



CsPOM1, a DYRK Family Kinase, Plays Diverse Roles in Fungal Development, Virulence, and Stress Tolerance in the Anthracnose Pathogen *Colletotrichum scovillei*

Jong-Hwan Shin, Hee-Yeong Kim, Teng Fu, Kwang-Ho Lee and Kyoung Su Kim*

Division of Bio-Resource Sciences, Bio-Herb Research Institute, and Interdisciplinary Program in Smart Agriculture, Kangwon National University, Chuncheon, South Korea

OPEN ACCESS

Edited by:

Yong-Sun Bahn,
Yonsei University, South Korea

Reviewed by:

Ci Fu,
University of Toronto, Canada
Hee-Soo Park,
Kyungpook National University,
South Korea

*Correspondence:

Kyoung Su Kim
kims@kangwon.ac.kr

Specialty section:

This article was submitted to
Fungal Pathogenesis,
a section of the journal
Frontiers in Cellular and
Infection Microbiology

Received: 25 January 2022

Accepted: 23 March 2022

Published: 26 April 2022

Citation:

Shin J-H, Kim H-Y, Fu T, Lee K-H and Kim KS (2022) CsPOM1, a DYRK Family Kinase, Plays Diverse Roles in Fungal Development, Virulence, and Stress Tolerance in the Anthracnose Pathogen *Colletotrichum scovillei*. *Front. Cell. Infect. Microbiol.* 12:861915. doi: 10.3389/fcimb.2022.861915

Colletotrichum scovillei is the major anthracnose fungus of sweet pepper and chili pepper (*Capsicum annuum* L.), causing significant losses in the yield and quality of the pepper fruits. Molecular mechanisms governing development and pathogenicity have been widely studied in many foliar fungal pathogens, but the information on fruit diseases is still limited. In this study, we determined the functional roles of the dual-specificity tyrosine phosphorylation-regulated kinase CsPOM1 in *C. scovillei*. Knockout mutant for CsPOM1 gene was obtained via homology-dependent gene replacement. The $\Delta Cspom1$ mutant exhibited a reduction in vegetative growth on osmotic stress, surface hydrophobicity, and conidiation compared with wild-type. Conidia of the $\Delta Cspom1$ mutant were already two-celled before inoculation on an induction surface, indicating that CsPOM1 negatively regulates conidial cell division. The $\Delta Cspom1$ mutant, similar to wild-type, formed appressoria on the plant surface, but was significantly reduced on hydrophobic coverslips, probably due to a defect in the recognition of surface hydrophobicity. Treatment of conidia with cutin monomers restored appressorium formation on hydrophobic coverslips in the $\Delta Cspom1$ mutant. On pepper fruits, the $\Delta Cspom1$ mutant exhibited delayed penetration and invasive growth, leading to significantly reduced virulence. Collectively, the results showed that CsPOM1 is important for stress tolerance, conidiation, surface hydrophobicity, appressorium formation, and virulence in *C. scovillei*.

Keywords: cell division, DYRK, *Colletotrichum scovillei*, pepper anthracnose, Pom1

INTRODUCTION

The molecular mechanisms governing fungal development and pathogenicity of many foliar fungal pathogens have been extensively studied through functional genomics (Asakura et al., 2009; Wilson and Talbot, 2009). However, compared with foliar diseases, there is still limited information on fruit diseases, such as anthracnose caused by *Colletotrichum* species. The genus *Colletotrichum* is a major

group of phytopathogenic fungi in the filamentous ascomycetes, causing anthracnose disease in many vegetables and fruits. The number of species within the genus ranges from 29 to more than 700, depending on taxonomic interpretation (Cannon et al., 2007; Dean et al., 2012). Due to the wide host range of *Colletotrichum* species, which included important crops such as apple, banana, coffeeberry, mango, and pepper, the genus is considered the eighth most important group of phytopathogenic fungi in the world (Dean et al., 2012). Anthracnose of sweet pepper and chili pepper (*Capsicum annuum* L.) caused by several *Colletotrichum* species, including *C. scovillei*, *C. truncatum*, *C. fructicola*, *C. dematium*, *C. gloeosporioides*, *C. coccodes*, and *C. capsici*, leads to significant losses in the yield and quality of pepper fruits in both pre- and postharvest periods (Caires et al., 2014; Kanto et al., 2014; Liu et al., 2016; Zhao et al., 2016; Diao et al., 2017). Recent advances in sequencing technology have revealed that *C. scovillei*, which belongs to the *C. acutatum* species complex is the dominant fungal pathogen of pepper anthracnose disease worldwide (Caires et al., 2014; Zhao et al., 2016; Diao et al., 2017; Noor and Zakaria, 2018; Khalimi et al., 2019). As *C. scovillei* was shown to be the most dominant fungal pathogen in pepper anthracnose in many countries, whole-genome sequencing, annotation, and genetic transformation methods for this pathogen have been established to understand fungal biology and pathogenicity (Han et al., 2016; Shin et al., 2019).

Fungi possess conserved signal transduction pathways, such as mitogen-activated protein kinase (MAPK) signaling pathways and the cyclic adenosine monophosphate (cAMP)-dependent protein kinase signaling pathway, which regulates developmental processes, stress responses, and virulence in many fungi (Xu and Hamer, 1996; Adachi and Hamer, 1998; Takano et al., 2000; Hagiwara et al., 2009; Mehrabi et al., 2009). Studying the mechanisms of fungal signaling systems provides information important for understanding fungal development and pathogenicity. One of the best-studied MAPK pathways is the Pmk1 MAPK pathway, which is required for appressorium formation and plant infection in many phytopathogenic fungi (Xu and Hamer, 1996; Zhao et al., 2007; Liang et al., 2019; Fu et al., 2022). The high osmolarity glycerol (HOG) MAPK pathway is also one of the well-characterized MAPK pathways that mediate cellular adaptation to hyperosmotic stress (Van Wuytswinkel et al., 2000; Tatebayashi et al., 2006; Wu et al., 2006; Hohmann, 2009; Duran et al., 2010; Saito and Posas, 2012). Recently, it has been revealed that a DYRK-family kinase PomA (a homolog of Pom1) is involved in the negative regulation of the HOG MAPK pathway in *Aspergillus nidulans*, affecting the phosphorylation level of Hog1, suggests important roles for PomA in the regulation of HOG MAPK pathway (Zhou et al., 2019).

DYRKs are serine/threonine protein kinases that autophosphorylate a tyrosine residue in their activation loop and phosphorylate their substrates on serine and threonine residues (Kentrup et al., 1996; Becker et al., 1998). The DYRK family can be classified into three subfamilies, i.e., DYRK kinases, homeodomain-interacting protein kinases, and pre-mRNA

processing protein 4 kinases, based on the homology within the kinase domain (Aranda et al., 2011). Among them, Yak1 and Pom1 are well-characterized DYRK kinases in yeast (Garrett et al., 1991; Bähler and Pringle, 1998). Yak1, the first DYRK kinase discovered, acts as a negative regulator of growth in the budding yeast *Saccharomyces cerevisiae* (Garrett et al., 1991). Pom1 is well studied in the fission yeast *Schizosaccharomyces pombe*, in which Pom1 provides positional information for both growth and division (Bähler and Pringle, 1998). However, the functional roles of Pom1 kinase in fungal development and virulence remain largely unknown in filamentous fungi. This prompted us to investigate the functional roles of Pom1 in *C. scovillei*.

In this study, we identified *C. scovillei* POM1 as a homolog of *S. pombe* POM1, which was designated CsPOM1. This study aimed to functionally characterize the roles of CsPOM1 in fungal development and pathogenicity. We generated a targeted deletion mutant of *CsPOM1* and performed phenotypic analysis. The *CsPOM1* deletion mutant exhibited defects in stress tolerance, hydrophobicity, cell division, conidiation, appressorium formation, and virulence. In addition, we found that the expression of glycogen debranching and class II hydrophobin genes was significantly altered in the *CsPOM1* deletion mutant, which might affect the phenotypic defects of the mutant.

MATERIALS AND METHODS

Fungal Strains and Culture Conditions

Colletotrichum scovillei strain KC05 (Han et al., 2016) was used as the wild-type (WT) strain in this study. Transformants were selected on TB3 agar (200 g of sucrose, 3 g of yeast extract, 3 g of casamino acids, 10 g of glucose, and 8 g of agar/L) containing 200 µg/ml of hygromycin B (EMD Millipore, USA) or 400 µg/ml of G418 geneticin (Gibco, USA). Complete agar (CMA, 10 g of sucrose, 6 g of yeast extract, 6 g of casamino acids, and 15 g of agar/L) was used for vegetative growth. V8 agar (V8A, 80 ml of V8 juice, 310 µl 10 N NaOH, and 15 g of agar/L) and oatmeal agar (OMA, 50 g oatmeal and 25 g agar/L) were used for conidiation. Experiments were conducted in triplicates and repeated three times. All data were processed with the SigmaStar statistical software package (SPSS Science, Chicago, IL).

Bioinformatics Analysis

All sequence information used in this study was obtained from the online database CFGP (<http://cftp.snu.ac.kr>). Protein sequences of putative glycogen debranching enzyme (CAP_006079), class II hydrophobin (CAP_004537), and dual-specificity tyrosine phosphorylation-regulated kinase Pom1 (CAP_000731) in *C. scovillei* were obtained through BLAST searches conducted using the protein sequences of Gdb1 (AJW05075) in *Saccharomyces cerevisiae*, Mhp1 (MG01173) in *Magnaporthe oryzae*, and Pom1 (NP_592974) in *Schizosaccharomyces pombe*, respectively. Protein sequence

alignment of CsPOM1 with related proteins from fungi was performed using the ClustalW program in MEGA 6.0 (Thompson et al., 1994; Tamura et al., 2013). Phylogenetic analysis of obtained protein sequences was performed using a neighbor-joining method (10,000 bootstrap replicates) (Tamura et al., 2013). Domain structure analysis was performed using InterPro Scan v83.0 (<http://www.ebi.ac.uk/interpro/>) (Mulder et al., 2005). Oligonucleotide primers used in this study were designed using PrimerQuest® Design Tool (<http://sg.idtdna.com/site>) and synthesized by Bioneer (Daejeon, South Korea).

