Jasmonate regulates plant resistance to *Pectobacterium brasiliense* by inducing indole glucosinolate biosynthesis

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*Pectobacterium brasiliense* (*P. brasiliense*) is a necrotrophic bacterium that causes the soft rot disease in *Brassica rapa*. However, the mechanisms underlying plant immune responses against necrotrophic bacterial pathogens with a broad host range are still not well understood. Using a *flg22*-triggered seedling growth inhibition (SGI) assay with *455 Brassica rapa* inbred lines, we selected six *B. rapa* flagellin-insensitive lines (*Brfin*2–7) and three *B. rapa* flagellin-sensitive lines (*Brfs*1–3). *Brfin* lines showed compromised *flg22*-induced immune responses (oxidative burst, mitogen-activated protein kinase (MAPK) activation, and seedling growth inhibition) compared to the control line R-o-18; nevertheless, they were resistant to *P. brasiliense*. To explain this, we analyzed the phytohormone content and found that most *Brfin* lines had higher *P. brasiliense*-induced jasmonic acid (JA) than *Brfs* lines. Moreover, MeJA pretreatment enhanced the resistance of *B. rapa* to *P. brasiliense*. To explain the correlation between the resistance of *Brfin* lines to *P. brasiliense* and activated JA signaling, we analyzed pathogen-induced glucosinolate (GS) content in *B. rapa*. Notably, in *Brfin*7, the neoglucobrassicin (NGBS) content among indole glucosinolates (IGS) was significantly higher than that in *Brfs*2 following *P. brasiliense* inoculation, and genes involved in IGSs biosynthesis were also highly expressed. Furthermore, almost all *Brfin* lines with high JA levels and resistance to *P. brasiliense* had higher *P. brasiliense*-induced NGBS levels than *Brfs* lines. Thus, our results show that activated JA-mediated signaling attenuates *flg22*-triggered immunity but enhances resistance to *P. brasiliense* by inducing indole glucosinolate biosynthesis in *Brassica rapa*. This study provides novel insights into the role of JA-mediated defense against necrotrophic bacterial pathogens within a broad host range.

**KEYWORDS**

*Brassica rapa*, *flg22*-triggered immunity, jasmonic acid, necrotrophic bacteria, bacterial soft rot, indole glucosinolate, neoglucobrassicin, *Pectobacterium brasiliense*
Introduction

Plants have developed complex defense systems against invading pathogens. The first line of plant defense is activated by the perception of pathogen-associated molecular patterns (PAMPs) such as flagellin, lipopolysaccharide, peptidoglycan, and EF-Tu, which are recognized by plant pattern recognition receptors (PRRs) (Abramovitch et al., 2006; Jones and Dangl, 2006; Zipfel, 2014). PAMP-triggered immunity (PTI) leads to a series of host responses, including oxidative burst (Jones and Dangl, 2006; He et al., 2007), stimulation of mitogen-activated protein kinase (MAPK) cascades (Asai et al., 2002), transcriptional reprogramming, and cell wall reinforcement via callose deposition (Nishimura et al., 2003). Plants perceive effectors through resistance (R) proteins and activate a robust and rapid defense response, namely effector-triggered immunity (ETI) (Alfano and Collmer, 1997), which leads to programmed cell death in the local tissue (Dangl et al., 1996). Moreover, plants undergo transcriptional reprogramming upon the expression of defense genes (Caplan et al., 2008; Bhattacharjee et al., 2013).

*P. brasiliense*, which is a necrotrophic bacterial pathogen from the family Pectobacteriaceae (Onkendi et al., 2014), is responsible for several serious pre- and post-harvest diseases of various plant types worldwide (Toth et al., 2003). Resistance to *P. brasiliense*, which has a broad host range, may be explained by PTI rather than by the expression of single resistance genes. The phytopathogenicity of *P. brasiliense* is largely related to its ability to synthesize and secrete plant cell wall-degrading enzymes (PCWDEs), including pectinases, cellulases, and proteases (Pirhonen et al., 1993; Heikinheimo et al., 1995; Mae et al., 1995; Marits et al., 1999). Typical symptoms of *P. brasiliense* infections include maceration and rotting of the leaves and other plant organs (Davidsson et al., 2013). Plant cell wall fragments released by PCWDEs secreted by *P. brasiliense* can act as danger-associated molecular patterns (DAMPs) that are recognized by PRRs to activate PTI in response to an invading pathogen (Mengiste, 2012). However, ETI has not been reported for necrotrophic bacterial pathogens, and host cell death is not expected to restrict necrotrophic pathogen growth (Glazebrook, 2005).

Phytohormones are crucial regulators of plant immune responses. Both ETI and PTI involve signaling pathways associated with common phytohormones, including salicylic acid (SA), jasmonic acid (JA), and ethylene (ET) (Glazebrook, 2005; Tsuda and Katagiri, 2010; Pieterse et al., 2012; Yi et al., 2014). The JA pathway generally protects against necrotrophic pathogens, whereas the SA pathway is associated with plant resistance to biotrophic pathogens (Glazebrook, 2005; Bari and Jones, 2009; Ali et al., 2017). Regarding *Pectobacterium* species, JA/ET- and SA-mediated signaling positively affect defense responses (Palva et al., 1994; Vidal et al., 1997; Norman-Setterblad et al., 2000; Li et al., 2004; Liu et al., 2019; Tsers et al., 2020). Abscisic acid (ABA), an abiotic stress signal, is important for modulating diverse plant-pathogen interactions. Several studies have shown that ABA biosynthesis is required for effective disease resistance against necrotrophic fungal pathogens (Ton and Mauch-Mani, 2004; Adie et al., 2007; Garcia-Andrade et al., 2011).

Chemical β-aminobutyric acid (BABA) enhances *Arabidopsis thaliana* resistance to hemibiotrophic bacteria by priming the salicylic acid (SA) defense response (Zimmerli et al., 2000; Ton et al., 2005). BABA also primes the PTI response upon necrotrophic bacterial *P. brasiliense* infection BABA primed the expression of the PTI-responsive genes FLG22-INDUCED RECEPTOR-LIKE KINASE 1 (FRK1), Arabidopsis Non-Race Specific Disease Resistance Gene (NDR1)/Hairpin-Induced Gene (HIN1)-LIKE 10 (NHL10), and Cytochrome P450, Family 81 (CYP81F2); callose deposition; and boosted Arabidopsis stomatal immunity to *P. brasiliense*. (Po-Wen et al., 2013). Vitamin B6 is a potent antioxidant that helps plants cope with both biotic and abiotic stress conditions (Vanderschuren et al., 2013). VitB6 and its de novo and salvage biosynthetic pathways positively regulate defense responses against *P. brasiliense* by modulating cellular antioxidant capacity (Chandrasekaran and Chun, 2018). Plant ferredoxin-like protein is a ferredoxin-I protein that is involved in the hypersensitive response (HR) and plant immune response to bacterial pathogens (You et al., 2003). A recent report showed that PFLP intensifies disease resistance against bacterial soft rot through the MAPK pathway in PAMP-triggered immunity (Hong et al., 2018).

