The experiments were repeated three times with similar results. (C) The accumulation of *B. cinerea β-tubulin* transcripts. RNA was isolated from spray-inoculated plants at 0 and 4 dpi, and the biomass of *B. cinerea* was analyzed in the corresponding plants. Error bars indicate the SD of three independent experiments. In (A) and (C), the different letters above columns indicate significant differences (one-way ANOVA; $P < 0.05$).

JAZ9 degradation compared to darkness (Fig. 3D). Moreover, the light-promoted degradation of JAZ9 was decelerated in the *coi1-16* background (Fig. 3D), implying that JAZ degradation upon light irradiation is dependent on COI1. The *coi1-16* mutation involves a C-to-T substitution that introduces the L245F change in the encoded protein. The mutated protein may remain partially functional, as suggested by the fact *coi1-16* plants were fertile at 16 °C, unlike the *coi1-1* plants, which were infertile because of a premature termination during protein expression (Ellis and Turner, 2002). These findings may help to explain why JAZ9 may still be slightly degraded upon white light irradiation in plants with the *coi1-16* background. Considered together, these results indicated that the phyB-mediated light signaling can promote JAZ degradation by enhancing JA-Ile concentration upon white light irradiation.

3.4. *phyB-mediated defense is dependent on JA signaling pathway*

JA plays crucial roles during plant defense against necrotrophic fungal pathogens, such as *B. cinerea* and *Alternaria brassicicola*. The mutation of COI1, a receptor required for JA signaling, makes plants more susceptible to *B. cinerea* (Méndez-Bravo et al., 2011). To assess the relationship between phyB-mediated light signaling and JA signaling in defense responses to *B. cinerea*, we generated *coi1-16*/*phyB-OX* plants via cross. The decreased lesion size and enhanced resistance of the *phyB-OX* plants were mostly suppressed by *coi1-16* (Fig. 4A). Furthermore, overexpression of *JAZ9* in the *phyB-OX* background also dramatically decreased the resistance of *phyB-OX* plants (Fig. 4B and C). According to these data, we assumed that the phyB-mediated light signaling may be coordinated with the JA signaling in defense responses to *B. cinerea.*

3.5. *pifq largely rescues the enhanced susceptibility of phyb mutant plants*

The PIFs function as the central regulators of the transduction of light signals perceived by phyB (Castillon et al., 2007; Leivar and Quail, 2011). An earlier study revealed that *pifq* can significantly rescue the long hypocotyl phenotype of *phyb* plants (Leivar et al., 2012). Recently, PIFs have been reported to redundantly control JA/ET signaling to regulate plant resistance against *B. cinerea* (Xiang et al., 2020). Thus, we assessed the relationship between PIFs and phyB in controlling JA-regulated defense response. The *phyb* plants were crossed with *pifq* plants to generate *phyb*/*pifq* quintuple mutants. The *phyb*/*pifq* plants were more resistant to *B. cinerea* than the *phyb* plants (Fig. 5A and B), suggesting *pifq* can rescue the diseased phenotype of *phyb* plants. It has been shown that the *phyb* mutants or plants grown under simulated shade accumulate lower level of *ORA59* and *PDF1.2* transcripts upon MeJA treatment (Moreno et al., 2009; Cerrudo et al., 2017), and that supplemental FR radiation reduces *Botrytis*-induced expression of these genes in plants (Cerrudo et al., 2012). Furthermore, we analyzed their expression in *phyb*, *phya/phyb* and *phyb/pifq* plants. As shown in Fig. 5C and D, *phyb* and *phya/phyb* plants had significantly reduced expression of *ORA59* and *PDF1.2* compared to wild type upon