Vector Construction for Fluorescence Microscopy

Plasmid pIGPAPA containing a *GFP* gene under the control of the *Neurospora crassa* lyase gene promoter and a geneticin resistance gene (*gen*) cassette under the control of the *A. nidulans trpC* promoter was provided from the Fungal Plant Pathology Laboratory of Seoul National University, Seoul, South Korea (Horwitz et al., 1999; Lee et al., 2008). To generate a CsPOM1-GFP vector, a 5.8-kb PCR product including 1.7 kb of the 5'-flanking promoter region and *CsPOM1* gene sequence was amplified from WT genomic DNA using the primers GFP_Pom1_F/GFP_Pom1_R (Supplementary Table S1). The pIGPAPA vector containing a *GFP* gene was linearized by PCR amplification using the primers GFP_Pom1_VF/GFP_Pom1_VR. The amplified 5.8-kb PCR product was cloned into the linearized pIGPAPA vector using an overlap DNA Cloning Kit (Elpis Biotech, Daejeon, South Korea).

Generation of Knockout Mutant and Gene Expression Analysis

Oligonucleotide primers used for PCR reactions are listed in Supplementary Table S1. Genomic DNA was extracted according to a standard method (Sambrook et al., 1989). Approximately 1.5 kb fragments of upstream and downstream of *CsPOM1* were amplified from WT genomic DNA using primers 5F/5R and 3F/3R, respectively. The amplified upstream and downstream fragments of *CsPOM1* were fused to the 1.5-kb *hyg* cassette amplified from plasmid pBCATPH via the double-joint PCR method (Yu et al., 2004; Choi et al., 2009). The resultant fusion was finally amplified using primers NF/NR and used as a knockout construct. Protoplast generation and transformation of *C. scovillei* were carried out according to previous work (Shin et al., 2019). Targeted gene deletion was confirmed with Southern blot hybridization and RT-PCR. For complementation, *CsPOM1* fragments including 1.9 kb upstream and 600 bp downstream were amplified from WT genomic DNA using primers CsPom1_cmF/R. The amplified fragments were transformed into protoplasts of *CsPOM1* deletion mutant with plasmid pII99 containing a geneticin resistance gene (*gen*) cassette (Yi et al., 2009).

For quantitative PCR (qPCR), total RNA was extracted using the Easy-Spin Total RNA Extraction Kit (Intron Biotechnology, Seoul, South Korea) from frozen fungal tissues according to the manufacturer's instructions. First-strand cDNA was synthesized with 5 µg of total RNA using the oligo (dT) primer with the

SuperScript™ III First-Strand Synthesis System kit (Invitrogen™ Life Technologies, CA, USA). qPCR was performed on the StepOne Real-Time PCR System (Applied Biosystems, CA, USA) using the HIPI Real-Time PCR 2x Master Mix (Elpis, Daejeon, South Korea) as previously described (Han et al., 2015). Ct values were normalized to that of *β-tubulin* (CAP_007327) and expressed as relative values, with 1 corresponding to WT (Kim et al., 2009). Experiments were conducted in triplicate and repeated three times.

Microscopy Methods

C. scovillei photographs were routinely obtained using a Carl Zeiss Axio Image A2 microscope (Carl Zeiss Microscope Division, Oberkochen, Germany) equipped with differential interference contrast (DIC) and fluorescence optics. Iodine solution (60 mg of KI and 10 mg of I₂/ml of sterile distilled water) was used to stain the glycogen in conidia (Thines et al., 2000). To stain nuclei, diamidino-2-phenylindole (DAPI) (Sigma-Aldrich, USA) was dissolved in sterile distilled water to yield a 10-µg/ml staining solution (10×) (Miyakawa et al., 1984). Conidial septa were stained with a combination of Calcofluor White (1 mg of Calcofluor White M2R and 0.5 mg of Evans blue) (Sigma-Aldrich, USA) and 10% potassium hydroxide (KOH) in a 1:1 ratio.

Phenotype Analysis

Vegetative growth was evaluated by measuring the diameter of colonies at 6 days postinoculation on CM agar. To assess the role of *CsPOM1* in hydrophobicity of aerial hyphae, 10 µl drops of water or 0.1% sodium dodecyl sulfate (SDS), respectively, were placed on the surface of the *CsPOM1* deletion mutant grown on V8 agar for 5 days in the dark. Conidiation was measured by counting the number of conidia harvested with 5 ml of sterile distilled water from 6-day-old V8 agar under continuous light, using a hemocytometer. Conidial morphology was observed under a microscope, and the conidial length was measured using the ZEN imaging software. Conidial germination and appressorium formation were measured after 6 and 16 h postinoculation of conidial drops (5×10^4 conidia/ml) on coverslips, respectively. To observe appressorium formation from hyphal tips, mycelial agar plugs were obtained from 3-day-old OMA plates and placed on slide glasses. The mycelial agar plugs were covered with coverslips and incubated for 3 days in a moistened box.

Infection Assays

For appressorial penetration and invasive growth assays, conidial drops (5×10^4 conidia/ml) collected from 5-day-old OMA were inoculated onto the surface of green pepper fruits and incubated in a moistened box. To visualize invasive hyphae, we used GFP-expressing strains generated with the pIGPAPA vector. The fruit surface was peeled off using a razor blade as thin as possible, and the appressorial penetration and invasive growth were examined under a microscope. For the disease development assay, conidial drops (15×10^4 conidia/ml) collected from 5-day-old OMA were inoculated onto the surface of pepper fruits and incubated for 14

days in a moistened box. All experiments were repeated three times with three replicates.

RESULTS

Identification of *CsPOM1* in *C. scovillei* KC05

We identified *CsPOM1* (locus CAP_000731), a homolog of *Schizosaccharomyces pombe* *POM1*, through BLAST searches conducted using the Comparative Fungal Genomics Platform (Bähler and Pringle, 1998; Park et al., 2006). The *CsPOM1* gene was predicted to encode a protein of 1,345 amino acids with a putative protein kinase catalytic domain near the C-terminus, and the protein shared 67.4% (positives 80.9%) amino acid identity with the *S. pombe* *Pom1* in their kinase domains. *Pom1* is a DYRK subfamily member (Aranda et al., 2011). Several members of the DYRK subfamily, including DYRK1A, DYRK1B (Mirk), DYRK2, DYRK3 (REDK), DYRK4, Yak1, and *Pom1*, have been identified in eukaryotes (Garrett et al., 1991; Bähler and Pringle, 1998; Goyard et al., 2008; Aranda et al., 2011). Among them, *Pom1* and *Yak1* were found in fungi (Garrett et al., 1991; Bähler and Pringle, 1998; Goyard et al., 2008). Phylogenetic analysis of *CsPOM1* and related proteins in other fungal species revealed that *Pom1* homologs form a separate lineage distinct from *Yak1* homologs (Supplementary Figure S1A). *Pom1* homologs of *C. higginsianum*, *Fusarium oxysporum*, *M. oryzae*, *N. crassa*, *Botrytis cinerea*, *A. nidulans*, and *S. pombe* showed 98.0%, 93.3%, 97.7%, 95.3%, 90.6%, and 84.9% amino acid identity, respectively, to *CsPOM1* in their kinase domains (Supplementary Figure S1B). Domain structure analysis confirmed that all DYRKs contain a DYRK homology (DH) box that regulates kinase activity (Becker and Joost, 1998) in the N-terminal of the catalytic domain (Supplementary Figure S1B).

Generation of the $\Delta CsPom1$ mutant

We generated targeted gene deletion mutant $\Delta CsPom1$ to characterize the functions of *CsPOM1* in fungal development and pathogenicity (Supplementary Figure S2A). Approximately 120 putative deletion transformants for the *CsPOM1* gene were selected on TB3 agar containing hygromycin B (200 ppm). Among the putative transformants, five candidates were selected by screening PCR, and the $\Delta CsPom1$ mutant was finally selected based on Southern blot hybridization (Supplementary Figure S2B). RT-PCR confirmed that the expression of the *CsPOM1* gene was completely abolished in the deletion mutant (Supplementary Figure S2C). The expression of the *CsPOM1* gene was restored in the complemented strain.

Vegetative Growth of the $\Delta CsPom1$ Mutant

To investigate the roles of *CsPOM1* in the vegetative growth of *C. scovillei*, we measured the vegetative growth of the wild-type (WT) and $\Delta CsPom1$ mutant on CM agar. After 6 days of incubation, the $\Delta CsPom1$ mutant exhibited reduced vegetative

growth compared with WT (20% reduction in average) (Figure 1A). Tolerance to environmental stress is important for the normal growth of fungi (Huang et al., 2017). To investigate whether *CsPOM1* is involved in stress tolerance, we inoculated mycelial agar plugs of the WT and mutant on CM agar supplemented with an osmotic stress agent, followed by incubation for 6 days. Relative growth was compared with WT on CM agar without supplements. The vegetative growth of mutant on CM agar supplemented with 0.5 M NaCl or 0.5 M KCl (osmotic stress) was significantly reduced compared with WT (38% and 41% reduction, respectively), which exhibits more severe reductions than that on CM agar without supplements (20% reduction) (Figure 1A). The results indicate that *CsPOM1* is involved in the tolerance to osmotic stress of *C. scovillei*.