Glucosinolates (GS), which are unique secondary metabolites found in Brassicaceae species, have long been considered to contribute to plant-microbe interactions (Brader et al., 2001; Halkier and Gershenzon, 2006). Various molecular and genetic studies on Arabidopsis have identified most structural genes and transcription factors involved in GS biosynthesis (Hitomi et al., 2007; Pfalz et al., 2009; Sonderby et al., 2010). Several studies have confirmed that tryptophan pathway genes involved in indole glucosinolate (IGS) biosynthesis are upregulated in *F. oxysporum*-infected plants (Kidd et al., 2011; Zhu et al., 2013), and the virulence of *Colletotrichum gloeosporioides* and *Colletotrichum orbiculare* is restricted by IGL in *B. rapa* (Hiruma et al., 2011). Bacterial pathogen inoculation also triggers upregulation of GS biosynthesis (Liu et al., 2019; Tinte et al., 2020). Furthermore, CYP79 overexpressed in Arabidopsis showed increased GS synthesis and enhanced resistance to *P. brasiliense*, suggesting that altering GS profiles can modulate disease resistance in plants (Brader et al., 2006). Recent studies have shown that MYB34, MYB51, and MYB122 are involved in regulating IGS biosynthesis (Gigolashvili et al., 2007; Hirai et al., 2007; Gigolashvili et al., 2008; Sonderby et al., 2010) and mediate the biosynthesis of plant hormones involved in plant defense (Frigermann and Gigolashvili, 2014). Additionally, Wiesner...
et al. (Wiesner et al., 2013) reported enhanced production of IGS by JA or MeJA treatment in *Brassica rapa*.

*P. brasiiliense* is a necrotrophic bacterium that causes the soft rot disease in *Brassica rapa*. The effect of plant flg22 perception on *P. brasiiliense* interactions remains unclear. Pretreatment of Arabidopsis with flagellin enhances the disease resistance of plants against hemibiotrophic bacterial pathogens (*Pst DC3000*) (Zipfel et al., 2004). However, it remains unclear whether flg22-triggered immunity is correlated with the extent of resistance to necrotrophic bacterial pathogens. The most *Br* fln lines selected through the SGI assay (the lines most insensitive to flg22) were relatively resistant to *P. brasiiliense*. Furthermore, we revealed that pathogen-induced changes in the contents of JA and IGS were critical determinants of plant immunity in *B. rapa* infected with *P. brasiiliense*. Our study demonstrated that flg22-triggered immunity differs between necrotrophic (e.g., *P. brasiiliense*) and hemibiotrophic bacterial pathogens (*Pst DC3000*). Based on these results, the relative resistance of *Br* fln lines and accumulation of high JA against *P. brasiiliense* may be explained by activated JA signaling, which suppresses flg22-triggered responses, whereas the tolerance of *B. rapa* to *P. brasiiliense* is dependent on JA signaling.

**Results**

**Growth inhibition by flg22 in a collection of diverse *B. rapa* inbred lines**

Host plants deploy different defense mechanisms and appropriate immune responses to defend themselves against necrotrophic pathogenic bacteria with a broad host range. Resistance to these pathogens is complex and does not appear to involve one resistance gene alone. *P. brasiiliense* is a flagellated gram-negative bacterium. A potent PAMP, flg22, triggers immune responses in multiple plant species after it is detected by PRR FLAGELLIN-SENSING2 (FLS2). However, it is unknown whether flg22 detection by FLS2 in *B. rapa* leads to enhanced basal immunity (PAMP-triggered immunity, PTI) during *P. brasiiliense* infection. To analyze the effect of flg22 on the interaction between *P. brasiiliense* and *B. rapa*, we conducted a seedling growth inhibition (SGI) assay, one of the most sensitive and convenient assays for activating FLS2 following treatment with flg22. Among the 455 inbred *B. rapa* inbred in our collection, Chifu (http://brassicadb.cn), R-o-18 (Stephenson et al., 2010) and Kenshin (Vanjildorj et al., 2009) have published genome information or functional studies (Figures 1A–C). We selected these three lines and used them to establish a system of SGI testing for *B. rapa*. We examined 455 *B. rapa* inbred lines to determine variations in flg22-induced growth inhibition. In most cases, the addition of flg22 adversely affected seedling growth (Figure 1). The flg22 treatment affected the root, leaf, and cotyledon growth of various *B. rapa* inbred (Figure 1A), resulting in a substantial decrease in fresh weight (Figure 1B). The inhibitory effect depended on the flg22 dose, with ~ 10 µM of flg22 leading to a half-maximal decrease in growth (Figure 1C). This high-throughput assay may be useful for the large-scale quantitative analysis of PTI in *B. rapa*. Of the 455 Chinese cabbage inbred lines, 280 lines, representing more than 60%, had flg22-induced SGI rates of 30–40%, as opposed to the untreated control plants (Figure 1D). *B. rapa* genotype R-o-18 is rapidly cycling and self-compatible. The flg22-triggered SGI rate of R-o-18 was also ~ 30–40% (Figure 1B); hence, this line was set as the control line for the SGI assay system and used for comparison when selecting a line with an altered flg22-triggered SGI rate. Notably, seven *B. rapa* accessions were highly insensitive to flg22, with an SGI rate of less than 10% (Figure 1D). These seven lines were named *B. rapa* flagellin-insensitive (*Br*fln1-*Br*fln7). In contrast, three *B. rapa* accessions were highly sensitive to flg22, with an SGI rate of > 80% (Figure 1D). These lines were named *B. rapa* flagellin-sensitive (*Br*fls1-*Br*fls3). Figure 2A shows the repetitive significantly altered flg22-triggered growth inhibition rates of nine *B. rapa* inbred lines (three *Br* and six *Br*fln lines) among the lines selected in the first SGI assay. However, the flg22 insensitivity in *Br*fln1 was not reproduced in the second test, and the SGI rate was similar to that of the control line R-o-18.

**Flg22-insensitive lines exhibited increased resistance to *P. brasiiliense***

To investigate whether flg22 sensitivity is correlated with plant resistance to *P. brasiiliense*, the responses of *Br*fs and *Br*fln lines to inoculation with *P. brasiiliense* (KACC 10225) were evaluated (Figure 2B). To assess the severity of the disease in *Br*fs and *Br*fln seedlings infected with *P. brasiiliense*, macerated leaf lesions were scored according to a modified version of a previously described method (Lee et al., 2020) (see Materials and Methods). The disease index of R-o-18 inoculated with *P. brasiiliense* was 2.3 (Figures 2A, B). Unexpectedly, six *Br*fln lines, which were most insensitive to flg22, had a disease index of 0.7–1.9. Among them, five *Br*fln lines tested in this experiment were significantly more resistant to *P. brasiiliense* than to R-o-18 (Figures 2A, B). Additionally, the disease index of the three *Br*fs lines for *P. brasiiliense* inoculation was > 3.5. Flg22 sensitive lines were significantly more susceptible to *P. brasiiliense* than *Br*fln lines (Figures 2A, B). In the case of the interaction between *Pst DC3000* and Arabidopsis, it has been reported that flg22 perception induces PTI, and the level of flg22-response sensitivity is associated with the enhancement of basal resistance (Trujillo et al., 2008). However, our results revealed that during *P. brasiiliense* and Chinese cabbage interaction, sensitivity to flg22 was not related to the improvement of the basal resistance of plants.
Comparison of phytohormone contents among selected lines (Brfs, Brfin lines, and R-o-18)

