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Transcriptional Changes of Plant Defense-Related Genes in Response to *Clavibacter* Infection in Pepper and Tomato

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Pepper and tomato plants infected with two *Clavibacter* species, *C. capsici* and *C. michiganensis* have shown different patterns of disease development depending on their virulence. Here, we investigated how pepper and tomato plants respond to infection by the high-virulent or low-virulent *Clavibacter* strains. For this, we chose two strains of each *Clavibacter* species to show different virulence level in the host plants. Although low-virulent strains showed less disease symptoms, they grew almost the same level as the high-virulent strains in both plants. To further examine the response of host plants to *Clavibacter* infection, we analyzed the expression patterns of plant defense-related genes in the leaves inoculated with different strains of *C. capsici* and *C. michiganensis*. Pepper plants infected with high-virulent *C. capsici* strain highly induced the expression of *CaPR1*, *CaDEF*, *CaPR4b*, *CaPR10*, and *CaLOX1* at 5 days after inoculation (dai), but their expression was much less in low-virulent *Clavibacter* infection. Expression of *CaSAR8.2* was induced at 2 dai, regardless of virulence level. Expression of *GluA*, *Pin2*, and *PR2* in tomato plants infected with high-virulent *C. michiganensis* were much higher at 5 dai, compared with mock or low-virulent strain. Expression of *PR1a*, *Osmotin-like*, *Chitinase*, and *Chitinase class 2* was increased, regardless of virulence level. Expression of *LoxA* gene was not affected by *Clavibacter* inoculation. These results suggested that *Clavibacter* infection promotes induction of certain defense-related genes in host plants and that differential expression of those genes by low-virulent *Clavibacter* infection might be affected by their endophytic lifestyle in plants.

Keywords : bacterial canker, *Clavibacter capsici*, *Clavibacter michiganensis*, plant defense-related genes, wilting

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The genus *Clavibacter* belongs to actinobacteria carrying genomic DNA with high G + C content (Eichenlaub and Gartemann, 2011). Two species, *C. michiganensis* and *C. capsici* have been shown to cause bacterial canker in tomato and pepper plants, respectively (Thapa et al., 2019). In particular, *C. capsici* was first isolated from infected pepper leaves in Korea and reported in 1999 (Lee et al., 1999). Since then, it has been considered as *C. michiganensis*, but recently it was reclassified as a separate species (Li et al., 2018; Oh et al., 2016). A recent study reported that *C. capsici* strains with different levels of virulence exist in Korea and that this virulence depends on the plasmid carrying the virulence genes (Hwang et al., 2020). The small plasmid, pCM1$_{Cc}$ of *C. capsici* carries at least five virulence genes, and these genes play a role in virulence during infection into host plants (Hwang et al., 2018).

C. michiganensis has been used as a representative species as Gram-positive bacterium for research on virulence genes and mechanisms in plants (Eichenlaub and Gartemann, 2011). Although, in Korea, this pathogen resided in xylem vessels was first observed in 1997, it was reported in 2008 because it became severe in tomato production (Myung et

al., 2008). *C. michiganensis* also carries two plasmids like *C. capsici*, and it was shown that both plasmids play an important role in virulence. The plasmid-borne *celA* (cellulase) gene in pCM1 and *pat-1* (putative serine protease) gene in pCM2 are critical for the virulence of *C. michiganensis* in tomato (Hwang et al., 2019; Jahr et al., 2000). In addition, a pathogenicity island region located in the chromosome of *C. michiganensis* also contains several virulence genes for putative serine proteases and enzymes that seem to be involved in disease development (Eichenlaub and Gartemann, 2011; Gartemann et al., 2008). Importantly, in both *C. capsici* and *C. michiganensis*, there was no positive correlation between bacterial growth and virulence level (Hwang et al., 2020; Thapa et al., 2017), meaning that both the high-virulent and low-virulent strains of both bacteria could grow well similarly inside host plants.

During the long-term battle between plants and pathogens, plants have developed defense mechanisms to protect themselves from pathogen attack, and these interactions have been intensively studied. For successful colonization inside the plants, molecules secreted by pathogens have to overcome physical barriers such as the cell wall. The plant also responds by the recognition of invading pathogens, and plant defense responses were promoted via activation of defense-related genes and production of defensive compounds (Abramovitch et al., 2006; Dodds and Rathjen, 2010; Jones and Dangl, 2006). Infection of *Xanthomonas campestris* pv. *vesicatoria* resulted in induced expression of many defense-related genes, such as *CaPR1*, *CaDEF*, *CaSAR8.2*, and *CaPR4b*, and these gene transcripts were accumulated at various time point in plants (Choi and Hwang, 2015; Hong et al., 2017; Lee and Hwang, 2006). Infection with a soil-borne pathogen *Phytophthora capsici* also triggered the expression of defense genes in leaves and roots, and these expressed genes might be involved in defense responses (Jin et al., 2015; Wang et al., 2013). Defense-related genes mentioned above have been shown to be differentially expressed depending on the virulence level of *X. campestris* pv. *vesicatoria* and *P. capsici* in pepper plants. The expression of the pepper defense-related genes by infection with incompatible pathogens were significantly and strongly induced at early time point in plants, compared to compatible pathogens (Choi and Hwang, 2015; Kim et al., 2010; Wang et al., 2013).