Surface Hydrophobicity of the $\Delta CsPom1$ Mutant

To assess the role of *CsPOM1* in the surface hydrophobicity of *C. scovillei*, 10 μ l drops of water or 0.1% SDS, respectively, were placed on the surface of WT and $\Delta CsPom1$ mutant grown on V8 agar for 5 days. We found that the drops of water and 0.1% SDS were suspended on the aerial hyphae of the WT, whereas they were immediately soaked into the mycelia of $\Delta CsPom1$ (Figure 1B). We identified a putative class II hydrophobin gene *CsMHP1* (CAP_004537) in *C. scovillei*, a homolog of *M. oryzae* *MHP1* (MG01173) sharing 46.1% amino acid identity (Kim et al., 2005), and compared the relative gene expression by quantitative PCR (qPCR). We found that expression of the *CsMHP1* gene was significantly reduced in the $\Delta CsPom1$ mutant compared to WT (Figure 1C). Collectively, these results indicate that *CsPOM1* is required for surface hydrophobicity, which is likely associated with the expression of the *CsMHP1* gene.

CsPOM1 Is Important for Conidial Production, Conidial Morphology, and Glycogen Metabolism

Conidial production of the $\Delta CsPom1$ mutant was quantified on V8 agar. Quantitative analysis of conidial production showed that the $\Delta CsPom1$ mutant produced significantly fewer conidia than WT (Figure 2A). This phenotype of the $\Delta CsPom1$ mutant was rescued in complemented strain *CsPom1c*. Next, we collected conidia of the mutants from 10-day-old OMA and examined the conidial morphology. The $\Delta CsPom1$ mutant produced longer conidia (15.3 ± 0.17 in length) than those of the WT (10.3 ± 0.16 μ m in length) (Figure 2B). In addition, approximately 34% of the $\Delta CsPom1$ mutant conidia were slightly bent, while most of the WT conidia were straight in shape (Figures 2B, C). Collectively, these data indicate that *CsPOM1* is involved in the regulation of conidial production and conidial size.

We also examined glycogen deposits in conidia. Iodine staining of 10-day-old conidia revealed that only 71% of the $\Delta CsPom1$ mutant conidia contained glycogen, compared with more than 90% of WT conidia (Figures 3A, B). Longer incubation (up to 20 days) further decreased the rate of glycogen-containing conidia, down to 29% in the $\Delta CsPom1$

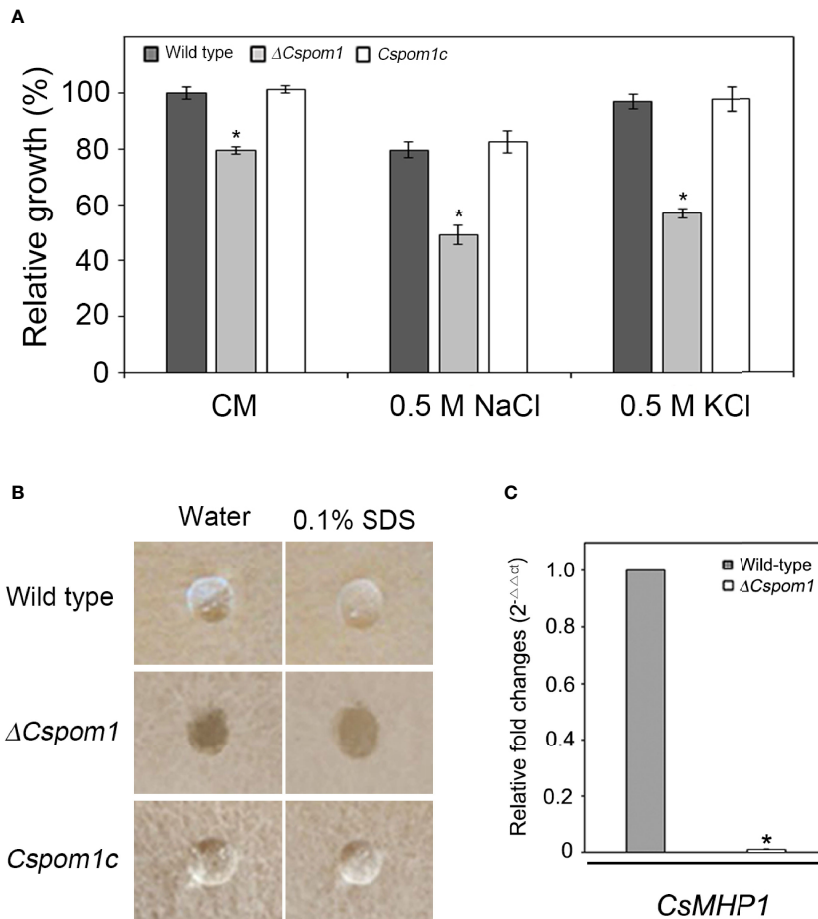


FIGURE 1 | Vegetative growth and surface hydrophobicity of the $\Delta Cspom1$ mutant. **(A)** Fungal colonies were grown on CM agar with and without an osmotic stress agent (0.5 M NaCl or KCl) for 6 days at 25°C in the dark. Relative growth was compared with WT grown on CM agar. Asterisk indicates significant difference between WT and mutant according to Tukey's test at $p < 0.05$. **(B)** First, 10 μ l water or 0.1% SDS were placed on the surface of each strain grown on V8 agar for 5 days in the dark. **(C)** Measurement of the expression of the *CsMHP1* gene encoding a putative class II hydrophobin (CAP_004537) in the $\Delta Cspom1$ mutant. Expression of the *CsMHP1* gene was measured by qPCR, normalized to β -tubulin, and expressed as a relative value of 1 in the WT.

mutant, while most of the WT conidia still contained glycogen (**Figures 3A, B**). We identified a putative glycogen debranching gene in *C. scovillei*, i.e., *CsGDB1*, which is a homolog of *S. cerevisiae GDB1* (AJW05075) sharing 47.0% amino acid identity, and compared the relative gene expression during vegetative growth in CM broth by qPCR. The qPCR analysis confirmed that expression of the glycogen debranching gene (CAP_006079) was significantly higher in the $\Delta Cspom1$ mutant compared with WT (**Figure 3C**). Taken together, these results indicate that *CsPOM1* is involved in the regulation of glycogen metabolism in a conidial age-dependent manner.

***CsPOM1* Regulates Conidial Cell Division and Is Localized in the Region of Septum Formation**

Pom1 kinase regulates cell division in the fission yeast *S. pombe* (Bähler and Pringle, 1998). Therefore, we examined conidial septation and nuclear division during appressorium formation

on hydrophobic coverslips. The WT conidia were aseptate and uninucleate at 0 h, which was visualized by Calcofluor White (**Figure 4A**) and DAPI staining (**Figure 4B**), respectively. By 2 h, the conidia formed two uninuclear cells separated by a septum, and a single germ tube emerged (**Figure 4B**). The tip of the germ tube began to swell and produced an appressorium. A second round of nuclear division started, resulting in another nucleus in the appressorium by 8 h (**Figure 4B**). However, approximately 32% of the $\Delta Cspom1$ conidia were already septate (**Figure 4A**) and two-celled (**Figure 4B**) at 0 h, in which most of the two-celled conidia were slightly bent in shape. These results suggest that *CsPOM1* may regulate conidial cell division in *C. scovillei*.

Next, we tagged the 3' end of *CsPOM1* with sequences encoding a green fluorescent protein (GFP) and transformed the construct into WT protoplasts to investigate the intracellular localization of *CsPOM1*. Conidia of the WT transformant were inoculated on hydrophobic coverslips, and the GFP-tagged Pom1 was monitored. The fluorescent analysis demonstrated

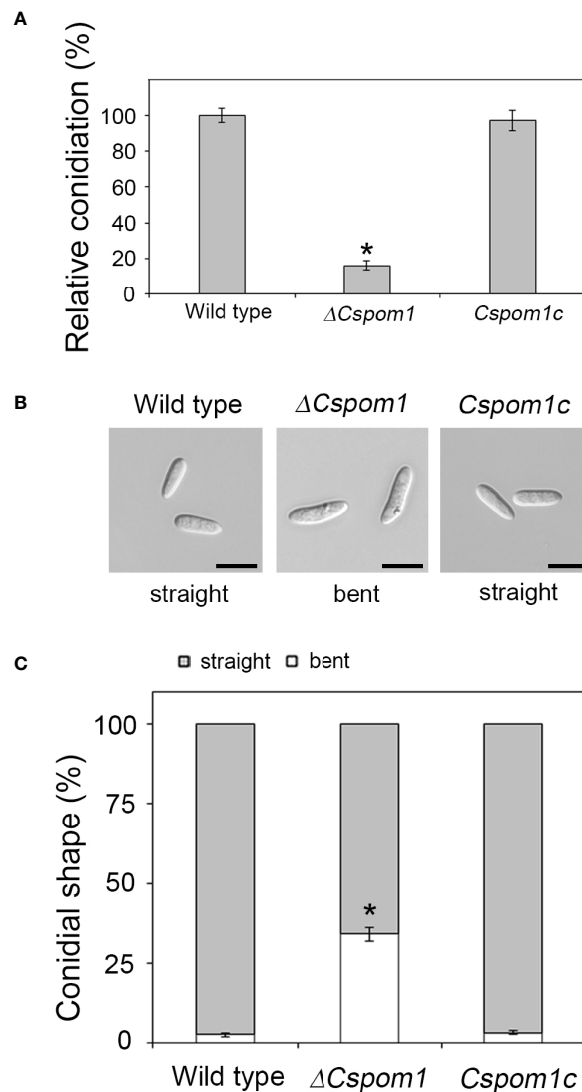


FIGURE 2 | Abnormal conidial production of the $\Delta Cspom1$ mutant. **(A)** Quantitative measurement of conidia at 6 days postinoculation on V8 agar at 25°C under continuous light. Asterisk indicates a significant difference at $p < 0.05$ according to Tukey's test. **(B)** Conidial morphology of the $\Delta Cspom1$ mutant. Scale bars, 10 μ m. **(C)** Quantitative comparison of abnormal conidia (bent shape). Conidia were harvested from 10-day-old OMA. The shape of the conidia was observed under a microscope. Asterisk indicates a significant difference at $p < 0.05$ according to Tukey's test.

that CsPom1-GFP was localized to the nucleus in conidia (**Figure 5A**). The GFP signal at the nucleus in conidia became weaker during germination (**Figure 5B**). CsPOM1-GFP was also observed in the region of septum formation in conidia at 30 min postinoculation (**Figure 5B**). The GFP signal was very faint and became weaker during germination (**Figure 5B**).