To help identify whether activated plant hormone signaling pathways contribute to the enhanced resistance of the Brfin lines to P. brasiliense (Figure 2C and Supplementary Figure S1), we analyzed the levels of plant hormones in Brfs, Brfin lines, and R-o-18. The ABA, SA, and JA contents were measured in B. rapa plants before and 24 h after P. brasiliense inoculation via LC-MS/MS analysis (Figure 2C and Supplementary Figure S1). The P. brasiliense-induced JA levels in the Brfin lines were ~ 140%–250% of those in R-o-18 (Figure 2C). ABA levels in Brfin lines (except Brfin3) were also higher than those in Brfs lines following P. brasiliense inoculation (Supplementary Figure S1). The relatively high pathogen-induced JA and ABA levels in most Brfin lines may explain the enhanced resistance of these lines to P. brasiliense. However, the P. brasiliense-induced SA levels in both the Brfs and Brfin lines were less than 50% of that of R-o-18, except for Brfin2 (Supplementary Figure S1). Additionally, we analyzed the basal levels of endogenous phytohormones before P. brasiliense inoculation. The basal levels of SA and JA were similar among Brfs, Brfin, and R-o-18, whereas the ABA level was more than 6-times higher in R-o-18 than in both Brfs and Brfin lines, except Brfin2 (Figure 2C and Supplementary Figure S1). These results suggest that endogenous basal levels of phytohormones (SA, JA, and ABA) may not affect plant basal resistance to P. brasiliense.

Suppressed PTI responses in Brfin6 and Brfin7

Brfs1 and Brfs2 were the most sensitive lines in the flg22-induced SGI assay of 455 B. rapa inbred lines, whereas Brfin6 and Brfin7 were the most insensitive lines. As R-o-18 was moderately sensitive to flg22 (Figure 1B), it was selected as the control line in the subsequent flg22 response assays. Brfin6, Brfin7, Brfin1, and Brfin2 plants were obtained from the doubled haploid (DH) production system, a protocol used to generate homozygous B. rapa plants by culturing microspores isolated from the young flower buds of each F1 plant (Broughton et al., 2014). We further analyzed whether these four selected Brfs and
Brfin lines increased or inhibited other flg22-induced responses compared to R-o-18. Since MAPK phosphorylation and ROS production are rapid and transient responses associated with two parallel flg22 signaling pathways, we compared these activities among the selected Brfs and Brfin lines and R-o-18.

We measured the flg22-triggered oxidative burst in selected B. rapa accessions using an L-012-based chemiluminescence detection system (Yi et al., 2014). Our assays revealed a substantial decrease in flg22-induced ROS production in Brfin 6 and Brfin7 (Figure 3A). For the Brfs1 and Brfs2 lines, we

**FIGURE 2**
Phenotypes of Brfs and Brfin lines during flg22- or P. brasiliense- triggered signaling. (A) Effects of flg22 on the growth of Brfs and Brfin lines. Six-day-old seedlings Brfs, Brfin lines and R-o-18 were incubated for an additional 6 days in plates containing Murashige and Skoog (MS) agar medium with or without 10 µM flg22. (B) Relatively strong resistance of Brfin lines to the soft rot pathogen P. brasiliense. 24-day-old B. rapa plants were inoculated with P. brasiliense KACC 10225 by drenching the proximal plant parts with a bacterial suspension (1 × 10⁶ CFU/mL). At 7 days post-inoculation, disease severity was evaluated on a 0–4 scale and then converted to a percentage. Error bars represent standard deviations of 30 replications. Similar results were obtained in at least two independent experiments. Different letters indicate significant differences among plant genotypes (α = 0.01, one-way ANOVA and Duncan test; SPSS software). (C) Endogenous and P. brasiliense-induced phytohormone contents in B. rapa inbred lines. The JA contents in the control and infected leaves were quantified at 24 h post-infection. Error bars represent standard deviations of five replications. Similar results were obtained in at least two independent experiments. Different letters indicate significant differences among plant genotypes (α = 0.05, one-way ANOVA and Duncan test; SPSS software).
expected a high level of flg22-triggered oxidative burst, but similar to that of R-o-18 (Figure 3A). To analyze flg22-induced MAPK activation, *B. rapa* seedlings were treated with flg22 or water and analyzed by immunoblotting with total protein extracts and an anti-phospho-p44/p42 antibody that specifically recognizes the phosphorylated forms of MPK3 and MPK6 (Flury et al., 2013). We expected robust flg22-induced MPK3 and MPK6 activation in Brfs1 and Brfs2 lines, while no clear change in flg22-induced MAPK activation was observed in Brfs lines compared to R-o-18 (Figure 3B). However, activation of MPK3 and MPK6 was significantly suppressed in Brfin6 and Brfin7 (Figure 3B). Hypersensitivity of Brfs lines to flg22 was observed only in SGI, but Brfin lines showed consistent characteristics in almost all tested flg22 responses. The Brfin6 and Brfin7 lines were insensitive to flg22.

Expression analysis of PAMP-induced genes (PIGs) in Brfs and Brfin lines following flg22 treatment

Since MAPK activation is linked to PAMP-induced transcriptional reprogramming (Zipfel et al., 2004; Füll et al., 2009), we analyzed PAMP-induced gene (PIGs) expression in *B. rapa* seedlings using PTI marker genes. We selected three marker genes, *WRKY33*, *WRKY40*, and *NHL10*, because their...

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**FIGURE 3**
Phenotypes of Brfs and Brfin lines during flg22-triggered signaling. (A) Flg22-induced ROS generation in liquid-grown intact seedlings of *B. rapa* inbred lines treated with 1 µM flg22. Error bars represent standard deviations of 24 independent samples. Similar results were obtained in two independent experiments. (B) Dual phosphorylation of the TEY motif in MPK3 and MPK6 in leaf discs. Phosphorylated MAPKs corresponding to MPK3 and MPK6 are indicated. Activated MAPKs were detected by immunoblotting using an antibody against Phospho-p44/42 MAPK (Erk1/2) (Cell Signaling Technology). The experiment was performed three times with similar results. Before transferring proteins to the PVDF membrane, equal protein loading was confirmed by comparing the fluorescence intensity of Rubisco in stain-free gels. (C) Transcript levels of PAMP-induced genes in R-o-18, Brfs, and Brfin seedlings. Twelve-day-old *B. rapa* seedlings were treated with 1 µM flg22 for 60 min, after which *BrWRKY33*, *BrWRKY40*, and *BrNHL10* transcript levels were determined using qRT-PCR. Gene transcript levels were normalized to ACT2 transcript levels. Error bars represent the standard deviation of three replicates. Similar results were obtained in at least two independent experiments. Different letters indicate significant differences among plant genotypes (α = 0.05, one-way ANOVA and Duncan test; SPSS software).
Expression analysis of pathogen-induced genes in Brfs and Brfin lines following P. brasiliense inoculation