Infected tomato plants with *Ralstonia solanacearum* induced expression of defense-related genes, such as *PR1a*, *GluA*, *Pin2*, *LoxA*, *Osmotin-like*, *PR2*, *Chitinase,* and *Chitinase class 2* (Milling et al., 2011). Expression of *PR1* gene was detected in tomato plants infected with *Pseudomonas syringae* pv. *tomato* under greenhouse condition (Herman et al., 2008). Despite of extensive researches on pathogen-induced defense responses in plants, including pepper and tomato, how transcriptional changes in plants after inoculation with *Clavibacter* species can protect plants remains unknown. The only finding that infection with *C. michiganensis* activates tomato basal defense responses and defense-related hormones by expression of defense-related genes, implying that plants may also activate defense responses against *Clavibacter* infection (Balaji et al., 2008).

In this study, we focused on the defense responses during infection of *Clavibacter* species in their host plants. In particular, we used the high-virulent and low-virulent strains of *C. capsici* and *C. michiganensis* in pepper and tomato, respectively, which have no positive correlation between virulence level and bacterial growth in host plants. Our study provides a detailed description of transcriptional changes induced by various *Clavibacter* strains infection in host plants.

Materials and Methods

Plant materials and growth conditions. Pepper (*Capsicum annuum* 'Nockwang') and tomato (*Solanum lycopersicum* cv. 'Betatini') plants used in this study were grown in a growth chamber at 26°C with a 14 h:10 h light:dark photoperiod and 50% relative humidity. For all inoculation experiments, 3-week-old pepper plants and 2-week-old tomato plants were used.

Bacterial strains. For pepper plant inoculation, the high-virulent strains *C. capsici* PF008 and 822 and the low-virulent strains PF008ΔpCM1$_{Cc}$ and 1101 (lacking pCM1$_{Cc}$) (Hwang et al., 2020) and for tomato plant inoculation, the high-virulent strains *C. michiganensis* LMG7333 (Hwang et al., 2019) and KACC16995 and the low-virulent strain LMG3685 (Yim et al., 2012; Oh et al., 2016) were used in this study.

Virulence assay and *in planta* bacterial growth. For virulence assay, bacteria were incubated in KB (King's B) medium (proteose peptone no. 3; 20 g, K_2HPO_4; 1.5 g, 1 M $MgSO_4$; 6 ml, and 50% glycerol; 16 ml in total volume 1 L) at 26°C, after single colony propagation. Cultured bacteria were then harvested and resuspended in 10 mM $MgCl_2$. Bacterial suspension adjusted to an optical density of 0.1 at 600 nm was infiltrated into leaves of pepper plants with the needleless syringe. OD_{600} = 0.4 of *C. michiganensis* bacterial suspension was prepared in 10 mM $MgCl_2$, and inoculated into tomato leaves by leaf clipping method using scis-

sors. Ten mM MgCl$_2$ was used as a mock inoculation.

After pepper inoculation with *C. capsici* strains, disease symptoms on inoculated leaves were scored at 7 days after inoculation (dai) based on the following 0 to 5 scales: 0, no visible symptoms; 1, <5% leaf area affected; 2, 6-25% leaf area affected; 3, 26-50% leaf area affected; 4, 51-75% leaf area affected; 5, >75% leaf area affected (Hwang et al., 2018). Tomato plants infected with *C. michiganensis* were scored at 23 dai. Disease severity was evaluated by scoring of wilting symptoms, using an index ranging from 0 to 5: 0, no symptoms; 1, one or two leaves showed wilting symptoms; 2, less than 25% leaves wilted; 3, more than 25% but less than 50% leaves wilted; 4, 50 to 75% leaves showed wilting symptoms; 5, more than 75% leaves wilted or plants dead (Hwang et al., 2019). All experiments were repeated at least 3 times independently.

In planta bacterial growth of *Clavibacter* strains was measured by harvesting samples after inoculation with 10^8 colony forming units (cfu)/ml of *C. michiganensis*, and 4 × 10^8 cfu/ml of *C. capsici* at various time points. *C. capsici*–inoculated pepper plants and *C. michiganensis*–inoculated tomato plants were collected at 0, and 5 dai, and at 3, 7, and 10 dai, respectively. Three independent samples were ground, plated on KB medium plates, and then incubated at 26°C for 2 days. After incubation, the number of cfu per leaf discs (cfu/cm^2) or leaf weight (cfu/g) by multiplying the average number of the colony were calculated.