CsPOM1 Is Essential for Appressorium Formation in Conidia and Hyphal Tips

To investigate the functional role of CsPOM1 in appressorium formation, we collected conidia of the WT and mutants (5×10^5 conidia/ml) from 6-day-old OMA plates and inoculated conidial drops on coverslips. The experiment revealed that the $\Delta Cspom1$

mutant was normal in germination but defective in appressorium formation on hydrophobic coverslips (**Figures 6A, B**). At 16 h postinoculation, approximately 90% of the WT conidia formed melanized appressoria (**Figures 6B, C**). However, only 8% of the $\Delta Cspom1$ mutant conidia formed appressoria (**Figures 6B, C**). Similarly, appressorium formation from hyphal tips was significantly reduced in the $\Delta Cspom1$ mutant (**Figure 6D**). These results suggest that CsPOM1 is essential for appressorium formation from germ tube and hyphal tips. Treatment of conidia with synthetic cutin monomers or exogenous cyclic adenosine monophosphate (cAMP) is a method to induce appressorium formation in some fungi (Lee and Dean, 1994; Fu et al., 2021). We treated $\Delta Cspom1$ mutant conidia with 10 μ M cutin monomers and 5

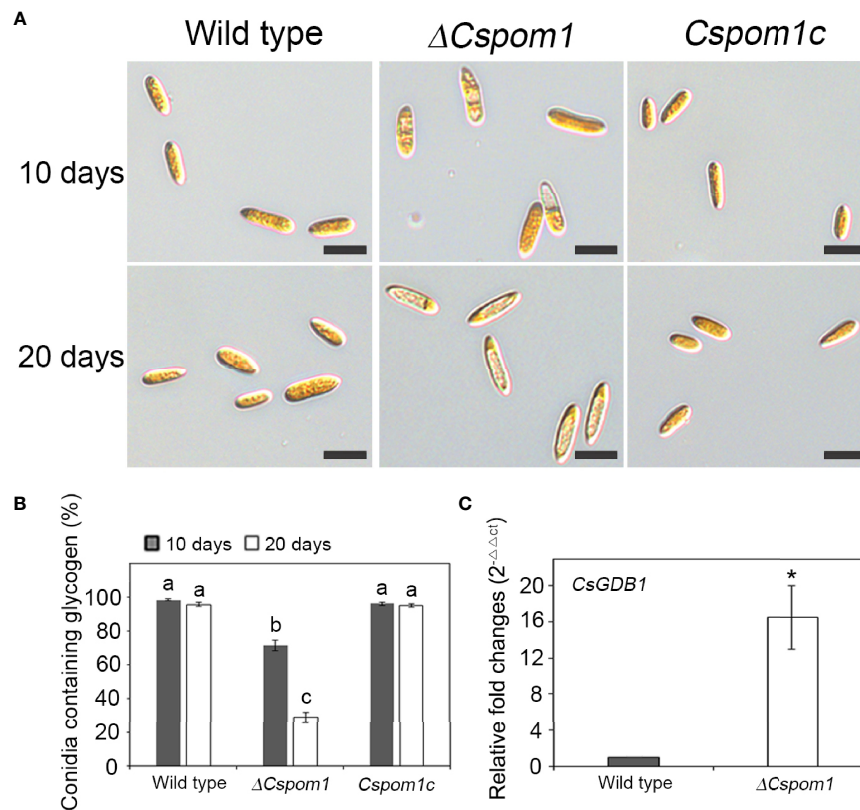


FIGURE 3 | Glycogen metabolism of the $\Delta Cspom1$ mutant in conidia. **(A)** Conidia harvested from 10- or 20-day-old OMA were stained with iodine solution (2 \times , 60 mg of KI and 10 mg of I₂/ml of sterile distilled water). Scale bars, 10 μ m. **(B)** Quantitative measurement of conidia containing glycogen. Different letters on bars indicate significant differences at $p < 0.05$ according to Tukey's test. **(C)** Measurement of the expression of the *CsGDB1* gene encoding a putative glycogen debranching enzyme (CAP_006079) in the $\Delta Cspom1$ mutant. Expression of the *CsGDB1* gene was measured by qPCR, normalized to β -tubulin, and expressed as a relative value of 1 in the WT. Asterisk indicates a significant difference between the WT and mutant at $p < 0.05$ according to two-tailed *t*-test.

mM exogenous cAMP, respectively. The cutin monomer treatment partially restored appressorium formation of the $\Delta Cspom1$ mutant (Figures 6B, C). In contrast, the cAMP treatment did not restore appressorium formation of the $\Delta Cspom1$ mutant (Supplementary Figure S3), which indicates that CsPOM1 functions downstream of cAMP or in a cAMP-independent manner.

CsPOM1 Is Required for Early Infection

Next, we investigated the roles of *CsPOM1* in fungal virulence on pepper fruit. We inoculated conidial drops (15×10^4 conidia/ml) collected from 5-day-old OMA plates onto the surface of pepper fruits (*C. annuum* L. cv. Gilsang) and incubated them in a moistened box. After 2 weeks, the WT caused typical anthracnose lesions on the pepper fruits, while the $\Delta Cspom1$ mutant produced tiny lesions on the fruits (Figure 7A). To investigate initial pepper fruit infection by the mutant, we generated GFP-expressing transformants and inoculated conidia on pepper fruits. Most of the WT conidia (>90%) formed appressoria on the pepper fruits, penetrated the plant cuticle by 24 h (Figure 7B), and formed a primary hypha in the plant epidermal cell by 72 h (Figure 7C). Unlike

the appressorium formation on coverslips (Figure 6B), most of the $\Delta Cspom1$ conidia (>80%) formed appressoria on the pepper fruits by 24 h, although there was a slight reduction in appressorium formation compared with the WT (Supplementary Figure S4). However, the mutant conidia exhibited delayed penetration and invasive growth during infection compared with WT (Figures 7B, C). Collectively, these results suggest that *CsPOM1* is involved in penetration and invasive growth during early infection on pepper fruits.

DISCUSSION

The DYRK kinase Pom1 is well-known to provide positional information for cell division in the fission yeast *S. pombe*, but the roles of Pom1 in filamentous fungi are not well characterized. In this study, we functionally characterized *CsPOM1*, a homolog of *S. pombe* *POM1*, in the anthracnose fungus *C. scovillei*. The $\Delta Cspom1$ mutant exhibited reduced vegetative growth on CM agar compared with the WT (Figure 1A). In the fission yeast *S. pombe*, deletion of *POM1* results in failure of switching from unipolar to bipolar growth (Bähler and Pringle, 1998). In the

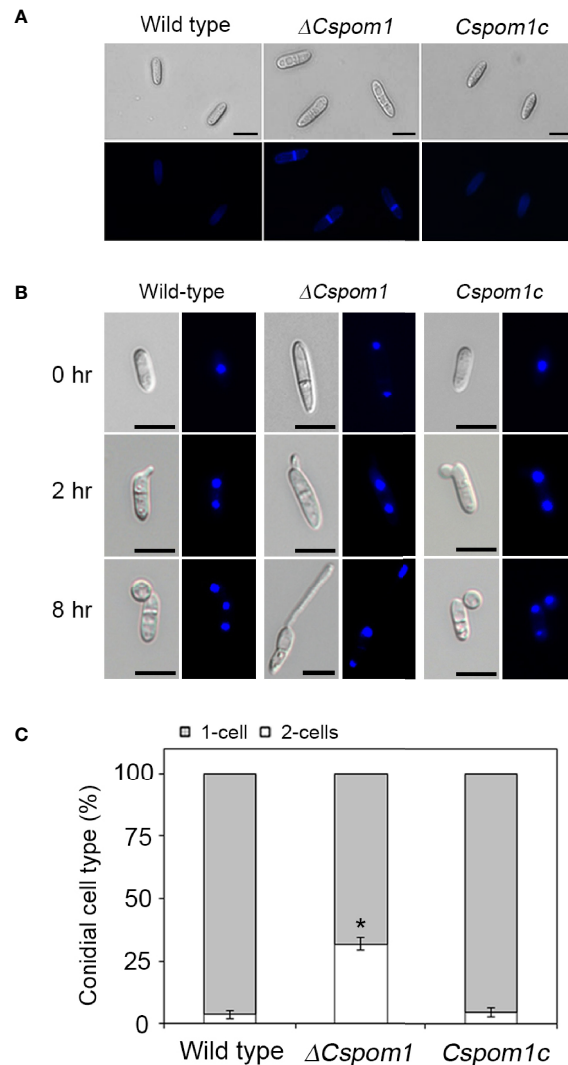


FIGURE 4 | Conidial cell division of the $\Delta Cspom1$ mutant. **(A)** Septation of conidia on coverslips (0 h). The conidial septa were stained with Calcofluor White (1 mg Calcofluor White M2R and 0.5 mg Evans blue). Scale bars, 10 μ m. **(B)** Nuclear division during conidial germination and appressorium development on coverslips. The conidial nuclei were stained with DAPI (1 μ g/ml). Scale bars, 10 μ m. **(C)** Quantitative comparison of the number of conidial cells (one- or two-celled) between the WT and mutant. Experiments were conducted in triplicate and repeated three times ($n \geq 100$ conidia per strain). Asterisk indicates a significant difference according to Tukey's test at $p < 0.05$.

filamentous fungus *F. graminearum*, the vegetative growth of the *Fgpom1* mutant was slightly reduced on potato dextrose agar media (Wang et al., 2011). Thus, our results agree with previous reports that *POM1* is involved in cell growth. In addition, the growth defect of the $\Delta Cspom1$ mutant became more severe when it was exposed to osmotic stress (NaCl or KCl). This suggests that *CsPOM1* is involved in osmoregulation, which might affect the basic growth phenotype of the $\Delta Cspom1$ mutant.