To analyze how altered flg22 responses affect the defense signaling of Brfs or Brfin lines against P. brasiliense, we first compared the expression patterns of several defense marker genes in selected B. rapa. Brfs2, a line sensitive to flg22 and having the lowest P. brasiliense-induced JA accumulation level, and Brfin7, a line insensitive to flg22 and having high JA production, were selected to compare the levels of P. brasiliense-induced marker gene expression (Figure 4). We used Arabidopsis JAZ5 and JAZ10, which are JA- or pathogen-responsive genes (Chung et al., 2008; Demianski et al., 2012; Valenzuela et al., 2016), as JA signaling markers. In the expression analysis of BrJAZ5 and BrJAZ10 (Figure 4), we observed differences in the expression levels of R-o-18, Brfs2, and Brfin7 before P. brasiliense infection. Interestingly, in the case of Brfin7, the expression levels of BrJAZ5 and BrJAZ10 were significantly higher than those in the other two lines before P. brasiliense infection. In particular, the steady-state expression level of BrJAZ5 in the Brfin7 line before inoculation was prolonged to 24 h after P. brasiliense infection. Furthermore, the Brfs2 line had the lowest P. brasiliense-induced BrJAZ5/BrJAZ10 expression level among the three tested lines (Figure 4), which was consistent with the P. brasiliense-induced JA level (Figure 2C). Arabidopsis WRKY33 is a key positive regulator of resistance to the necrotrophic fungi Alternaria brassicicola and Botrytis cinerea (Zheng et al., 2006; Birkenbihl et al., 2012). Arabidopsis WRKY18 and WRKY40 act redundantly to negatively regulate resistance to the hemibiotrophic pathogen Pseudomonas syringae but positively regulate resistance to B. cinerea (Xu et al., 2006). In Arabidopsis, NHL10 is abundantly expressed in senescing leaves and during the hypersensitive response caused by exposure to an avirulent cucumber mosaic virus (Zheng et al., 2004). Next, we compared the changes in the expression of BrWRKY33, BrWRKY18, BrWRKY40, and BrNHL10 among R-o-18, Brfs2, and Brfin7 plants before and after P. brasiliense inoculation. P. brasiliense inoculation significantly suppressed the induced expression of BrWRKY33, BrWRKY18, BrWRKY40, and BrNHL10 in the P. brasiliense susceptible plant, Brfs2, compared to Brfin7 (Figure 4). In response to P. brasiliense, the expression levels of BrWRKY18 and BrWRKY40 were greater in the resistant plant Brfin7 than in R-o-18 at 24 h post-inoculation. These results indicate that the JA signaling pathway plays a crucial role in plant defense against P. brasiliense.

Exogenous JA suppressed the disease development of B. rapa infected with P. brasiliense

Previous results (Figures 2, 5) showed that the P. brasiliense-induced JA level positively affected defense-related gene expression. Line Brfin7 exhibited suppressed PTI. However, their P. brasiliense-induced defense-related gene expression levels and JA content were higher than that of R-o-18. In contrast, the flg22 sensitive line, Brfs2, had significantly lower P. brasiliense-induced defense-related gene expression levels and JA accumulation than Brfin7. Therefore, we speculated that the resistance of the Brfin lines to P. brasiliense was associated with considerable accumulation of JA. We investigated whether the application of exogenous JA could decrease the susceptibility of Brfs2 to P. brasiliense. An earlier investigation demonstrated that the defense response of calla lily to P. brasiliense involves the JA/Et signaling pathway (Luzzatto et al., 2007). To evaluate the disease severity of JA-pretreated B. rapa inoculated with P. brasiliense, leaf lesions were scored according to a modified version of a previously reported method (Luzzatto et al., 2007). Leaf discs were inoculated with P. brasiliense 24 h after treatment with MeJA or water. The necrotic area and infection rate were recorded 24 h later (48 h after hormone pretreatment). Application of 1 mM MeJA suppressed disease development (Figure 5). Specifically, MeJA pretreatment significantly decreased the soft rot disease development in Brfs2 from 100% to 30% (Figure 5). Moreover, the disease symptoms on Brfs2 leaves pretreated with MeJA were less severe than those on the leaves of Brfin7, which were resistant to P. brasiliense. These results suggest that activated JA signaling positively affects the resistance to P. brasiliense in B. rapa.

Brfin7 had higher P. brasiliense-induced neoglucobrassicin levels than Brfs2

JA is a plant hormone involved in chemical and physiological defense responses. Although JA does not directly affect plant-pathogen interactions, it contributes to an intracellular signaling cascade that induces the production of secondary metabolites that
Transcript levels of pathogen-induced genes in R-o-18, Brfs2, and Brfin7 plants. Four-week-old B. rapa plants were inoculated with P. brasiiliense and sampled at 24 h post-inoculation. The BrJAZ25, BrJAZ10, BrWRKY18, BrWRKY40, BrNHL10 and BrWRKY33 transcript levels were determined using qRT-PCR. Gene transcript levels were normalized to the BrACT2 transcript levels. Error bars represent the standard deviation of three replicates. Similar results were obtained in at least two independent experiments. Different letters indicate significant differences among plant genotypes ($\alpha = 0.01$, one-way ANOVA and Duncan test; SPSS software).
are important for plant defenses (Campos et al., 2014). In this study, activated JA signaling was revealed to be important for restricting the infection of *B. rapa* by *P. brasiliense* (Figures 5, 6). As JA is an elicitor that stimulates IGL biosynthesis (Ku et al., 2014), we analyzed *B. rapa* glucosinolate contents to elucidate the effect of JA accumulation on defense signaling in response to *P. brasiliense*. Individual glucosinolates were analyzed in the R-o-18, Brfs2, and Brfin7 lines before and after *P. brasiliense* inoculation (Figure 6A). As expected, *P. brasiliense* inoculation significantly influenced the IGL contents (Figure 6A), and the glucobrassicin (GBS) and neoglucobrassicin (NGBS) contents increased over 2-fold after R-o-18 was inoculated with *P. brasiliense*. However, there were no pathogen-related changes in the content of either aliphatic (progoitrin, sinigrin, and gluconapoleiferin) or aromatic (gluconasturtiin) glucosinolates (Figure 6A). Interestingly, the Brfin7 line with a high JA biosynthesis level exhibited *P. brasiliense*-induced NGBS accumulation twice as high as that of R-o-18, whereas in Brfs2 with a low pathogen-induced JA accumulation level, *P. brasiliense* inoculation did not affect the NGBS level. Our results (Figures 5-7) suggested that *P. brasiliense*...
resistance observed in Brfin lines was strongly correlated with activated JA signaling. We wondered whether other Brfin lines that produce relatively high amounts of pathogen-induced JA also have relatively high \textit{P. brasiliense}-induced NGBS levels, similar to Brfin7. Thus, we compared NGBS content in three Brfs lines, six Brfin lines, and R-o-18 24 h after \textit{P. brasiliense} inoculation (Figure 6B). Almost all Brfin lines (except Brfin5) accumulated significantly higher \textit{P. brasiliense}-induced NGBS than the R-o-18 line. In contrast, all Brfs lines showed significantly lower \textit{P. brasiliense}-induced NGBS accumulation compared to R-o-18. In the Brfs lines, NGBS levels were almost unaffected by \textit{P. brasiliense} inoculation.