Confirmation of *Clavibacter* species. With primer sets to amplify virulence genes or plasmid back-borne genes specific to *Clavibacter* species, PCR was performed to confirm the used strains. Type strains of each species, which are *C. capsici* PF008 and *C. michiganensis* LMG7333, were used as a positive control. Primers used in this study are listed in Supplementary Table 1. All PCR assay was performed using BioFACT 2× *Taq* PCR Master Mix 2 (BioFACT, Daejeon, Korea).

Gene expression profiling in *Clavibacter*-infected plants by quantitative real-time PCR (qRT-PCR). Pepper defense-related genes, *CaPR1* (accession no. AF053343), *CaDEF* (accession no. AF442388), *CaPR4b* (accession no. HM581975), *CaPR10* (accession no. AF244121), *CaLOX1* (accession no. FJ377808), and *CaSAR8.2* (accession no. AF112868) and tomato defense-related genes, *GluA* (accession no. M80604), *PR1a* (accession no. M69247), *Pin2* (accession no. AY129402), *LoxA* (accession no. U09026), *PR2* (accession no. BT013355), *Osmotin-like* (accession no. M21346), *Chitinase* (accession no. BG629612), and *Chitinase class 2* (accession no. U30465) were targeted to

check their gene expression. Total RNA was isolated from infected plant samples at 0 (right after inoculation), 2, and 5 dai by EZ Total RNA Miniprep Kit (Enzynomics, Daejeon, Korea), and then reverse-transcribed to generate cDNA by TOPscript III Reverse Transcriptase (Enzynomics). Using these synthesized cDNA, qRT-PCR using TOPreal qPCR 2× PreMIX (Enzynomics) was performed, and relative expression of target genes was normalized with *CaActin* (accession no. GQ339766) for pepper plants and *SlActin* (accession no. BT013524) for the tomato plants. The primer sets to amplify plant defense-related genes are listed in Supplementary Table 1.

Statistical analysis. Statistical analysis was performed using StatistiXL 2 (statistiXL, Broadway, Australia). Disease severity data were analyzed by the non-parametric ANOVA (Kruskal-Wallis test), and other data were analyzed by Duncan's multiple range test ($P < 0.05$). All experiments were repeated a minimum of three times with similar results.

Results

Characterization of high-virulent and low-virulent strains of *C. capsici*. In the previous study, we reported *C. capsici* natural isolates with differential virulence level in pepper plants (Hwang et al., 2020). To explore the role of defense mechanism in pepper plants, especially the expression of defense-related genes depending on virulence difference of *C. capsici* strains, we selected *C. capsici* type strain PF008 and natural isolate 822 as high-virulent strains and strain PF008ΔpCM1$_{Cc}$ and natural isolate 1101 as low-virulent strains due to the lack of the small plasmid pCM1$_{Cc}$ with important virulence genes such as *chpE* and *chpG* (Hwang et al., 2018). The virulence level of these selected strains were confirmed by inoculation into pepper leaves. Pepper leaves inoculated with strain 822 showed severe necrotic symptoms as observed in PF008-inoculated leaves. The disease symptoms by both strains in pepper leaves started to appear prior to 5 dai, and the entire inoculated leaves displayed necrotic symptoms at 7 to 10 dai (Fig. 1A). In contrast, PF008ΔpCM1$_{Cc}$, and 1101 caused barely visible and weak necrotic symptoms at 7 dai. Their disease severity was quantified as shown in Supplementary Fig. 1.

We further checked the bacterial number of *C. capsici* strains in the infiltrated leaves. The result showed that bacterial numbers of low-virulent *C. capsici* strains were almost equivalent to high-virulent strains in pepper leaves, indicating that there is little correlation between the virulence level and bacterial population of these strains in