Hydrophobins are small, hydrophobic proteins that provide a hydrophobic coating on aerial hyphae and conidia in fungi (Wessels et al., 1991; Kim et al., 2005; Pham et al., 2016). The hydrophobic surfaces of fungal pathogens are associated with conidial production, and the conidia surfaces are coated to

reduce wettability and promote surface interaction (Pham et al., 2016). In *M. oryzae*, deletion of *MHP1*, a class II hydrophobin gene, results in a loss of surface hydrophobicity and reduction in conidiation (Kim et al., 2005). In this study, the $\Delta Cspom1$ mutant exhibited a loss of hydrophobicity, reduced conidiation, and reduced expression of *CsMHP1* compared with WT (**Figures 1, 2**). Thus, we suggest that *CsPOM1* is involved in the transcriptional regulation of the *CsMHP1* gene in *C. scovillei*, in which the reduced expression of the *CsMHP1* gene caused the defects in surface hydrophobicity and conidiation in the $\Delta Cspom1$ mutant.

Conidia of *C. scovillei* were uninucleated single cells that became two-celled just before germination and differentiated

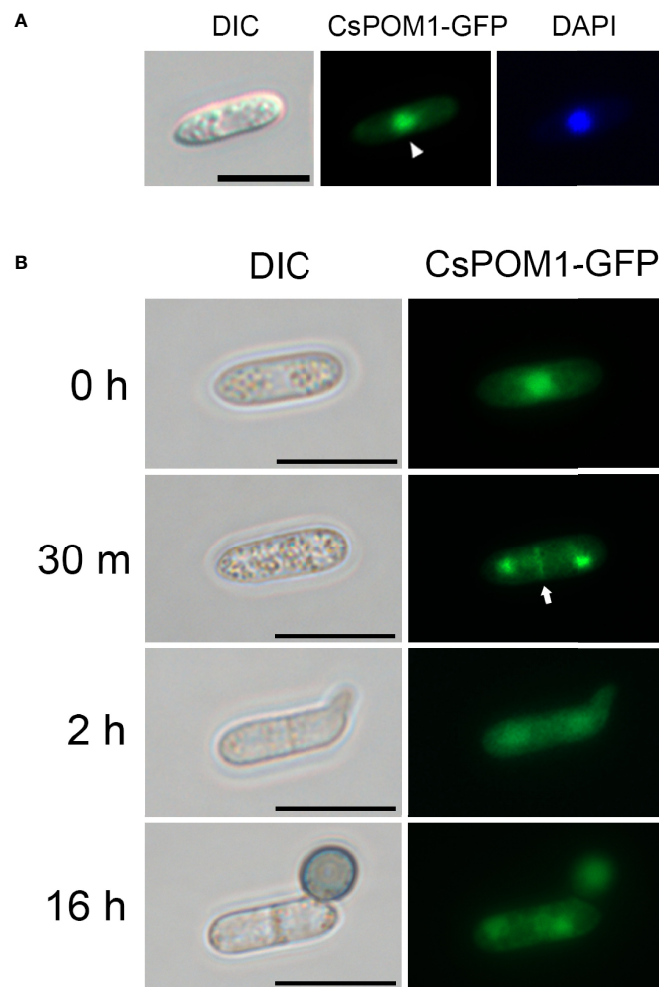


FIGURE 5 | Intracellular localization of CsPom1-GFP fusion. Wild-type conidia expressing CsPom1-GFP fusion were inoculated on coverslips and incubated in a moistened box. **(A)** Nuclear localization of CsPOM1-GFP fusion. The conidial nuclei were stained with DAPI (1 μg/ml). Scale bar, 10 μm. **(B)** Localization of CsPOM1-GFP fusion during conidial germination and appressorium formation. Arrow indicates the region of septum formation in conidia. Scale bars, 10 μm.

into an appressorium with one nucleus (**Figures 4A, B**). In the fission yeast *S. pombe*, Pom1 forms a polar gradient at the cell ends inhibiting Cdr2 activity, which causes a delay in mitotic entry (Moseley et al., 2009; Gerganova et al., 2019). Deletion of *POM1* in *S. pombe* results in premature entry into mitosis (Moseley et al., 2009). Consistently, approximately 32% of the conidia produced by the $\Delta Cspom1$ mutant were already two-celled before inoculation of the conidia on an inductive surface (**Figure 4A–C**). Thus, we suggest that *CsPOM1* has an important role in the negative regulation of cell division in *C. scovillei*. In *S. pombe*, Pom1 is localized at both cell ends and also localized to the cell division site (Bähler and Pringle, 1998). As cell division proceeds, the concentration of Pom1 at both cell ends decreases while the concentration at the cell center increases (Bähler and Pringle, 1998; Celton-Morizur et al., 2006). In our study, we found that CsPOM1-GFP fusion is localized in the region of septum formation in conidia after a 30-min incubation on hydrophobic coverslips, which is approximately the time at

which nuclear division starts (**Figure 5B**). Consistent with *S. pombe* Pom1, this suggests that CsPOM1 provides positional information for cell division in *C. scovillei*. Unlike the *S. pombe* Pom1, the localization of CsPOM1-GFP fusion in the nuclei of ungerminated conidia were noticeable (**Figure 5A**), although some DYRK family kinases, including DYRK1A and Yak1, are localized to the nuclei (Moriya et al., 2001; Kaczmarek et al., 2014; Han et al., 2015). This may reflect divergent roles for *CsPOM1* in the filamentous fungus, as shown in pleiotropic defects of the $\Delta Cspom1$ while the cellular roles of the protein await elucidation.

Glycogen is one of the major energy stores in fungi (Talbot, 1995). Glycogen is utilized during conidial germination and appressorium development, and is involved in the supply of energy during infection in fungi (Thines et al., 2000; Badaruddin et al., 2013). In our study, glycogen deposits in conidia of the $\Delta Cspom1$ mutant were already degraded before conidial germination (**Figures 3A, B**). In addition, the mutant exhibited

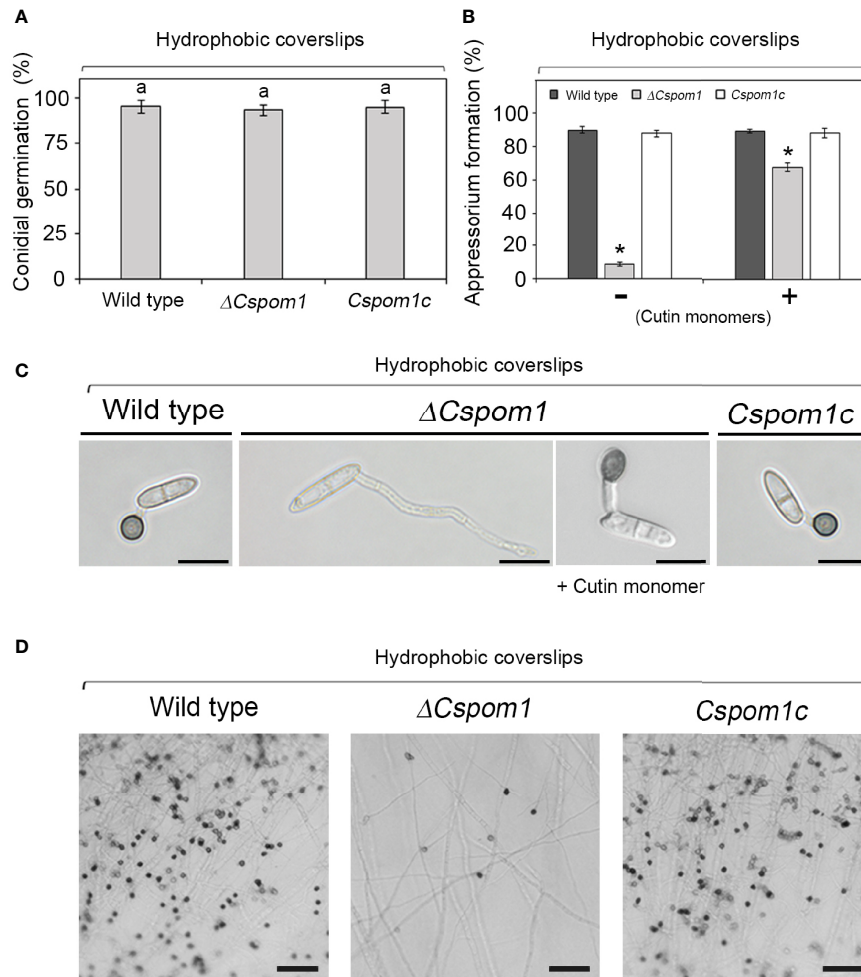


FIGURE 6 | Appressorium formation of the $\Delta Cspom1$ mutant. **(A)** Quantitative measurement of conidial germination at 6 h postinoculation on coverslips. Experiments were conducted in triplicate and repeated three times ($n \geq 100$ conidia per strain). The same letters on bars indicate there was no significant difference according to Tukey's test. **(B)** Quantitative measurement of appressorium formation of conidia with or without 10 μM cutin monomers at 16 h postinoculation on coverslips. Experiments were conducted in triplicate and repeated three times ($n \geq 100$ conidia per strain). Asterisks indicate significant differences according to Tukey's test at $p < 0.05$. **(C)** Appressorium formation of conidia on coverslips. Scale bars, 10 μm . **(D)** Appressorium formation from hyphal tips. Mycelial agar plugs from 3-day-old OMA were placed on slide glasses and covered with coverslips. Photographs were taken at 3 days postinoculation on coverslips. Scale bars, 50 μm .

delayed plant penetration, resulting in tiny and restricted lesions on pepper fruits (Figure 7). We found that expression of the *CsGDB1* gene in the $\Delta Cspom1$ mutant was significantly increased compared with WT (Figure 3C). Thus, it is likely that *CsPOM1* acts as a negative regulator inhibiting the expression of the glycogen debranching gene, *CsGDB1* in *C. scovillei*, which is likely associated with the abnormal glycogen degradation and reduced virulence in the $\Delta Cspom1$ mutant.