\textbf{Brfin7 accumulates more pathogen-induced glucosinolate biosynthesis genes than Brfs2}

The upregulated expression of glucosinolate biosynthesis genes is associated with an increased abundance of individual glucosinolates in plants (Robin et al., 2016; Yi et al., 2016; Robin et al., 2017a; Robin et al., 2017b). Thus, we analyzed the correlations among the IGL profiles of the R-o-18, Brfs2, and Brfin7 lines and the expression of IGL biosynthesis genes following infection with \textit{P. brasiliense}. MYB transcription factor MYB51 regulates IGL biosynthesis (Celenza et al., 2005; Gigolashvili et al., 2008; Malitsky et al., 2008). \textit{Arabidopsis} SULFOTRANSFERASE 5A (ST5a) encodes a desulfoglucosinolate sulfotransferase that is involved in the final step of glucosinolate core structure biosynthesis (Klein et al., 2006). In \textit{Arabidopsis}, CYP81F4 catalyzes the conversion of I3M to 1OH-I3M, which in turn is converted to 1MO-I3M (NGBS) by indole glucosinolate methyltransferase 1 (IGMT1) or IGMT2 (Pfalz et al., 2009; Pfalz et al., 2011). \textit{P. brasiliense}-induced gene expression in R-o-18, Brfs2, and Brfin7 leaf tissues were analyzed at 0, 6, and 24 h after infection. Since a previous report demonstrated that the most critical defense regulation period against \textit{P. brasiliense} in Chinese cabbage was from 6–12 h after infection (Liu et al., 2019), we included an early time point, 6 h after infection. Expression of IGL biosynthesis genes was detected at different time points after \textit{P. brasiliense} infection. The
results showed that BrMYB51, BrST5a, and BrIGMT1 expression was strongly induced by P. brasilienise infection in R-o-18 at 6 h after infection and then decreased at 24 h after infection. In contrast, BrCYP81F4 was strongly induced in R-o-18 at 24 h post-inoculation. For BrMYB51 and BrIGMT1, a distinct difference in P. brasilienise-induced expression was observed between R-o-18, Brfs2, and Brfin7 6 h after P. brasilienise inoculation; whereas in the case of BrCYP81F4, features between lines were observed 24 h after P. brasilienise inoculation. Taken together, expression analysis revealed that the upregulation of key biosynthetic genes of IGL (BrMYB51, BrCYP81F4, and BrIGMT1) was significantly compromised in Brfs2 compared with that in R-o-18 after inoculation with P. brasilienise (Figure 7). Meanwhile, the P. brasilienise response expression of IGL biosynthesis genes in Brfin7 was significantly higher than that in R-o-18. These results suggested that higher IGL biosynthesis gene expression levels may be related to a more significant accumulation of NGBS in Brfin7 than in Brfs2.

Discussion
Genetic requirements for individual PAMP responses may differ among B. rapa inbred lines

P. brasilienise causes the destructive soft rot disease in many economically important vegetables, including B. rapa. However, little is known about the mechanism underlying this molecular battle between plant immunity and P. brasilienise virulence. In this study, we analyzed whether flg22-induced immunity affects the development of the soft rot disease following infection with P. brasilienise. Flagellin perception restricts bacterial infection and contributes to plant disease resistance. Arabidopsis ecotypeWs-0 rapidly develops severe disease symptoms after being sprayed with Pst DC3000 because of a natural deficiency in flagellin perception (Zipfel et al., 2004). Under natural conditions, the hemibiotrophic bacterial pathogen Pst DC3000
enters host plants through wounds or natural openings (e.g., stomata) and then multiplies, resulting in high population densities in intercellular spaces (Beattie and Lindow, 1995). In contrast, *P. brasilense* is an aggressive necrotrophic bacterium that produces PCWDEs as its primary virulence determinant. Therefore, it is necessary to analyze whether flg22-triggered immunity positively affects the resistance of plants to *P. brasilense*, as in the *Ps* DC3000 and Arabidopsis interaction.

Chinese cabbage (*Brassica rapa* subsp. *pekinesis*) is the most widely grown vegetable crop in Asia. Therefore, there is a substantial abundance of genetic and genomic resources available for the improvement of Brassica crops. Furthermore, fundamental research on Arabidopsis may apply to *B. rapa* because both species belong to the family Brassicaceae. Previous research revealed – 80% amino acid sequence identity and 90% amino acid sequence similarity between the *Brassica* and Arabidopsis FLS2 LRR domains as well as the functionality of the LRR domains of *Brassica* FLS2 homologs in Arabidopsis (Dunning et al., 2007). This high degree of conservation is indicative of the importance of this receptor for *B. rapa* defense against pathogens (Kim et al., 2020). To clarify the defense-related signaling in *B. rapa* induced by *P. brasiliense*, we conducted a forward genetics screening to isolate *B. rapa* inbred lines exhibiting impaired flg22-induced SGI (Figure 1). Of the 455 *B. rapa* lines screened, three flagellin-sensitive (*Br*fs) lines and six flagellin-insensitive (*Brfin*) lines had reproducibly significant alterations in their flg22-induced responses compared to R-o-18 (Figure 2A).

We focused on *Brfin6* and *Brfin7*, in which almost all of the examined flg22-triggered responses were severely suppressed, with the exception of PIG expression (Figure 3C). Flg22-induced rapid activation of MAPK cascades is one of the critical components that regulate transcriptional changes in elicited cells. For example, in Arabidopsis protoplasts, MPK6 and MPK3 are phosphorylated upon flg22 treatment and activate WRKY transcription factors (TFs) (Nuhse et al., 2000; Asai et al., 2002). Fifteen WRKY TF genes were strongly induced 30 min after flg22 treatment in Arabidopsis seedlings including WRKY18, WRKY33, and WRKY40 (Zipfel et al., 2004). In the current study, we also observed strong expression of WRKY33 and WRKY40 together with MAPK activation, following flg22 elicitation in R-o-18 (Figure 3). Interestingly, there was no correlation between the level of MAPK activation and the transcript levels of WRKY TF genes in *Brfs* and *Brfin* lines (Figure 3C). These results suggest that to regulate PIGs induction, another signaling component is required in addition to the phosphorylation of MPK3/MPK6 in *B. rapa*.

We also analyzed the early flg22 responses of *Brfs1* and *Brfs2*, which were selected as the lines most sensitive to flg22 in the SGI assay, along with the *Brfin* lines. Interestingly, the level of flg22-triggered oxidative burst and phosphorylation of MAPKs was similar to that of R-o-18, whereas the expression level of PIGs was significantly suppressed compared to that of R-o-18 (Figure 3). Previous reports have suggested that genetic requirements vary among individual PAMP responses. For example, ethylene sensing is required for flg22-induced ROS production and callose deposition but not for flg22-triggered MAP kinase activation, seedling growth arrest, and induced resistance (Zipfel et al., 2004; Adams-Phillips et al., 2008; Clay et al., 2009; Mersmann et al., 2010). Therefore, although *Brfin6* and *Brfin7* were minimally responsive to flg22, their PIGs expression levels were relatively high. In an earlier investigation involving Arabidopsis, flg22-induced callose deposition was undetectable in cyp81F2-1 and cyp81F2-2 mutants, in which the PIG CYP81F2 had been mutated (Clay et al., 2009). In the current study, compared with R-o-18, flg22-induced *BrCYP81F2* expression was lower and callose deposition was suppressed in *Brfin7* (Supplementary Figure S2). In contrast, we observed strong flg22-induced *BrCYP81F2* expression and callose deposition in *Brfin6* cells (Supplementary Figure S2). Hence, some of the examined flg22-induced responses varied between *Brfin6* and *Brfin7*, implying that the genetic requirements for individual PAMP responses differed between the two *Brfin* lines.