A
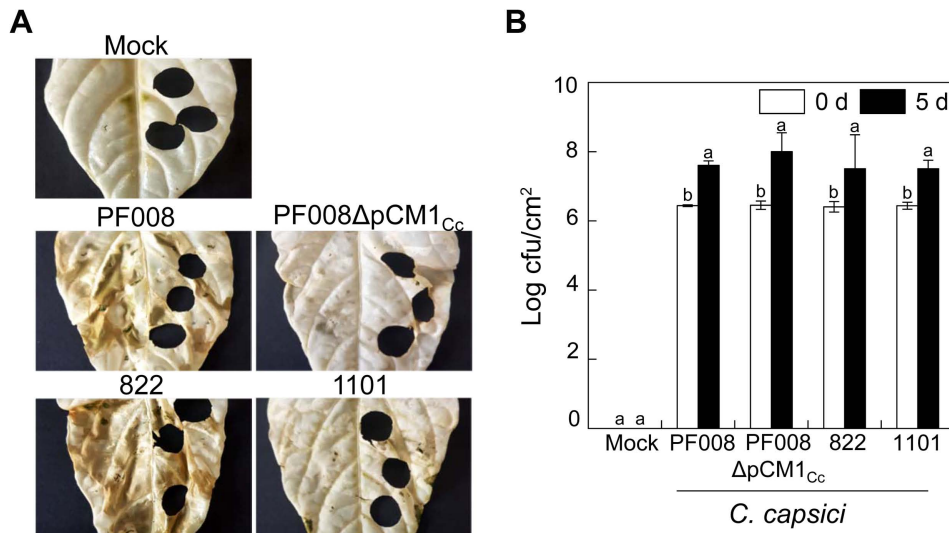
Mock

PF008

PF008ΔpCM1$_{Cc}$

822

1101

B

Fig. 1. Virulence assay in pepper leaves with *Clavibacter capsici* natural isolates. (A) Virulence phenotypes on pepper leaves in response to *C. capsici*. The pepper leaves was bleached with ethanol to make symptom more obvious, and the picture was taken at 7 days after inoculation (dai). (B) Bacterial growth of *C. capsici* strains in plants at 0 and 5 dai. The data represent means ± standard deviation (n = 3). The different letters indicate statistically significant differences as determined by Duncan's multiple range test ($P < 0.05$).

plants (Fig. 1B). Collectively, these results imply that both high-virulent and low-virulent strains of *C. capsici* can survive and grow in plants regardless of the virulence level.

Expression profiles of defense-related genes triggered by *C. capsici* inoculation in pepper. As we mentioned above, the high-virulent strains of *C. capsici* caused severe necrotic symptoms on pepper leaves within 7 dai. The expression of the virulence genes in high-virulent and/or low-virulent *C. capsici* strains was analyzed in the previous report (Hwang et al., 2020), showing that the transcript level of plasmid-borne virulence genes was differentially induced by the presence or the absence of the small plasmid, pCM1$_{Cc}$. In particular, we found that the expression of most plasmid-borne virulence genes tended to decrease in low-virulent strains after inoculation in plants. So far, however, very little research has focused on changes in defense-related genes in *Clavibacter*-inoculated plants. Thus, we analyzed how pepper defense-related genes respond to *C. capsici* infection. The expression profiles of defense-related genes such as *CaPR1*, *CaDEF*, *CaPR4b*, *CaPR10*, *CaLOX1*, and *CaSAR8.2*, which have been shown to respond to bacterial and/or fungal infection or various abiotic stresses (Choi and Hwang, 2015), were determined by qRT-PCR with high-virulent or low-virulent *C. capsici* strains at 0, 2 and 5 dai before the disease looks very severe.

Expression of three PR genes, *CaPR1*, *CaPR4b*, and *CaPR10*, was induced from 2 dai, unlike those genes were induced immediately after inoculation in pepper with Gram-negative bacteria, such as *X. campestris* pv. *vesicatoria* (Choi and Hwang, 2015). They displayed stronger and higher induction in plants inoculated with high-virulent strains, PF008 and 822, at 5 dai, which is the

time when the disease symptom began to appear (Fig. 2). Although their expression significantly increased toward the later time points of infection with low-virulent strains, PF008ΔpCM1$_{Cc}$ and 110, it was not strong as much as that in plant leaves inoculated with high-virulent strains at 5 dai. Expression of *CaDEF* and *CaLOX1* could significantly be detected right after inoculation, and their expression was highly induced at 5 dai (Fig. 2). Like PR genes, higher expression was observed in leaves inoculated with high-virulent strains than in leaves with low-virulent strains. While induction of these 5 genes after infection with *C. capsici* strains was notably increased during infection until 5 dai, expression of *CaSAR8.2* was temporarily increased only at 2 dai regardless of virulence level of inoculated strains (Fig. 2). These results indicate that all examined defense-related genes exhibited increased expression during infection of *C. capsici* and the magnitude was bigger during infection of high-virulent strains than low-virulent strains.