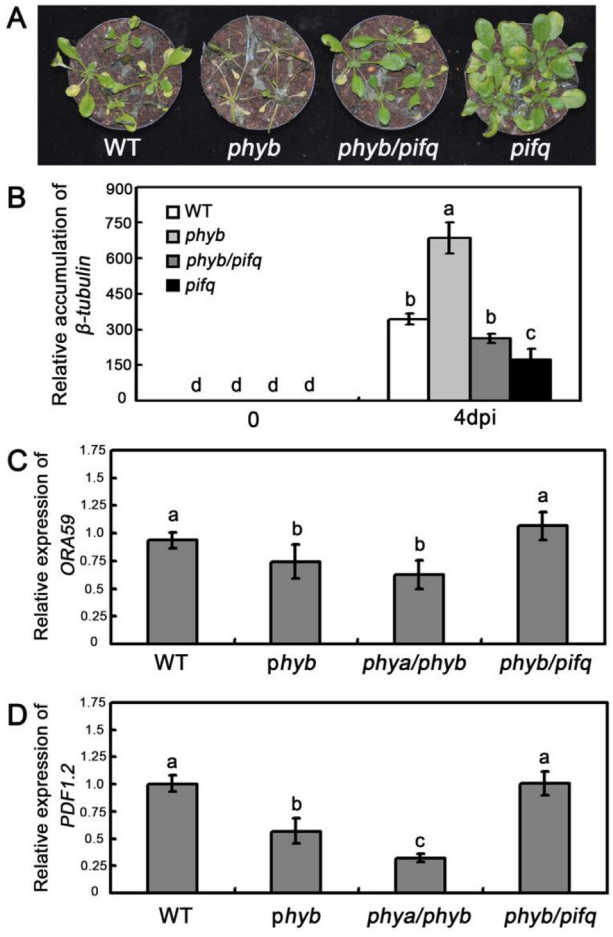

Fig. 5. PIF proteins are involved in phyB-mediated defense response. (A) Phenotypic analysis upon *Botrytis* infection. The WT, *phyb*, *phyb*/*pifq* and *pifq* plants grown on soil for 28 days under 12 h light/12 h dark conditions were sprayed with 5×10^5 spores/ml of *Botrytis cinerea*, and representative photograph was taken at 5 dpi. Similar results were obtained from three biological repeats. (B) qPCR analysis of *B. cinerea β-tubulin* mRNA accumulation. RNA was isolated from plants inoculated with 5×10^5 spores/ml of *B. cinerea* at 0 and 4 dpi, and the biomass of *B. cinerea* was analyzed. (C) and (D) The expression of *ORA59* and *PDF1.2* in WT, *phyb*, *phya/phyb* and *phyb*/*pifq* plants upon *B. cinerea* infection. The 21-day-old soil grown plants were spray-inoculated with *B. cinerea* (5×10^5 spores/ml). The relative expression of *ORA59* and *PDF1.2* was examined at 24h after inoculation. For (B), (C) and (D), error bars indicate the SD of three independent experiments. The different letters above columns indicate significant differences (one-way ANOVA; $P < 0.05$).

B. cinerea inoculation; and the compromised expression of these genes in *phyb* mutant was also rescued by *pifq* plants, biologically relevant to the enhanced resistance of *phyb/pifq* plants when compared to *phyb* mutant. Therefore, we concluded that phyB-mediated light signaling is essential for plant resistance regulated by JA signaling against *B. cinerea* and that PIFs function downstream in this process.

4. Discussion

Light is a crucial environmental cue that represents an energy source for photosynthesis and also helps regulate plant development. Photoreceptor-mediated light signaling was recently reported to be involved in plant defense responses. For example, the blue light receptor cryptochrome CRY1 modestly regulates SA accumulation and SA-induced *PR-1* expression to promote R protein-mediated plant resistance to *P. syringae* (Wu and Yang, 2010). Demkura and Ballaré (2012) confirmed that UV-B radiation can promote *A. thaliana* resistance to *B. cinerea* by enhancing sinapate production in a UVR8-dependent manner. Additionally, the low red/far-red ratios of environmental light conditions increase the susceptibility of plants to *B. cinerea* because of the decreased production of glucosinolates and camalexin (Cargnel et al., 2014). Moreover, the red and far-red light photoreceptor phyB reportedly regulates defense responses to *B. cinerea* by influencing JAZ stability (Leone et al., 2014; Chico et al., 2014), implying that phyB-mediated light signaling may crosstalk with JA signaling during defense responses to this pathogen.