**Relationship between phytohormones and early flg22 responses in *B. rapa***

Previous studies have indicated that PAMP-induced responses are independent of phytohormone signaling (Zipfel et al., 2004; Ferrari et al., 2007). However, the oxidative burst is reportedly diminished in ethylene-insensitive mutants (Mersmann et al., 2010). Our previous report also showed that a clear increase in ROS production was detected in *fad7/fad8*, *coi1*, and *jar1* mutants, which have impaired JA biosynthesis and signaling (Yi et al., 2014). Overall, ET signaling had a positive effect on flg22-triggered oxidative burst, but JA signaling tended to inhibit ROS production. Additionally, PAMPs have been reported to stimulate JA and ethylene (ET) production (Doares et al., 1995; Simpson et al., 1998; Kunze et al., 2004), as well as upregulate genes encoding proteins involved in the biosynthesis of JA and ET (Moscatiello et al., 2006) or pathogenesis-related proteins linked to SA-mediated responses (Gomez-Gomez et al., 1999). In the present study, we analyzed the levels of specific phytohormones (ABA, SA, and JA) in *B. rapa* before and after *P. brasiliense* inoculation and revealed that the JA level was clearly higher in the *Brfin* lines than in *Brfs* lines (Figure 2C). Although the endogenous basal level of JA in the *Brfin* lines was not higher than that in the *Brfs* lines, five *Brfin* lines had high *P. brasiliense*-induced JA levels, which may be involved in the fast and strong activation of the JA-dependent signaling pathway in *Brfin* lines (Figure 2C). These results may correlate with the highly suppressed flg22-triggered oxidative
burst in Brtn6 and Brtn7 lines, which accumulate relatively high JA (Figure 3A). Consistent with our results, a study by Denoux et al. (Denoux et al., 2008) showed that treatment with flg22 triggers a fast response in the early stages of multiple defense signaling pathways mediated by SA, JA, and ET. Early responses are associated with JA, and late responses are mediated mainly by SA. In addition, ET biosynthesis has been detected in flg22-treated Arabidopsis (Denoux et al., 2008). These results suggest that in addition to defense responses that are activated independently of defense hormone signaling, activated plant hormone signaling may also stimulate flg22 responses.

In Arabidopsis, the NADPH oxidase responsible for the PAMP-triggered oxidative burst is the plasma membrane-localized RBOHD (Nuhse et al., 2007; Zhang et al., 2007). The Arabidopsis rbohD mutant shows impaired PAMP-induced ROS burst and stomatal closure (Macho et al., 2012; Marino et al., 2012). The extent of flg22-induced activation of MPK3, MPK4, and MPK6 was similar to that in the rbohD mutant, suggesting that the ROS burst is not required for MAPK activation (Zhang et al., 2007; Xu et al., 2014). Furthermore, another study confirmed that flg22-induced MPK3/MPK6 activation is similar between wild-type plants and dde2 ein2 pad4 sid2 quadruple mutants, implying that MAPK activation occurs independently of the SA, JA, and ET signaling pathways (Tsuda et al., 2008). However, Mine et al. (Mine et al., 2017) reported that ABA and JA mediate inactivation of the immune-associated MAP kinases, MPK3 and MPK6, in Arabidopsis italic. In our study, Brtn lines had relatively high P. brasilienne-induced JA or ABA (Figure 2C and Supplementary Figure S1) compared to R-o-18, and they represented suppressed flg22-induced MAPK activation compared to R-o-18 (Supplementary Figure S3). These results are consistent with those described in a published report (Mine et al., 2017) and may help explain the traits in the Brtn lines related to suppressed flg22 responses (Supplementary Figure S3 and Figure 3B).

Relationships between activated JA signaling and defense-related gene expression by P. brasilienne

In Arabidopsis, although WRKY33, WRKY40, and NHL10 are early flg22 response genes (Navarro et al., 2004; Zipfel et al., 2004), several studies have shown that pathogens also induce the expression of these genes (Zheng et al., 2004; Xu et al., 2006; Zheng et al., 2006; Birkenbihl et al., 2012). WRKY40 and WRKY33 transcription factors modulate the SA and JA pathways and function as activators of JA-dependent defense pathways and repressors of SA signaling (Xu et al., 2006; Zheng et al., 2006). Arabidopsis WRKY33 is a key transcriptional regulator of hormonal and metabolic activities that protect plants from B. cinerea strain 2100 (Birkenbihl et al., 2012). During this plant-pathogen interaction, WRKY33 positively regulates the expression of target genes involved in camalexin biosynthesis as well as JA/ET-related downstream signaling, while negatively regulating ABA-dependent signaling (Liu et al., 2015). As P. brasilienne is also a necrotrophic pathogen like B. cinerea, we predicted that the WRKY33 and WRKY40 genes have similar expression patterns in response to P. brasilienne. However, following P. brasilienne inoculation, BrWRKY33 expression levels were lower in the P. brasilienne-resistant line Brtn7 than in R-o-18, whereas BrWRKY40 was more highly expressed in the Brtn7 line than in R-o-18 (Figure 4). Therefore, the lower P. brasilienne-induced BrWRKY33 expression levels in Brtn7 than in R-o-18 imply that although both B. cinerea and P. brasilienne are necrotrophic pathogens, their infection of host plants involves different hormonal and metabolic processes. A previous report also revealed that different B. cinerea strains employ diverse strategies to invade and colonize plants (Derckel et al., 1999; Kleibeusten et al., 2005). During infection with B. cinerea strain B05.10, WRKY33-mediated host defenses are suppressed by the pathogen. Ectopic expression of WRKY33 leads to elevated ABA levels and results in plants that are completely resistant to B. cinerea strain B05.10 (Li et al., 2017).

Relationships between activated JA signaling and defense response to P. brasilienne