Characterization of high-virulent and low-virulent strains of *C. michiganensis*. *C. michiganensis* type strain LMG7333 known as a high-virulent strain against tomato plants was first selected as a high-virulent strain (Hwang et al., 2019). Then, two more *C. michiganensis* strains KACC16995 and LMG3685 were obtained, and their identities were checked by PCR with primers specific to *C. michiganensis* (Supplementary Fig. 2). To examine differences in virulence of these two *C. michiganensis* strains, tomato plants were inoculated by the leaf clipping method, and symptom development was observed for 4 weeks after inoculation. Tomato plants inoculated by strains either LMG7333 or KACC16995 developed wilting symptoms approximately 2 weeks after inoculation, whereas plants
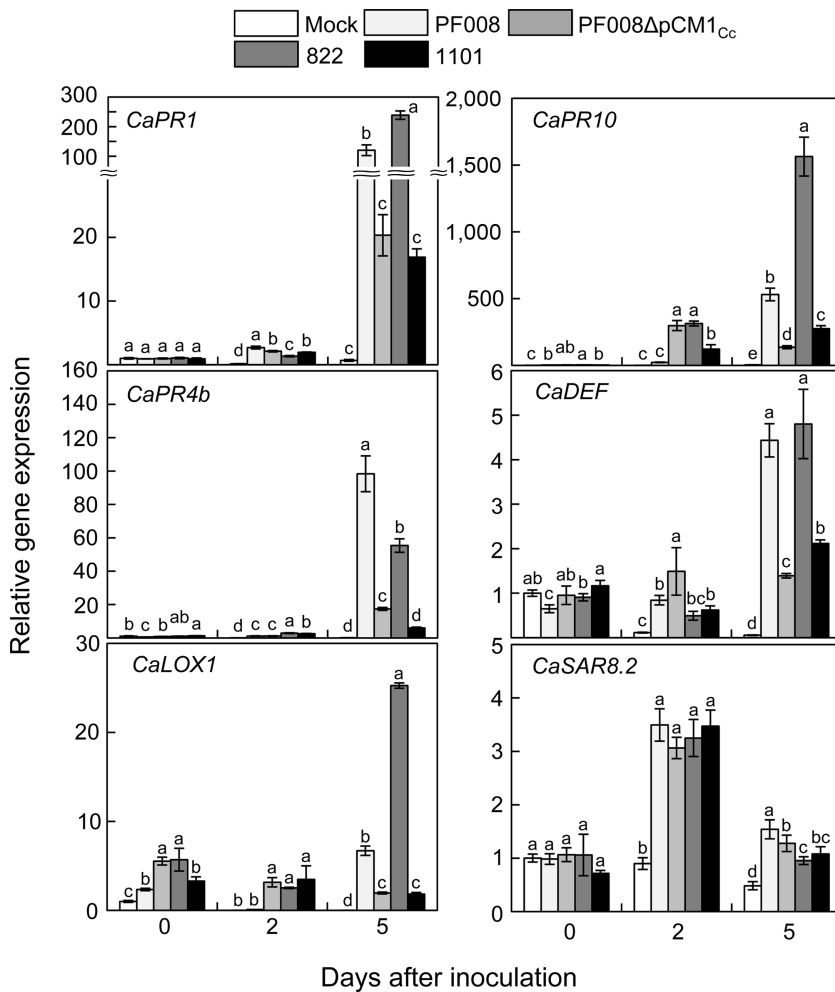
Fig. 2. Transcriptional changes of defense-related host genes in pepper plants after inoculation with less- or high-virulent isolates of *Clavibacter capsici*. Quantitative real-time PCR analysis of the indicated defense-related genes in pepper plants were measured at 0, 2, and 5 days after inoculation. The data represent means ± standard deviation (*n* = 3). The different letters indicate statistically significant differences as determined by Duncan's multiple range test (*P* < 0.05). Pepper *Actin* gene was used to normalize the expression of defense-related genes.

inoculated by LMG3685 remained mostly healthy over the two-week. After 3 weeks, strains LMG7333 and KACC16995 caused severe wilting and bacterial canker on their stems, resulting in eventual death of the plants (Fig. 3A and B). However, LMG3685-inoculated plants exhibited mild or no visual symptoms at the same time point (Fig. 3A and B). Therefore, KACC16995 and LMG3685 were categorized and selected as high-virulent and low-virulent strains, respectively.

To determine if the virulence of these strains is directly related to the bacterial population in plants, the bacterial growth assay was performed *in planta*. Regardless of virulence level of bacterial strains, all strains grew well, and there was no difference in bacteria titers even at 10 dai (Fig. 3C). These are consistent with those of *C. capsici* in pepper leaves (Fig. 1B). These results support that the disease severity caused by two *Clavibacter* species is not correlated with bacterial titers in plants, as shown in our previous report (Hwang et al., 2019).