Many phytopathogenic fungi recognize various host signaling factors including surface hydrophobicity, cutin monomers, and leaf waxes to develop appressoria (Adachi and Hamer, 1998; Kang et al., 1999; Dickman et al., 2003). In this study, the $\Delta Cspom1$ mutant exhibited significantly reduced appressorium formation on hydrophobic coverslips, but the defect was restored on the plant surface (Supplementary Figure S4) and by cutin

monomer treatment (Figure 6). Therefore, we think that *CsPOM1* contributes to the recognition of hydrophobic surface for appressorium formation in *C. scovillei*. In rice blast fungus *M. oryzae*, targeted deletion of class I hydrophobin *MPG1* or class II hydrophobin *MHP1* results in reduced appressorium formation on hydrophobic surface, and infectious growth in plant cells is significantly limited (Talbot et al., 1993; Kim et al., 2005). The addition of cAMP restored appressorium formation in the *MPG1* deletion mutant, but not in the *MHP1* deletion mutant, indicating that Mhp1 may function downstream of cAMP for appressorium formation in *M. oryzae* (Talbot et al., 1993; Kim et al., 2005). In our study, the addition of cAMP did not restore appressorium formation in the $\Delta Cspom1$ mutant (Supplementary Figure S3). Considering that the $\Delta Cspom1$ mutant exhibited significantly reduced expression of the

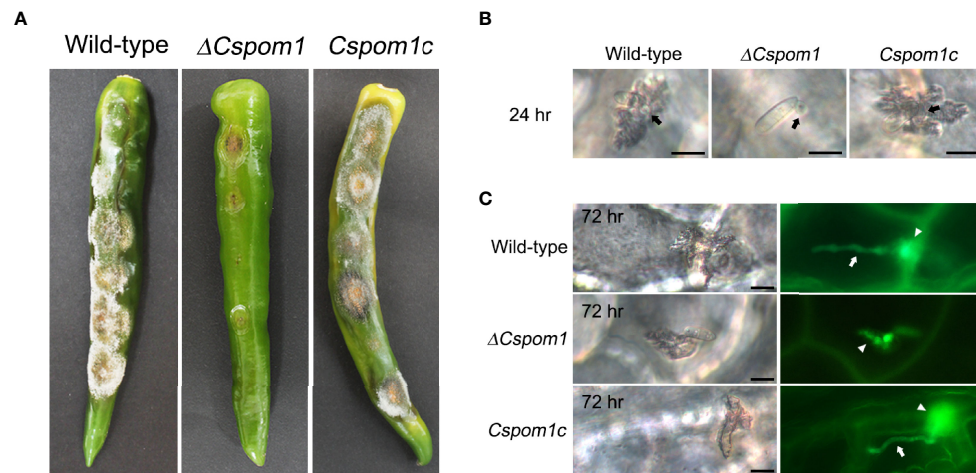


FIGURE 7 | CsPom1 is important for plant penetration and invasive growth. **(A)** Conidial drops (15×10^4 conidia/ml) of WT and $\Delta Cspom1$ mutant were inoculated onto the surface of green pepper fruits (*Capsicum annuum* L. cv. Gilsang). Photographs were taken at 14 days postinoculation. **(B)** Green pepper fruits were inoculated with conidial drops (5×10^4 conidia/ml) of the WT and $\Delta Cspom1$ mutant. Photographs were taken at 24 h postinoculation **(B)** and 72 h postinoculation **(C)**. Black arrows indicate appressorium. White arrows indicate primary hypha in a plant epidermal cell. White arrowheads indicate fungal hypha in plant cuticle. Scale bars, 10 μ m.

CsMHP1 gene, *CsPOM1* may function downstream of cAMP or a cAMP-independent manner for appressorium formation in *C. scovillei*. On pepper fruits, the $\Delta Cspom1$ mutant was able to form appressoria, probably *via* the sensing of signals on the plant surface, but the mutant showed a delay in penetration and infectious growth in plant cells (Figure 7). Therefore, the reduction of $\Delta Cspom1$ in virulence may be related to the defects in plant penetration and growth of invasive hyphae after penetration. *POM1* has not been studied in the context of appressorium formation in other fungi, but the *YAK1* kinase gene, belonging to the DYRK subfamily with *POM1*, is required for appressorium formation on the hydrophobic surface in *M. oryzae* (Han et al., 2015). Similar to the $\Delta Cspom1$ mutant, the $\Delta Moyak1$ mutant in *M. oryzae* was able to form appressoria on the plant surface, but the appressoria were defective in plant penetration. Thus, these two genes belonging to the DYRK subfamily appear to have similar roles in the appressorium formation of phytopathogenic fungi.

Based on the pleiotropic roles and nuclear localization of *CsPOM1*, we speculate that *CsPOM1* may play diverse roles in phosphorylating regulators in signaling pathways for fungal development and metabolism. Supportively, a recent study reported that PomA regulates the HOG MAPK pathway in *A. nidulans* (Zhou et al., 2019). Two HOG MAPKs, SakA and MpkC, regulate the expression of *gdbA*, a glycogen debranching gene, during glycogen metabolism in *A. fumigatus* (de Assis et al., 2018). Our study revealed that *CsPOM1*, a DYRK kinase gene, plays important roles in fungal morphogenesis and disease development in *C. scovillei*, which provide fundamental basis to understand anthracnose diseases on fruits caused by *Colletotrichum* species.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

Conceived and designed the experiments: JHS, HYK, TF, KHL, KSK. Performed the experiments: JHS, HYK, TF, KHL. Analyzed the data: JHS, HYK, TF, KSK. Wrote the paper: JHS, KSK. Originated research leading up to this paper and provided guidance and review: JHS, HYK, KSK.

FUNDING

This study was supported by Basic Science Research Program through the National Research Foundation of Korea grant (NRF-2020R1A2C100550700) funded by the Ministry of Education, Science and Technology.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fcimb.2022.861915/full#supplementary-material>