The resistance of *A. thaliana* to necrotrophic pathogens depends on the activation of JA signaling (Glazebrook, 2005). Low red/far-red ratios cannot further increase the susceptibility of *phyb* mutant plants to *B. cinerea* (Cerrudo et al., 2012). Another study revealed that *phyb* mutants exhibit down-regulated expression of JA-inducible genes, such as *HEL*, and *PDF1.2*, and compromised resistance to *B. cinerea* (Fig. 1; Moreno et al., 2009; Cerrudo et al., 2012; de Wit et al., 2013). Furthermore, it has been reported that a far-red light treatment, which inactivates phyB, can stabilize most JAZs (Chico et al., 2014). Interestingly, one recent research also showed that phyB signaling can contribute to the disease resistant of tomato plants against *B. cinerea* through JA-dependent modulation of soluble sugars (Courbier et al., 2020). These observations imply that there may be crosstalk between phyB-mediated light signaling and JA signaling. Our data further demonstrated that the light-induced expression of JA-synthesis-associated genes is inhibited in the *phyb* and *phya/phyb* plants, thereby preventing the increased production of JA-Ile (Figs. 2 and 3). Accordingly, phyA and phyB may function redundantly to control bioactive JA pool following light irradiation. Consistent with our results, several other studies also demonstrated that light can regulate both the expression of JA-synthesis genes (in rice and maize) and the synthesis of wound-induced JA-Ile (in lima bean) (Haga and Iino, 2004; He et al., 2005; Radhika et al., 2010). What's more, we also found that the enhanced susceptibility of *phyb* plants against *B. cinerea* can be rescued to wild type level by exogenous supplement of MeJA (Fig. S1).

The effect of phyB-mediated light signaling on JA pools might be caused by changes in JA biosynthesis or metabolism. Recently, light has been reported to regulate JA biosynthesis to promote photomorphogenesis (Yi et al., 2020). Considering that the significantly up-regulated expression of JA biosynthesis genes correlates well with the improved JA-Ile concentration in wild type plants under white light condition and that *phyb* and *phya/phyb* have much lesser JA-Ile pool under the same condition (Figs. 2 and 3), there is highly possible that phyB has an important effect on light-regulated JA biosynthesis. However, it has been reported most recently that *ST2a,* which is upregulated by low R:FR ratios and acts as a direct target of PIF, functions as a sulfotransferase to reduce the concentration of bioactive JA through catalyzing the conversion of OH-JA to HSO_4-JA under shade condition (Fernández-Milmanda et al., 2020), implying that phyB-mediated light signaling could also have a capability to control JA pools by modulating its metabolism. Collectively, these studies suggested that phyB modulates JA signaling by controlling its biosynthesis and metabolism.

Despite the expression of *JAZ9* was induced by red or white light treatment (Fig. S2), our protein stability analysis demonstrated that JAZ9 degradation increased following the exposures to red or white light in a COI1-dependent manner (Fig. 3D). On the basis of the increased concentration of JA-Ile and enhanced degradation of JAZ9 upon light irradiation, we deduced that light modulated JAZ stability by controlling JA pools. Furthermore, a genetic analysis revealed that phyB regulates plant resistance through JA signaling and that PIFs also were involved in the complex network (Figs. 4 and 5), implying that phyB-mediated light signaling may

coordinately function with the JA pathway to mediate plant defenses against necrotrophic pathogens.