Expression profiles of defense-related genes triggered by *C. michiganensis* in tomato. We hypothesized that the similar bacterial titers regardless of virulence level of *C. michiganensis* strains might affect to the expression of tomato defense-related genes during infection. To examine the change of the transcripts of defense-related genes in tomato plants inoculated with high-virulent or low-virulent *C. michiganensis* strains, we focused on the tomato genes, of which their expression was previously reported to be induced during *C. michiganensis* infection (Balaji et al., 2008). Among them, we selected *GluA*, encoding β-1,3 glucanase, and *PR1a* for the salicylic acid (SA) pathway, *PR2* and osmotin-like gene for the ethylene (ET) pathway, and *Pin2* (tomato protease inhibitor) and *LoxA* (lipoxygenase) genes for the jasmonic acid (JA) pathway.

Expression of *GluA*, *PR2*, and *Pin2* was begun to be induced in response to infection of the high-virulent *C. michiganensis* strains, LMG7333 and KACC16995 at 2 dai and dramatically increased at 5 dai (Fig. 4). However, although expression of those genes was slightly increased by
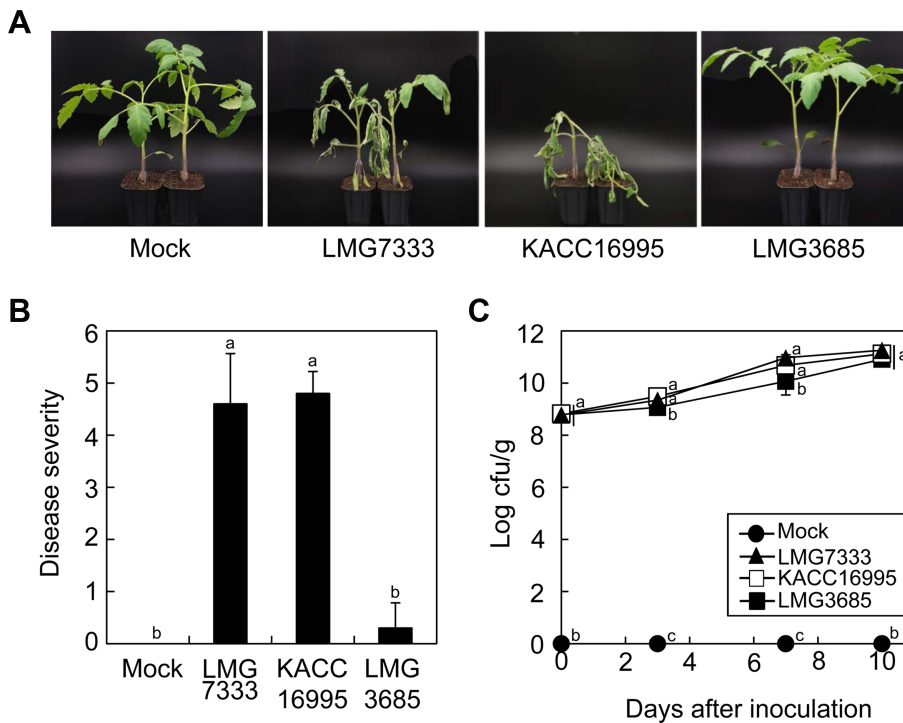
Fig. 3. Virulence assay in tomato plants with *Clavibacter michiganensis* isolates. (A) Phenotypes in tomato plants in response to *C. michiganensis* isolates. (B) Disease severity of *C. michiganensis* isolates in tomato plants at 14 days after inoculation. The data represent means ± standard deviation ($n = 9$). The different letters indicate statistically significant differences across treatments as per a non-parametric Kruskal-Wallis one-way ANOVA, followed by a post-hoc test (Dunnett's test, $P < 0.05$). (C) Bacterial growth of *C. michiganensis* isolates in tomato plants at the indicated time points. The data represent means ± standard deviation ($n = 3$). The different letters indicate statistically significant differences as determined by Duncan's multiple range test ($P < 0.05$).

the low-virulent strain, LMG3685, their expression level was much lower than those by the high-virulent strains at 5 dai. Expression of *PR1a* and osmotin-like genes was also begun to be induced at 2 dai, but those expression was increased at the similar level by both the high-virulent and the low-virulent strains (Fig. 4). Similarly, expression of two chitinase genes related to the ET pathway was increased from 2 dai by both the high-virulent and the low-virulent strains (Supplementary Fig. 3). Finally, expression of *LoxA* was not significantly different, compared to that of the control (Mock) plant, by both the high-virulent and the low-virulent strains (Fig. 4). These results indicate that both the high-virulent and low-virulent *C. michiganensis* strains could induce most of defense-related genes in tomato, but the expression level could be different due to the virulence level, but not because of bacterial titers inside plant tissues.