JA signaling mediates plant defenses against necrotrophic pathogens, including bacteria (e.g., Pectobacterium atrosepticum), fungi (e.g., A. brassicicola, B. cinerea, Pectosphaerella cucumerina, and Fusarium oxysporum), and oomycetes (e.g., Pythium spp.) (Campos et al., 2014; Yan and Xie, 2015). The P. brasilienne-induced JA content in Brtn lines was relatively higher than that in R-o-18 (Figure 2C). Accordingly, we hypothesized that changes in the regulation of phytohormones might affect the development of necrotrophic bacterial diseases in Brtn lines. Brtn6 and Brtn7 were relatively resistant to P. brasilienne (Figure 2). This result suggests that relatively high P. brasilienne-induced JA levels may contribute to reduced necrotic symptom development (Figure 2). Furthermore, MeJA pretreatment suppressed the development of necrotrophic symptoms by P. brasilienne, and these results support our hypothesis (Figure 5). The P. brasilienne susceptible line R-o-18 showed that more than 80% of the tested leaf discs were necrotic. However, MeJA pretreatment decreased the number of necrotic leaf discs from more than 80% to 50%, reflecting the inhibitory effect of MeJA on the development of the soft rot disease (Figure 5). Furthermore, MeJA pretreatment effectively inhibited necrosis development in the P. brasilienne hyper-susceptible line, Brbs2, and as a result, represented stronger resistance to P. brasilienne than Brtn7, the
resistant line (Figure 5). In other published reports, exogenous JA and MeJA treatments have been used to induce plant responses, resulting in pathogen resistance. For example, an investigation of the interaction between Arabidopsis and *P. brasilien*se revealed that MeJA stimulates IGL accumulation (Brader et al., 2001). Specifically, experiments involving plant hormone signaling-deficient mutants have indicated that JA, but not SA or ET, mediates pathogen-induced IGL biosynthesis. *P. brasilien*se-induced IGL accumulation was not observed in the JA-insensitive mutant *coi1-1*. However, similar to wild-type plants, *NahG* and ethylene-insensitive *ein2-1* mutant plants reportedly lack changes in IGL levels and MeJA- or elicitor-induced IGL accumulation (Brader et al., 2001). In this study, inoculation of R-o-18 plants with *P. brasilien*se increased GBS content by more than 6-fold. Interestingly, the *Brfn* lines with high *P. brasilien*se-induced JA levels had GBS contents that were half that of plants before inoculation. However, *P. brasilien*se-induced NGBS levels were almost 2-fold higher in *Brfn* lines than in R-o-18. These results suggest that NGBS may be more important than other *P. brasilien*se-inducible IGLs for the *P. brasilien*se resistance-related signaling pathway in *B. rapa* (Figure 6). We also analyzed the correlation between *P. brasilien*se-induced NGBS accumulation and IGL biosynthesis gene expression patterns. In response to *P. brasilien*se inoculation, all examined genes (*BrMYB51, BrST5a, BrCYP81F4, and BrIGMT1*) were more highly expressed in *Brfn7* than in R-o-18 (Figure 7). MYB transcription factors MYB34, MYB51, and MYB122 regulate IGL biosynthesis. Dominant mutants or lines overexpressing the genes encoding these transcription factors have increased IGL content and upregulated IGL biosynthesis gene expression levels, whereas the corresponding loss-of-function mutants have decreased IGL content and downregulated IGL biosynthesis gene expression levels (Celenza et al., 2005; Gigolashvili et al., 2008; Malitsky et al., 2008). In this study, we analyzed the *P. brasilien*se-induced expression pattern of *BrMYB51* and found that the expression level was significantly higher than that of R-o-18 at either 6 or 24 h of inoculation. In Arabidopsis, CYP81F4 belongs to a small cytochrome P450 monooxygenase family, which also includes CYP81F1, CYP81F2, and CYP81F3. CYP81F4 catalyzes the conversion of I3M to 1OH-I3M, which in turn is converted to 1MO-I3M (NGBS) by either indole glucosinolate methyltransferase 1 (IGMT1) or IGMT2 (Pfalz et al., 2009; Pfalz et al., 2011). Our expression analysis showed that the *P. brasilien*se-induced expression levels of *BrIGMT1*, a closely related gene involved in NGBS biosynthesis, were higher in *Brfn* than in R-o-18 6 h after infection (Figure 7). In the case of *BrCYP81F4*, the *P. brasilien*se response expression pattern differed from that of the other tested genes. In the control line R-o-18, the *P. brasilien*se-induced expression level was strongly increased after 24 h instead of 6 h, and the expression level of *BrCYP81F4* in *Brfn7* was significantly higher than R-o-18 in this time. Taken together, these results indicate that the *P. brasilien*se-induced expression levels of key biosynthetic genes of IGLs, which are closely related genes involved in NGBS biosynthesis, were higher in the *Brfn* lines than in R-o-18, which might correlate with high NGBS accumulation following *P. brasilien*se infection in *Brfn7* (Figure 7). The *P. brasilien*se hyper-susceptible line Brfs2 had the lowest *P. brasilien*se-induced JA and NGBS levels among the tested plant lines (Figures 2C, 7B). In addition, the induced expression of IGL biosynthesis genes after *P. brasilien*se inoculation of Brfs2 was very low, within 20% of that of *Brfn7* (Figure 7). As expected, *P. brasilien*se-induced NGBS accumulation in Brfs2 was very low, unaffected by *P. brasilien*se infection, or decreased slightly (Figure 6). These findings suggest a correlation between JA-mediated NGBS accumulation and *P. brasilien*se resistance in *B. rapa*.

### Materials and methods

#### Plant growth

*B. rapa* plants were grown in soil in a growth chamber at 23°C with a 16-h light/8-h dark photoperiod (i.e., long-day conditions) for 4 weeks. The third leaf from each plant was used for pathogen inoculation tests. To grow *B. rapa* seedlings on Murashige and Skoog (MS) medium, the seeds were surface-sterilized in 25% NaOCl solution for 2 min, washed thrice in sterile water, and sown on MS agar medium containing 3% sucrose. Seedlings were grown in a growth chamber at 23°C, 60% relative humidity, and long-day conditions under white light (140 μmol m⁻² s⁻¹).

#### Growth assay

Seedlings grown for five days on MS agar medium in plates were transferred to liquid MS medium supplemented with 10 μM flg22 (three seedlings per 6 mL medium in the wells of 6-well plates) or distilled water. The seedlings were photographed and weighed (i.e., fresh weight) after six days to determine the effect of flg22 treatment on seedling growth.

#### Pathogen infection assays

Because the taxonomy of *Pectobacterium* Genus updated (Portier et al., 2020) we confirmed the taxonomic status of KACC10225 (http://genebank.rda.go.kr/eng/uat/uaia/actionMain.do), used in this study as a plant necrotrophic bacterial pathogen. The housekeeping genes dnaX, *leuS* and *recA* of KACC10225 were amplified and sequenced. PCR protocols and primers were described in Portier et al. (Portier et al., 2020).
rapa plants were inoculated, as previously described (Liu et al., 2019). The GenBank accession numbers for dnaX, leuS, and recA genes of KACC10225 are OP328786, OP328785, and OP328784 respectively. Because three genes showed ~99% nucleotide sequence identity to those of Pectobacterium brasiliense strain BC1 (GenBank accession number: CP009769) we described KACC10225 as Pectobacterium brasiliense in this study. To induce P. brasiliense systemic infection, B. rapa plants were inoculated as previously described (Lee et al., 2020). Briefly, P. brasiliense isolate KACC 10225 was cultured in NB broth (Becton, Dickinson, and Co.) for 36 h in an incubator set at 30°C with continuous shaking (200 rpm). The bacterial culture was diluted in distilled water to an OD_{600} of 0.1 (1 × 10^6 CFU/mL) and then 24-day-old seedlings were inoculated with P. brasiliense KACC 10225 by drenching the proximal plant parts with the bacterial suspension. The inoculated plants were incubated in a dew chamber at 25°C for 24 h and then transferred to a growth room set at 25°C and 80% relative humidity with a 12-h photoperiod. Seven days after inoculation, the disease severity was assessed using the following scale: 0 (no symptoms), 1 (chlorosis or 1%–25% rotted), 2 (chlorosis or 25%–50% rotted), 3 (chlorosis or 50%–75% rotted), and 4 (chlorosis or 75%–100% rotted; the plant was dead), which was later converted to a percentage (Lee et al., 2020).

To analyze localized P. brasiliense infections, 4-week-old B. rapa plants were inoculated, as previously described (Liu et al., 2019). Briefly, the third leaf collected from each plant was cut into 2.3 cm diameter discs using a cork borer and placed on two layers of moistened filter paper in covered square Petri dishes (12.5 × 12.5 cm) to maintain high humidity. The leaf discs were then pierced at the center with a sterile tip, inoculated with 5 µL (12.5 × 12.5 cm) to maintain high humidity. The leaf discs were layered of moistened into 2.3 cm diameter discs using a cork borer and placed on two filter paper in covered square Petri dishes.

To analyze the effect of JA on the development of the soft rot disease, 4-week-old B. rapa plants were inoculated as previously described (Luzzatto et al., 2007; Liu et al., 2019). A 1 mM methyl jasmonate (MeJA; Sigma-Aldrich) solution was applied as a foliar spray 24 h before inoculation. Leaf discs were prepared and inoculated with P. brasiliense as described above. Disease symptoms were recorded 24 h later (48 h after MeJA treatment).