REFERENCES

- Adachi, K., and Hamer, J. E. (1998). Divergent cAMP Signaling Pathways Regulate Growth and Pathogenesis in the Rice Blast Fungus *Magnaporthe Grisea*. *Plant Cell* 10, 1361–1373. doi: 10.1105/tpc.10.8.1361
- Aranda, S., Laguna, A., and Luna, S. D. L. (2011). DYRK Family of Protein Kinases: Evolutionary Relationships, Biochemical Properties, and Functional Roles. *FASEB J.* 25, 449–462. doi: 10.1096/fj.10-165837
- Asakura, M., Ninomiya, S., Sugimoto, M., Oku, M., Yamashita, S. I., Okuno, T., et al. (2009). Atg26-Mediated Pexophagy is Required for Host Invasion by the Plant Pathogenic Fungus *Colletotrichum Orbiculare*. *Plant Cell* 21, 1291–1304. doi: 10.1105/tpc.108.060996
- Badaruddin, M., Holcombe, L. J., Wilson, R. A., Wang, Z.-Y., Kershaw, M. J., and Talbot, N. J. (2013). Glycogen Metabolic Genes are Involved in Trehalose-6-Phosphate Synthase-Mediated Regulation of Pathogenicity by the Rice Blast Fungus *Magnaporthe Oryzae*. *PLoS Pathog.* 9, e1003604. doi: 10.1371/journal.ppat.1003604
- Bähler, J., and Pringle, J. R. (1998). Pom1p, a Fission Yeast Protein Kinase That Provides Positional Information for Both Polarized Growth and Cytokinesis. *Genes Dev.* 12, 1356–1370. doi: 10.1101/gad.12.9.1356
- Becker, W., and Joost, H.-G. (1998). Structural and Functional Characteristics of Dyrk, a Novel Subfamily of Protein Kinases With Dual Specificity. *Prog. Nucleic Acid Res. Mol. Biol.* 62, 1–17. doi: 10.1016/S0079-6603(08)60503-6
- Becker, W., Weber, Y., Wetzel, K., Eirmbter, K., Tejedor, F. J., and Joost, H.-G. (1998). Sequence Characteristics, Subcellular Localization, and Substrate Specificity of DYRK-Related Kinases, a Novel Family of Dual Specificity Protein Kinases. *J. Biol. Chem.* 273, 25893–25902. doi: 10.1074/jbc.273.40.25893
- Caires, N. P., Pinho, D. B., Souza, J. S. C., Silva, M. A., Lisboa, D. O., Pereira, O. L., et al. (2014). First Report of Anthracnose on Pepper Fruit Caused by *Colletotrichum Scovillei* in Brazil. *Plant Dis.* 98, 1437–1437. doi: 10.1094/PDIS-04-14-0426-PDN
- Cannon, P., Damm, U., Johnston, P., and Weir, B. (2007). *Colletotrichum*—current Status and Future Directions. *Stud. Mycol.* 59, 129–145. doi: 10.3114/sim0014
- Celton-Morizur, S., Racine, V., Sibarita, J. B., and Paoletti, A. (2006). Pom1 Kinase Links Division Plane Position to Cell Polarity by Regulating Mid1p Cortical Distribution. *J. Cell Sci.* 119, 4710–4718. doi: 10.1242/jcs.03261
- Choi, J., Kim, Y., Kim, S., Park, J., and Lee, Y.-H. (2009). *MoCRZ1*, a Gene Encoding a Calcineurin-Responsive Transcription Factor, Regulates Fungal Growth and Pathogenicity of *Magnaporthe Oryzae*. *Fungal Genet. Biol.* 46, 243–254. doi: 10.1016/j.fgb.2008.11.010
- Dean, R., Van Kan, J. A., Pretorius, Z. A., Hammond-Kosack, K. E., Di Pietro, A., Spanu, P. D., et al. (2012). The Top 10 Fungal Pathogens in Molecular Plant Pathology. *Mol. Plant Pathol.* 13, 414–430. doi: 10.1111/j.1364-3703.2011.00783.x
- de Assis, L. J., Manfollí, A., Mattos, E., Fabri, J. H. M., Malavazi, I., Jacobsen, I. D., et al. (2018). Protein Kinase A and High-Osmolarity Glycerol Response Pathways Cooperatively Control Cell Wall Carbohydrate Mobilization in *Aspergillus Fumigatus*. *MBio* 9, e01952-18. doi: 10.1128/mBio.01952-18
- Diao, Y.-Z., Zhang, C., Liu, F., Wang, W.-Z., Liu, L., Cai, L., et al. (2017). *Colletotrichum* Species Causing Anthracnose Disease of Chili in China. *Pers.: Mol. Phylogeny. Evol. Fungi.* 38, 20. doi: 10.3767/003158517X692788
- Dickman, M. B., Ha, Y. S., Yang, Z., Adams, B., and Huang, C. (2003). A Protein Kinase From *Colletotrichum Trifolii* is Induced by Plant Cutin and is Required for Appressorium Formation. *Mol. Plant-Microbe Interact.* 16, 411–421. doi: 10.1094/MPMI.2003.16.5.411
- Duran, R., Cary, J. W., and Calvo, A. M. (2010). Role of the Osmotic Stress Regulatory Pathway in Morphogenesis and Secondary Metabolism in Filamentous Fungi. *Toxins* 2, 367–381. doi: 10.3390/toxins2040367
- Fu, T., Han, J. H., Shin, J. H., Song, H., Ko, J., Lee, Y. H., et al. (2021). Homeobox Transcription Factors are Required for Fungal Development and the Suppression of Host Defense Mechanisms in the *Colletotrichum Scovillei*-Pepper Pathosystem. *Mbio* 12, e01620-21. doi: 10.1128/mBio.01620-21
- Fu, T., Shin, J. H., Lee, N. H., Lee, K. H., and Kim, K. S. (2022). Mitogen-Activated Protein Kinase *CsPMK1* is Essential for Pepper Fruit Anthracnose by *Colletotrichum Scovillei*. *Front. Microbiol.* 13. doi: 10.3389/fmicb.2022.770119
- Garrett, S., Menold, M. M., and Broach, J. R. (1991). The *Saccharomyces Cerevisiae* YAK1 Gene Encodes a Protein Kinase That is Induced by Arrest Early in the Cell Cycle. *Mol. Cell. Biol.* 11, 4045–4052. doi: 10.1128/mcb.11.8.4045-4052.1991
- Gerganova, V., Floderer, C., Archetti, A., Michon, L., Carlini, L., Reichler, T., et al. (2019). Multi-Phosphorylation Reaction and Clustering Tune Pom1 Gradient Mid-Cell Levels According to Cell Size. *Elife* 8, e45983. doi: 10.7554/eLife.45983
- Goyard, S., Knechtle, P., Chauvel, M., Mallet, A., Prévost, M. C., Proux, C., et al. (2008). The Yak1 Kinase is Involved in the Initiation and Maintenance of Hyphal Growth in *Candida Albicans*. *Mol. Biol. Cell* 19, 2251–2266. doi: 10.1091/mbc.e07-09-0960
- Hagiwara, D., Asano, Y., Marui, J., Yoshimi, A., Mizuno, T., and Abe, K. (2009). Transcriptional Profiling for *Aspergillus Nidulans* HogA MAPK Signaling Pathway in Response to Fludioxonil and Osmotic Stress. *Fungal Genet. Biol.* 46, 868–878. doi: 10.1016/j.fgb.2009.07.003
- Han, J.-H., Chon, J.-K., Ahn, J.-H., Choi, I.-Y., Lee, Y.-H., and Kim, K. S. (2016). Whole Genome Sequence and Genome Annotation of *Colletotrichum Acutatum*, Causal Agent of Anthracnose in Pepper Plants in South Korea. *Genom. Data* 8, 45–46. doi: 10.1016/j.gdata.2016.03.007
- Han, J. H., Lee, H. M., Shin, J. H., Lee, Y. H., and Kim, K. S. (2015). Role of the *MoYAK1* Protein Kinase Gene in *Magnaporthe Oryzae* Development and Pathogenicity. *Environ. Microbiol.* 17, 4672–4689. doi: 10.1111/1462-2920.13010
- Hohmann, S. (2009). Control of High Osmolarity Signalling in the Yeast *Saccharomyces Cerevisiae*. *FEBS Lett.* 583, 4025–4029. doi: 10.1016/j.febslet.2009.10.069
- Horwitz, B. A., Sharon, A., Lu, S. W., Ritter, V., Sandrock, T. M., Yoder, O. C., et al. (1999). AG Protein Alpha Subunit From *Cochliobolus Heterostrophus* Involved in Mating and Appressorium Formation. *Fungal Genet. Biol.* 26, 19–32. doi: 10.1006/fgbi.1998.1094
- Huang, L., Zhang, S., Yin, Z., Liu, M., Li, B., Zhang, H., et al. (2017). *MoVrp1*, a Putative Verprolin Protein, is Required for Asexual Development and Infection in the Rice Blast Fungus *Magnaporthe Oryzae*. *Sci Rep* 7, 1–16. doi: 10.1038/srep41148
- Kaczmarek, W., Barua, M., Mazur-Kolecka, B., Frackowiak, J., Dowjat, W., Mehta, P., et al. (2014). Intracellular Distribution of Differentially Phosphorylated Dual-Specificity Tyrosine Phosphorylation-Regulated Kinase 1A (DYRK1A). *J. Neurosci. Res.* 92, 162–173. doi: 10.1002/jnr.23279
- Kang, S. H., Khang, C. H., and Lee, Y. H. (1999). Regulation of cAMP-Dependent Protein Kinase During Appressorium Formation in *Magnaporthe Grisea*. *FEMS Microbiol. Lett.* 170, 419–423. doi: 10.1111/j.1574-6968.1999.tb13403.x
- Kanto, T., Uematsu, S., Tsukamoto, T., Moriwaki, J., Yamagishi, N., Usami, T., et al. (2014). Anthracnose of Sweet Pepper Caused by *Colletotrichum Scovillei* in Japan. *J. Gen. Plant Pathol.* 80, 73–78. doi: 10.1007/s10327-013-0496-9
- Kentrup, H., Becker, W., Heukelbach, J., Wilmes, A., Schürmann, A., Huppertz, et al. (1996). Dyrk, a Dual Specificity Protein Kinase With Unique Structural Features Whose Activity is Dependent on Tyrosine Residues Between Subdomains VII and VIII. *J. Biol. Chem.* 271, 3488–3495. doi: 10.1074/jbc.271.7.3488
- Khalimi, K., Darmadi, A. A. K., and Suprpta, D. N. (2019). First Report on the Prevalence of *Colletotrichum Scovillei* Associated With Anthracnose on Chili Pepper in Bali, Indonesia. *Int. J. Agric. Biol.* 22, 363–368. doi: 10.17957/IJAB/15.1072
- Kim, S., Ahn, I. P., Rho, H. S., and Lee, Y. H. (2005). *MHP1*, a *Magnaporthe Grisea* Hydrophobin Gene, is Required for Fungal Development and Plant Colonization. *Mol. Microbiol.* 57, 1224–1237. doi: 10.1111/j.1365-2958.2005.04750.x
- Kim, S., Park, S.-Y., Kim, K. S., Rho, H.-S., Chi, M.-H., Choi, J., et al. (2009). Homeobox Transcription Factors are Required for Conidiation and Appressorium Development in the Rice Blast Fungus *Magnaporthe Oryzae*. *PLoS Genet.* 5, e1000757. doi: 10.1371/journal.pgen.1000757
- Lee, Y.-H., and Dean, R. A. (1994). Hydrophobicity of Contact Surface Induces Appressorium Formation in *Magnaporthe Grisea*. *FEMS Microbiol. Lett.* 115, 71–75. doi: 10.1111/j.1574-6968.1994.tb06616.x
- Lee, J., Leslie, J. F., and Bowden, R. L. (2008). Expression and Function of Sex Pheromones and Receptors in the Homothallic Ascomycete *Gibberella Zeae*. *Eukaryot. Cell* 7, 1211–1221. doi: 10.1128/EC.00272-07
- Liang, X., Wei, T., Cao, M., Zhang, X., Liu, W., Kong, Y., et al. (2019). The MAP Kinase *CsPMK1* is a Key Regulator of Pathogenesis, Development, and Stress