Discussion

In this study, to gain molecular insights about molecular responses of pepper and tomato plants to *Clavibacter* infection, we analyzed gene expression profiles of pepper or tomato defense-related genes after infection of the high-virulent and low-virulent strains of *Clavibacter* species. Previously, only a few studies have been reported to show plant responses against *C. michiganensis* infection in tomato. During high-virulent *C. michiganensis* infection, typical basal defense responses in tomato plants were activated by

induction of defense-related genes, production and scavenging of free oxygen radicals, enhanced protein turnover, and hormone synthesis (Balaji et al., 2008). Among the genes up-regulated by the high-virulent pathogen infection, *PR1a*, *Pin2*, Osmotin-like protein, *PR2*, and Chitinase class 2 were involved in the disease development (Balaji et al., 2008; Milling et al., 2011). Here, in common, infection with *C. capsici* and *C. michiganensis* in pepper and tomato plants, respectively, resulted in accumulation of transcripts of plant defense-related genes. Notably, we also found that host plants inoculated with the high-virulent *Clavibacter* had significantly higher expression of most defense-related genes and showed dramatically elevated transcript accumulation at 5 dai, compared to mock-inoculated plants (Figs. 2 and 4), although the expression of *CaSAR8.2* in pepper and *LoxA* in tomato in response to *Clavibacter* infection only increased at 2 dai or no induction. The fact that most of pepper defense-related genes, *CaPR1*, *CaDEF*, *CaPR4b*, *CaPR10*, and *CaLOX1*, in this study were strongly induced at 5 dai indicates that pepper defense-related genes might contribute to defense responses to protect itself.

Moreover, we also showed that tomato plants responded to the high-virulent *C. michiganensis* infection regulated defense marker genes for the SA, JA, and ET defense pathways, which are responsible for plant defense responses (Fig. 4). Except for *LoxA* gene, other defense-related genes were strongly expressed, and its expression was peaked at 5 dai. In agreement with the reported data, defense-related
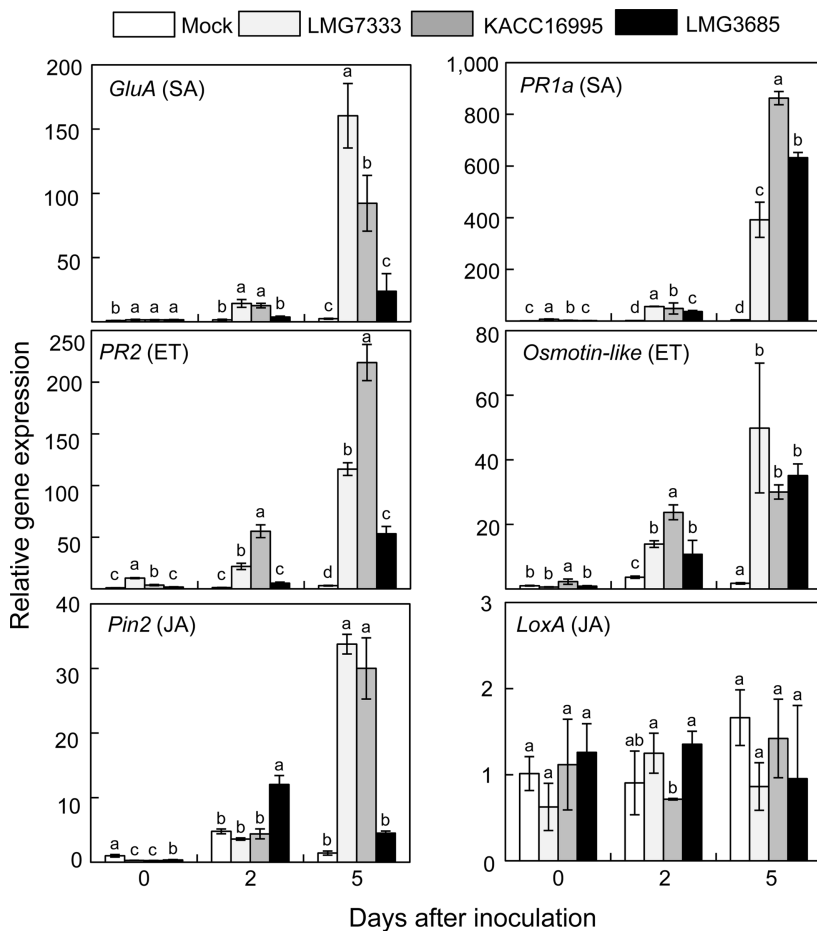
Fig. 4. Transcriptional changes of defense-related host genes in tomato plants after inoculation with *Clavibacter michiganensis* isolates. Quantitative real-time PCR analysis of the indicated defense-related genes in tomato plants were measured at 0, 2, and 5 days after inoculation. The data represent means ± standard deviation ($n = 3$). The different letters indicate statistically significant differences as determined by Duncan's multiple range test ($P < 0.05$). Tomato *Actin* gene was used to normalize the expression of defense-related genes.

genes in tomato were highly expressed during infection, indicating that tomato defense-related genes may participate in the activation of the defense responses against *C. michiganensis* infection through the expression of various defense-related genes related to plant hormone (Bari and Jones, 2009). Furthermore, when we analyzed the expression of the same defense-related genes during the low-virulent *Clavibacter* infection, the patterns were different from ones during the high-virulent *Clavibacter* infection, and it compromised accumulation of defense-related genes. In the pepper plants, except *CaSAR8.2*, all tested defense genes showed reduced expression, compared to the pepper inoculated with the high-virulent strains. In the tomato plants, some of them, which are *GluA*, *Pin2*, and *PR2*, failed to express as much as those in plants infected with the high-virulent strains.