5. Conclusions

In nature, light provides plants with energy for photosynthesis and also function as an important environmental cue to modulate both growth and defense response. It has been reported that phyB-mediated light signaling is involved in JA-regulated resistance against *Botrytis cinerea*, but the mechanisms underlying this process are not well understood. In this study, we provided compelling evidence to demonstrate that phyB-mediated light signaling participates in plant defense responses by promotion of JA biosynthesis. Our study greatly increased our understanding regarding the relationship between phyB-regulated defense and JA pathway.

Author contributions

LGC and DQY conceived the project and designed the experiments. SYX, SGW, and YFJ performed the experiments. LGC and SYX wrote the article. All authors interpreted and discussed the data.

Declaration of competing interest

The authors declare no conflict of interest.

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Appendix A. Supplementary data

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References

Ballaré, C.L., 2014. Light regulation of plant defense. Annu. Rev. Plant Biol. 65, 335–363.
Ballaré, C.L., Austin, A.T., 2019. Recalculating growth and defense strategies under competition: key roles of photoreceptors and jasmonates. J. Exp. Bot. 70, 3425–3434.
Bhosale, R., Jewell, J.B., Hollunder, J., et al., 2013. Predicting gene function from uncontrolled expression variation among individual wild-type Arabidopsis plants. Plant Cell 25 (8), 2865–2877.
Browse, J., 2009. Jasmonate passes muster: a receptor and targets for the defense hormone. Annu. Rev. Plant Biol. 60, 183–205.
Cargnel, M.D., Demkura, P.V., Ballaré, C.L., 2014. Linking phytochrome to plant immunity: low red: far-red ratios increase Arabidopsis susceptibility to Botrytis cinerea by reducing the biosynthesis of indolic glucosinolates and camalexin. New Phytol. 204 (2), 342–354.
Castillon, A., Shen, H., Huq, E., 2007. Phytochrome interacting factors: central players in phytochrome-mediated light signaling networks. Trends Plant Sci. 12 (11), 514–521.
Cerrudo, I., Keller, M.M., Cargnel, M.D., et al., 2012. Low red/far-red ratios reduce Arabidopsis resistance to Botrytis cinerea and jasmonate responses via a COI1-JAZ10-dependent, salicylic acid-independent mechanism. Plant Physiol. 158 (4), 2042–2052.
Cerrudo, I., Caliri-Ortiz, M.E., Keller, M.M., et al., 2017. Exploring growth-defence trade-offs in Arabidopsis: phytochrome B inactivation requires JAZ10 to suppress plant immunity but not to trigger shade-avoidance responses. Plant Cell Environ. 40 (5), 635–644.
Chehab, E.W., Kim, S., Savchenko, T., et al., 2011. Intronic T-DNA insertion renders Arabidopsis opr3 a conditional jasmonic acid-producing mutant. Plant Physiol. 156 (2), 770–778.
Chen, M., Chory, J., 2011. Phytochrome signaling mechanisms and the control of plant development. Trends Cell Biol. 21 (11), 664–671.
Chico, J.M., Fernández-Barbero, G., Chini, A., et al., 2014. Repression of jasmonate-dependent defenses by shade involves differential regulation of protein stability of MYC transcription factors and their JAZ repressors in *Arabidopsis*. Plant Cell 26 (5), 1967–1980.
Courbier, S., Grevink, S., Sluijs, E., et al., 2020. Far-red light promotes Botrytis cinerea disease development in tomato leaves via jasmonate-dependent modulation of soluble sugars. Plant Cell Environ. 43 (11), 2769–2781.
de Wit, M., Spoel, S.H., Sanchez-Perez, G.F., et al., 2013. Perception of low red:far-red ratio compromises both salicylic acid- and jasmonic acid-dependent pathogen defences in Arabidopsis. Plant J. 75 (1), 90–103.
Demkura, P.V., Ballaré, C.L., 2012. UVR8 mediates UV-B-induced Arabidopsis defense responses against Botrytis cinerea by controlling sinapate accumulation. Mol. Plant 5 (3), 642–652.
Ellis, C., Turner, J.G., 2002. A conditionally fertile coi1 allele indicates cross-talk between plant hormone signalling pathways in Arabidopsis thaliana seeds and young seedlings. Planta 215 (4), 549–556.