- Tolerance of *Colletotrichum Fruticola*. *Front. Microbiol.* 10. doi: 10.3389/fmicb.2019.01070
- Liu, F., Tang, G., Zheng, X., Li, Y., Sun, X., Qi, X., et al. (2016). Molecular and Phenotypic Characterization of *Colletotrichum* Species Associated With Anthracnose Disease in Peppers From Sichuan Province, China. *Sci. Rep.* 6, 1–17. doi: 10.1038/srep32761
- Mehrabi, R., Zhao, X., Kim, Y., and Xu, J.-R. (2009). “The cAMP Signaling and MAP Kinase Pathways in Plant Pathogenic Fungi,” in *Plant Relationships*, Vol. 5 (Berlin: Springer-Heidelberg), 157–172. doi: 10.1007/978-3-540-87407-2_8
- Miyakawa, I., Aoi, H., Sando, N., and Kuroiwa, T. (1984). Fluorescence Microscopic Studies of Mitochondrial Nucleoids During Meiosis and Sporulation in the Yeast, *Saccharomyces Cerevisiae*. *J. Cell Sci.* 66, 21–38. doi: 10.1242/jcs.66.1.21
- Moriya, H., Shimizu-Yoshida, Y., Omori, A., Iwashita, S., Katoh, M., and Sakai, A. (2001). Yak1p, a DYRK Family Kinase, Translocates to the Nucleus and Phosphorylates Yeast Pop2p in Response to a Glucose Signal. *Genes Dev.* 15, 1217–1228. doi: 10.1101/gad.884001
- Moseley, J. B., Mayeux, A., Paoletti, A., and Nurse, P. (2009). A Spatial Gradient Coordinates Cell Size and Mitotic Entry in Fission Yeast. *Nature* 459, 857–860. doi: 10.1038/nature08074
- Mulder, N. J., Apweiler, R., Attwood, T. K., Bairoch, A., Bateman, A., Binns, D., et al. (2005). InterPro, Progress and Status in 2005. *Nucleic Acids Res.* 33, D201–D205. doi: 10.1093/nar/gki106
- Noor, N. M., and Zakaria, L. (2018). Identification and Characterization of *Colletotrichum* Spp. Associated With Chili Anthracnose in Peninsular Malaysia. *Eur. J. Plant Pathol.* 151, 961–973. doi: 10.1007/s10658-018-1431-x
- Park, G., Xue, C., Zhao, X., Kim, Y., Orbach, M., and Xu, J.-R. (2006). Multiple Upstream Signals Converge on the Adaptor Protein Mst50 in *Magnaporthe Grisea*. *Plant Cell* 18, 2822–2835. doi: 10.1105/tpc.105.038422
- Pham, C. L., Rey, A., Lo, V., Soullès, M., Ren, Q., Meisl, G., et al. (2016). Self-Assembly of MPG1, a Hydrophobin Protein From the Rice Blast Fungus That Forms Functional Amyloid Coatings, Occurs by a Surface-Driven Mechanism. *Sci. Rep.* 6, 1–16. doi: 10.1038/srep25288
- Saito, H., and Posas, F. (2012). Response to Hyperosmotic Stress. *Genetics* 192, 289–318. doi: 10.1534/genetics.112.140863
- Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989). *Molecular cloning: A Laboratory Manual* (New York: Cold Spring Harbor Laboratory Press).
- Shin, J.-H., Han, J.-H., Park, H.-H., Fu, T., and Kim, K. S. (2019). Optimization of Polyethylene Glycol-Mediated Transformation of the Pepper Anthracnose Pathogen *Colletotrichum Scovillei* to Develop an Applied Genomics Approach. *Plant Pathol. J.* 35, 575. doi: 10.5423/PPJ.OA.06.2019.0171
- Takano, Y., Kikuchi, T., Kubo, Y., Hamer, J. E., Mise, K., and Furusawa, I. (2000). The *Colletotrichum Lagenarium* MAP Kinase Gene *CMK1* Regulates Diverse Aspects of Fungal Pathogenesis. *Mol. Plant-Microbe Interact.* 13, 374–383. doi: 10.1094/MPML.2000.13.4.374
- Talbot, N. J. (1995). Having a Blast: Exploring the Pathogenicity of *Magnaporthe Grisea*. *Trends Microbiol.* 3, 9–16. doi: 10.1016/S0966-842X(00)88862-9
- Talbot, N. J., Ebbole, D. J., and Hamer, J. E. (1993). Identification and Characterization of *MPG1*, a Gene Involved in Pathogenicity From the Rice Blast Fungus *Magnaporthe Grisea*. *Plant Cell* 5, 1575–1590. doi: 10.1105/tpc.5.11.1575
- Tamura, K., Stecher, G., Peterson, D., Filipowski, A., and Kumar, S. (2013). MEGA6: Molecular Evolutionary Genetics Analysis Version 6.0. *Mol. Biol. Evol.* 30, 2725–2729. doi: 10.1093/molbev/mst197
- Tatebayashi, K., Yamamoto, K., Tanaka, K., Tomida, T., Maruoka, T., Kasukawa, E., et al. (2006). Adaptor Functions of Cdc42, Ste50, and Sho1 in the Yeast Osmoregulatory HOG MAPK Pathway. *EMBO J.* 25, 3033–3044. doi: 10.1038/sj.emboj.7601192
- Thines, E., Weber, R. W., and Talbot, N. J. (2000). MAP Kinase and Protein Kinase A-dependent Mobilization of Triacylglycerol and Glycogen During Appressorium Turgor Generation by *Magnaporthe Grisea*. *Plant Cell* 12, 1703–1718. doi: 10.1105/tpc.12.9.1703
- Thompson, J. D., Higgins, D. G., and Gibson, T. J. (1994). CLUSTAL W: Improving the Sensitivity of Progressive Multiple Sequence Alignment Through Sequence Weighting, Position-Specific Gap Penalties and Weight Matrix Choice. *Nucleic Acids Res.* 22, 4673–4680. doi: 10.1093/nar/22.22.4673
- Van Wuytswinkel, O., Reiser, V., Siderius, M., Kelders, M., Ammerer, G., Ruis, H., et al. (2000). Response of *Saccharomyces Cerevisiae* to Severe Osmotic Stress: Evidence for a Novel Activation Mechanism of the HOG MAP Kinase Pathway. *Mol. Microbiol.* 37, 382–397. doi: 10.1046/j.1365-2958.2000.02002.x
- Wang, C., Zhang, S., Hou, R., Zhao, Z., Zheng, Q., Xu, Q., et al. (2011). Functional Analysis of the Kinome of the Wheat Scab Fungus *Fusarium Graminearum*. *PLoS Pathog.* 7, e1002460. doi: 10.1371/journal.ppat.1002460
- Wessels, J., De Vries, O., Asgeirsdottir, S., and Springer, J. (1991). The *Thn* Mutation of *Schizophyllum Commune*, Which Suppresses Formation of Aerial Hyphae, Affects Expression of the Sc3 Hydrophobin Gene. *Microbiology* 137, 2439–2445. doi: 10.1099/00221287-137-10-2439
- Wilson, R. A., and Talbot, N. J. (2009). Under Pressure: Investigating the Biology of Plant Infection by *Magnaporthe Oryzae*. *Nat. Rev. Microbiol.* 7, 185–195. doi: 10.1038/nrmicro2032
- Wu, C., Jansen, G., Zhang, J., Thomas, D. Y., and Whiteway, M. (2006). Adaptor Protein Ste50p Links the Ste11p MEKK to the HOG Pathway Through Plasma Membrane Association. *Genes Dev.* 20, 734–746. doi: 10.1101/gad.1375706
- Xu, J.-R., and Hamer, J. E. (1996). MAP Kinase and cAMP Signaling Regulate Infection Structure Formation and Pathogenic Growth in the Rice Blast Fungus *Magnaporthe Grisea*. *Genes Dev.* 10 (21), 2696–2706. doi: 10.1101/gad.10.21.2696
- Yi, M., Chi, M.-H., Khang, C. H., Park, S.-Y., Kang, S., Valent, B., et al. (2009). The ER Chaperone LHS1 is Involved in Asexual Development and Rice Infection by the Blast Fungus *Magnaporthe Oryzae*. *Plant Cell* 21, 681–695. doi: 10.1105/tpc.107.055988
- Yu, J.-H., Hamari, Z., Han, K.-H., Seo, J.-A., Reyes-Domínguez, Y., and Scazzocchio, C. (2004). Double-Joint PCR: A PCR-Based Molecular Tool for Gene Manipulations in Filamentous Fungi. *Fungal Genet. Biol.* 41, 973–981. doi: 10.1016/j.fgb.2004.08.001
- Zhao, X., Mehrabi, R., and Xu, J. R. (2007). Mitogen-Activated Protein Kinase Pathways and Fungal Pathogenesis. *Eukaryot. Cell* 6, 1701–1714. doi: 10.1128/EC.00216-07
- Zhao, W., Wang, T., Chen, Q., Chi, Y., Swe, T., and Qi, R. (2016). First Report of *Colletotrichum Scovillei* Causing Anthracnose Fruit Rot on Pepper in Anhui Province, China. *Plant Dis.* 100, 2168–2168. doi: 10.1094/PDIS-04-16-0443-PDN
- Zhou, X., Ye, J., Zheng, L., Jiang, P., and Lu, L. (2019). A New Identified Suppressor of Cdc7p/SepH Kinase, PomA, Regulates Fungal Asexual Reproduction via Affecting Phosphorylation of MAPK-HogA. *PLoS Genet.* 15, e1008206. doi: 10.1371/journal.pgen.1008206

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher’s Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Copyright © 2022 Shin, Kim, Fu, Lee and Kim. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.