Differentially expressed defense-related genes depending on the level of virulence were extensively studied in the host plants infected with Gram-negative plant pathogenic bacteria (Choi and Hwang, 2015; Ciardi et al., 2000; Potnis et al., 2015). So far, pepper plants have been used as a model plant for the study of *X. campestris* pv. *vesicatoria*-

host interactions and plant responses against bacterial infection. During infection with *X. campestris* pv. *vesicatoria*, the expression of *CaPR1* gene, the representative defense gene in pepper, was highly induced from 6 hours after inoculation (hai) and reached a maximum at 18 to 25 hai (Kim et al., 2004). *CaDEF* gene was also expressed during *X. campestris* pv. *vesicatoria* infection, especially the compatible interaction, in pepper plants (Do et al., 2004). Other defense-related genes, such as *CaPR4b*, *CaPR10*, *CaLOX1*, and *CaSAR8.2*, tested in this study were strongly expressed and peaked before 24 hai after *X. campestris* pv. *vesicatoria* infection (Choi and Hwang, 2015; Hwang and Hwang, 2010). Other model systems, such as the interaction between *Phytophthora capsici* and a pepper plant, also showed that defense-related genes, such as *CaPR1*, *CaDEF1*, and *CaSAR82*, were significantly induced in pepper plants infected with *P. capsici* at relatively late time point, compared to those in the plants infected with *X. campestris* pv. *vesicatoria* (Jin et al., 2015). In most cases, these defense-related genes were more strongly expressed at the early time points in the incompatible interaction of the pepper with *X. campestris* pv. *vesicatoria* than in the com-

patible interaction, suggesting that the expression of these genes occurred differentially in the level of virulence-dependent manner (Choi and Hwang, 2015; Han and Hwang, 2017).

These Gram-negative bacterial pathogens have strong weapons, known as effectors secreted by type III secretion system (T3SS) to attack their host plants, however, the Gram-positive pathogen like *Clavibacter* lacks effectors delivered by T3SS (Eichenlaub and Gartemann, 2011; Nandi et al., 2018). Therefore, instead of effector proteins, *Clavibacter* species seem to use proteases, and these proteases act as virulence factors that play an important role in the virulence of this pathogen (Hwang et al., 2019; Thapa et al., 2019). To move within the plant, *Clavibacter* without flagella moves into the target area through the xylem vessels, implying that the expression of plant defense-related genes seems to be related to the transport speed of *Clavibacter* and the ability to directly kill plant cells over time. Relatively late symptom development by *Clavibacter* pathogens compared to other Gram-negative bacterial pathogens with effectors led to the late transcript accumulation of defense-related genes in host plants. The unique lifestyle of this pathogen might prevent the plants from recognizing it, until plant cells were directly attacked, and interrupt the rapid turn-on of these defense-related genes. A notable finding that despite the reduced expression of plant defense gene during the interaction between the low-virulent *Clavibacter* and its host plants, the low-virulent *Clavibacter* can survive in the plants as an endophyte without restricting bacterial growth, suggesting that the presence of *Clavibacter* is difficult to detect by plants before this pathogen reveals its virulence, such as releasing virulence factors that can directly affect the plants. Otherwise, these low-virulent *Clavibacter* strains might suppress plant defense responses activated by defense gene expression for survival in plants.

Overall, we showed the transcriptional changes of plant defense-related genes in response to the infection of *Clavibacter* with high or low virulence. The results of this work demonstrated that pepper or tomato plants have triggered the expression of defense-related genes against *Clavibacter* infection, even though gene expression was begun from the late time points with symptom development. We also revealed that these transcriptional changes between the high-virulent and the low-virulent inoculated host plants may result in its xylem-limited lifestyle of *Clavibacter*. We still do not yet know whether this late defense-related gene expression plays a significant role in the defense responses to suppress symptom development by *Clavibacter* infection, but this research may help us develop a potential option for

understanding their interaction between *Clavibacter* and its host plants.

Acknowledgments

Electronic Supplementary Material

Supplementary materials are available at The Plant Pathology Journal website (http://www.ppjonline.org/).

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