Fernández-Milmanda, G.L., Crocco, C.D., Reichelt, M., et al., 2020. A light-dependent molecular link between competition cues and defence responses in plants. Native Plants 6 (3), 223–230.
Genoud, T., Buchala, A.J., Chua, N.H., et al., 2002. Phytochrome signalling modulates the SA-perceptive pathway in Arabidopsis. Plant J. 31 (1), 87–95.
Glazebrook, J., 2005. Contrasting mechanisms of defense against biotrophic and necrotrophic pathogens. Annu. Rev. Phytopathol. 43, 205–227.
Guo, Q., Yoshida, Y., Major, I.T., et al., 2018. JAZ repressors of metabolic defense promote growth and reproductive fitness in *Arabidopsis*. Proc. Natl. Acad. Sci. U. S. A. 115 (45), E10768–E10777.
Haga, K., Iino, M., 2004. Phytochrome-mediated transcriptional upregulation of ALLENE OXIDE SYNTHASE in rice seedlings. Plant Cell Physiol. 45 (2), 119–128.
Havko, N.E., Major, I.T., Jewell, J.B., et al., 2016. Control of carbon assimilation and partitioning by jasmonate: an accounting of growth-defense balance. Plants 5 (1), 7.
He, G., Tarui, Y., Iino, M., 2005. A novel receptor kinase involved in jasmonate-mediated wound and phytochrome signaling in maize coleoptiles. Plant Cell Physiol. 46 (6), 870–883.
Izaguirre, M.M., Mazza, C.A., Biondini, M., et al., 2006. Remote sensing of future competitors: impacts on plant defenses. Proc. Natl. Acad. Sci. U. S. A. 103 (18), 7170–7174.
Jiang, Z., Xu, G., Jing, Y., et al., 2016. Phytochrome B and REVEILLE1/2-mediated signalling controls seed dormancy and germination in Arabidopsis. Nat. Commun. 7, 12377.
Kami, C., Lorrain, S., Hornitschek, P., et al., 2010. Light-regulated plant growth and development. Curr. Top. Dev. Biol. 91, 29–66.
Kircher, S., Gil, P., Kozma-Bognár, L., et al., 2002. Nucleocytoplasmic partitioning of the plant photoreceptors phytochrome A, B, C, D, and E is regulated differentially by light and exhibits a diurnal rhythm. Plant Cell 14, 1541–1555.
Leivar, P., Quail, P.H., 2011. PIFs: pivotal components in a cellular signaling hub. Trends Plant Sci. 16 (1), 19–28.
Leivar, P., Monte, E., Cohn, M.M., et al., 2012. Phytochrome signaling in green Arabidopsis seedlings: impact assessment of a mutually negative phyB-PIF feedback loop. Mol. Plant 5 (3), 734–749.
Leone, M., Keller, M.M., Cerrudo, I., et al., 2014. To grow or defend? Low red: far-red ratios reduce jasmonate sensitivity in Arabidopsis seedlings by promoting DELLA degradation and increasing JAZ10 stability. New Phytol. 204 (2), 355–367.
Li, J.Y., Deng, X.G., Chen, L.J., et al., 2015. Involvement of PHYB in resistance to Cucumber mosaic virus in Nicotiana tabacum. Plant Growth Regul. 77, 33–42.
Méndez-Bravo, A., Calderón-Vázquez, C., Ibarra-Laclette, E., et al., 2011. Alkamides activate jasmonic acid biosynthesis and signaling pathways and confer resistance to *Botrytis cinerea* in *Arabidopsis thaliana*. PLoS One 6 (11), e27251.
Mengiste, T., 2012. Plant immunity to necrotrophs. Annu. Rev. Phytopathol. 50, 267–294.
Moreno, J.E., Tao, Y., Chory, J., et al., 2009. Ecological modulation of plant defense via phytochrome control of jasmonate sensitivity. Proc. Natl. Acad. Sci. U. S. A. 106 (12), 4935–4940.
Qi, J., Zhang, M., Lu, C., et al., 2018. Ultraviolet-B enhances the resistance of multiple plant species to lepidopteran insect herbivory through the jasmonic acid pathway. Sci. Rep. 8 (1), 277.
Radhika, V., Kost, C., Mithöfer, A., et al., 2010. Regulation of extrafloral nectar secretion by jasmonates in lima bean is light dependent. Proc. Natl. Acad. Sci. U. S. A. 17228–17233.
Robson, F., Okamoto, H., Patrick, E., et al., 2010. Jasmonate and phytochrome A signaling in Arabidopsis wound and shade responses are integrated through JAZ1 stability. Plant Cell 22 (4), 1143–1160.
Rowe, H.C., Walley, J.W., Corwin, J., et al., 2010. Deficiencies in jasmonate-mediated plant defense reveal quantitative variation in Botrytis cinerea pathogenesis. PLoS Pathog. 6 (4), e1000861.