**Transcript profiling**

For quantitative reverse transcription PCR (qRT-PCR) analysis, 8-day-old seedlings grown under sterile conditions were treated with 1 µM flg22 or H2O for 30 min. Four-week-old B. rapa plants grown in soil were inoculated with a P. brasiliense bacterial solution (OD_{600} = 0.1) and sampled at 0, 6, and 24 h post-inoculation. Total RNA was extracted, and residual DNA was digested using the RNeasy Plant Mini Kit and RNase-Free DNase Set (Qiagen, Hilden, Germany). Purified RNA (1 µg) served as the template for synthesizing cDNA using the ReverTra Ace-α kit with oligo-(dT) primers (Toyobo, Osaka, Japan). qRT-PCR analysis was performed using the TB Green Premix Ex Taq premix (Takara Bio, Shiga, Japan) and CFX96 Real-Time PCR system (Bio-Rad). The qRT-PCR primers used are listed in Supplementary Table S1.

**Reactive oxygen species measurements**

Leaf discs prepared from 12-day-old B. rapa plants were used to analyze ROS content as previously described (Yi et al., 2014), with minor modifications. Briefly, leaf discs were incubated in a 96-well microplate containing liquid MS medium supplemented with 0.1% (w/v) sucrose. An EnVision 2101 multi-label plate reader (Perkin Elmer, Waltham, MA, USA) was used to measure the L-012-derived chemiluminescence (counts per second) at an emission wavelength of 590 nm.

**MAPK phosphorylation assay and protein detection**

The MAPK activity of crude protein extracts from the cotyledons of 8-day-old seedlings treated with 1 µM flg22 for 1 h was determined as previously described (Flury et al., 2013). Specifically, the proteins from the crude extracts were separated by 10% SDS-PAGE and then transferred to a PVDF membrane (Bio-Rad; www.bio-rad.com) using a Mini-Protean II semi-dry electrobboning system (Bio-Rad). Activated MAPKs were detected following a 1 h incubation with Phospho-p44/42 MAPK (Erk1/2) rabbit monoclonal antibody (mAb) (1:2,000; Cell Signaling Technology, www.cellsignal.com) and a subsequent 1 h incubation with anti-rabbit-HRP secondary antibodies (Bio-Rad). The signals were visualized using a Clarity™ Western ECL system (Bio-Rad).

**Aniline blue staining, microscopic analysis, and callose quantification**

Cotyledons from 8-day-old B. rapa seedlings were treated with 1 µM flg22 or H2O; subsequently, they were treated with 95% ethanol, and stained with aniline blue as previously described (Gomez-Gomez et al., 1999) with minor modifications. Briefly, the cotyledons were incubated for at least 24 h in 95%–100% ethanol until the tissues were transparent. They were then washed with 0.07 M phosphate buffer (pH 9) and incubated for 1–2 h in 0.07 M phosphate buffer containing 0.01% (w/v) aniline blue (Sigma). At least ten cotyledons per condition per experiment were examined under
combined supernatants were used as the crude glucosinolate and the residue was re-extracted twice as described above. The min in a water bath. After centrifugation at 12,000 rpm at 4°C extracted with 1.5 ml of boiling 70% (v/v) MeOH at 70°C for 5 crude glucosinolates from 100 mg of freeze-dried powder were extracted from 100 mg freeze-dried B. rapa (Schafer et al., 2016), with some modi- 8050, Shimadzu Corp., Kyoto, Japan), according to the methods chromatography-triple quadrupole mass spectrometer (LCMS- were analyzed using an ultra-high-performance liquid chromatography (UPLC BEH, 1.7 µm particle size, 100 mm length × 2.1 µm inner diameter, Waters, Milford, MA, USA) and separated using an HPLC system (Agilent Technologies, Palo Alto, CA, USA) at 227 nm wavelength. The mobile phase consisted of ultrapure water (solvent A) and acetonitrile (solvent B), at a flow rate of 1 mL/ min. The gradient program was as follows (40 min): 7%–24% solvent B, 18 min; 24% solvent B, 14 min; 7% solvent B, 32.1 min; and 7% solvent B, 8 min. Individual glucosinolates were identified according to their HPLC peak area ratios and quantified based on their retention times, peak areas, and response factors. DesulfoSinigrin (Sigma-Aldrich Co. Ltd., St. Louis, MO, USA) was used as an external reference standard.

Analytical procedures

Fresh B. rapa leaves were harvested at 0 and 24 h post-inoculation, weighed, immediately frozen in liquid nitrogen, and stored at −80 °C. Samples were prepared and plant hormones were analyzed as previously described (Park et al., 2022). Briefly, to extract phytohormones, we used ethyl acetate (HPLC grade, Sigma-Aldrich, Saint Louis, MO, USA) spiked with labeled phytohormones as internal standards: D₆-ABA, D₄-SA, and D₆-JA in 20 ng µL⁻¹. Two steel balls of 3 mm diameter and 1 mL of spiked ethyl acetate were added to 100 mg of ground B. rapa leaf sample and vortexed using a Tissuelyser II (Qiagen, Hilden, Germany) for 2 min. Samples were centrifuged at 13,000 rpm and 4°C for 20 min, and supernatants were transferred to a new 2 mL tube each. This extraction process was repeated by adding ethyl acetate (0.5 mL) without an internal standard. Supernatants were evaporated at 30°C using a HyperVAC-MAX (Hanil Scientific Inc., Daejeon, Republic of Korea) until dry. Subsequently, 500 µL 70% methanol (HPLC grade, Sigma-Aldrich) was added to dissolve the dried pellet. After centrifugation at 13,000 rpm for 10 min at 4°C, 400 µL of the dissolved sample was transferred into an HPLC vial. The extracts were analyzed using an ultra-high-performance liquid chromatography-triple quadrupole mass spectrometer (LCMS-8050, Shimadzu Corp., Kyoto, Japan), according to the methods of (Schafer et al., 2016), with some modifications (Joo et al., 2021). Then, 2 µL of the extracts were injected into a C₁₈ column (UPLC BEH, 1.7 µm particle size, 100 mm length × 2.1 µm inner diameter, Waters, Milford, MA, USA) and separated using an HPLC system (Shimadzu Corp., Japan). Solvent A consisted of deionized water containing 0.1% (v/v) acetonitrile and 0.5% formic acid, whereas solvent B consisted of 100% methanol.

Transcript abundance, fresh weight, number of callose deposits, symptom development rate, phytohormone content, and individual glucosinolate contents were obtained from at least three independent biological replicates. Analysis of variance (ANOVA) was performed using IBM SPSS Statistics (version 26). One-way ANOVA followed by Tukey’s honestly significant difference (HSD) test or Duncan test was conducted to compare three or more groups.

Accession numbers

The sequence data included in this study are available from the Brassicaceae Database (BRAD) (http://brassicadb.cn) under the following accession numbers: BrACT2 (Bra022356), BrWRKY33 (Bra005104), BrWRKY40 (Bra035148), BrNHL10 (Bra017272), BrMYB51 (Bra016553), BrST5a (Bra008132), BrCYP81F4 (Bra010598), and BrIGMT1 (Bra012270).

Data availability statement

The data supporting the findings of this study are available from the corresponding author upon reasonable request.
Author contributions

Conceptualization, SY. Data curation, SY. Funding acquisition, SY and S-GK. Investigation, ML, SP, LL and GL. Methodology, SY and S-GK. Project administration, SY. Resources, YL. Visualization, SY. Writing the manuscript, SY and S-YK. All authors have read and agreed to the published version of the manuscript.

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Conflict of interest

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpls.2022.964092/full#supplementary-material
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