Sakamoto, K., Nagatani, A., 1996. Nuclear localization activity of phytochrome B. Plant J. 10 (5), 859–868.
Scalschi, L., Sanmartín, M., Camañes, G., et al., 2015. Silencing of OPR3 in tomato reveals the role of OPDA in callose deposition during the activation of defense responses against *Botrytis cinerea*. Plant J. 81 (2), 304–315.
Thines, B., Katsir, L., Melotto, M., et al., 2007. JAZ repressor proteins are targets of the SCF(COI1) complex during jasmonate signalling. Nature 448 (7154), 661–665.
Wang, J., Wu, D., Wang, Y., et al., 2019. Jasmonate action in plant defense against insects. J. Exp. Bot. 70 (13), 3391–3400.
Wu, L., Yang, H.Q., 2010. CRYPTOCHROME 1 is implicated in promoting R protein-mediated plant resistance to Pseudomonas syringae in Arabidopsis. Mol. Plant 3 (3), 539–548.
Xiang, S., Wu, S., Zhang, H., et al., 2020. The PIFs redundantly control plant defense response against Botrytis cinerea in Arabidopsis. Plants 9 (9), E1246.
Xie, D.X., Feys, B.F., James, S., et al., 1998. COI1: an Arabidopsis gene required for jasmonate-regulated defense and fertility. Science 280 (5366), 1091–1094.
Xie, X.Z., Xue, Y.J., Zhou, J.J., et al., 2011. Phytochromes regulate SA and JA signaling pathways in rice and are required for developmentally controlled resistance to Magnaporthe grisea. Mol. Plant 4 (4), 688–696.
Xu, L., Liu, F., Lechner, E., et al., 2002. The SCF(COI1) ubiquitin-ligase complexes are required for jasmonate response in Arabidopsis. Plant Cell 14 (8), 1919–1935.
Yamaguchi, R., Nakamura, M., Mochizuki, N., et al., 1999. Light-dependent translocation of a phytochrome B-GFP fusion protein to the nucleus in transgenic Arabidopsis. J. Cell Biol. 145 (3), 437–445.
Yan, J., Zhang, C., Gu, M., et al., 2009. The Arabidopsis CORONATINE INSENSITIVE1 protein is a jasmonate receptor. Plant Cell 21 (8), 2220–2236.
Yang, D.L., Yao, J., Mei, C.S., et al., 2012. Plant hormone jasmonate prioritizes defense over growth by interfering with gibberellin signaling cascade. Proc. Natl. Acad. Sci. U. S. A. 109 (19), E1192–E1200.
Yi, R., Yan, J., Xie, D., 2020. Light promotes jasmonate biosynthesis to regulate photomorphogenesis in Arabidopsis. Sci. China Life Sci. 63 (7), 943–952.