

A proper PiCAT2 level is critical for sporulation, sporangium function, and pathogenicity of *Phytophthora infestans*

Tu-Hong Wang¹ | Xiao-Wen Wang¹ | Xiao-Qiong Zhu¹ | Qun He² | Li-Yun Guo ¹

¹College of Plant Protection and Key Lab of Pest Monitoring and Green Management, MOA, China Agricultural University, Beijing, China

²State Key Laboratory of Agrobiotechnology and MOA Key Laboratory of Soil Microbiology, College of Biological Sciences, China Agricultural University, Beijing, China

Correspondence

Li-Yun Guo, College of Plant Protection and Key Lab of Pest Monitoring and Green Management, MOA, China Agricultural University, Beijing, China.
Email: ppguo@cau.edu.cn, ppguoly@126.com

Funding information

This work was funded in part by the Special Fund for Agro-Scientific Research in the Public Interest (201303018) and the Chinese Universities Scientific Fund (2017ZB001).

Abstract

Catalase is present in prokaryotic and eukaryotic organisms and is important for the protective effects of the antioxidant system against free radicals. Many studies have confirmed that catalase is required for the growth, development, and pathogenesis of bacteria, plants, animals, and fungi. However, there has been relatively little research on the catalases in oomycetes, which form an important group of fungus-like eukaryotes that produce zoospores. In this study, we detected two *Phytophthora infestans* genes encoding catalases, but only PiCAT2 exhibited catalase activity in the sporulation stage and was highly produced during asexual reproduction and in the late infection stage. Compared with the wild-type strain, the PiCAT2-silenced *P. infestans* transformants were more sensitive to abiotic stress, were less pathogenic, and had a lower colony expansion rate and lower PiMPK7, PiVPS1, and PiGPG1 expression levels. In contrast, the PiCAT2-overexpressed transformants were slightly less sensitive to abiotic stress. Interestingly, increasing and decreasing PiCAT2 expression from the normal level inhibited sporulation, germination, and infectivity, and down-regulated PiCdc14 expression, but up-regulated PiSDA1 expression. These results suggest that PiCAT2 is required for *P. infestans* mycelial growth, asexual reproduction, abiotic stress tolerance, and pathogenicity. However, a proper PiCAT2 level is critical for the formation and normal function of sporangia. Furthermore, PiCAT2 affects *P. infestans* sporangial formation and function, pathogenicity, and abiotic stress tolerance by regulating the expression of cell cycle-related genes (PiCdc14 and PiSDA1) and MAPK pathway genes. Our findings provide new insights into catalase functions in eukaryotic pathogens.

KEYWORDS

abiotic stress, appressorium, catalase, *Phytophthora infestans*, reactive oxygen species, sporulation

This is an open access article under the terms of the Creative Commons Attribution-NonCommercial License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited and is not used for commercial purposes.

© 2020 The Authors. *Molecular Plant Pathology* published by British Society for Plant Pathology and John Wiley & Sons Ltd

1 | INTRODUCTION

Hydrogen peroxide (H_2O_2) is a reactive oxygen species (ROS) that is constantly produced intracellularly as a normal metabolic by-product through the electron transport chain in aerobic microorganisms (Apel and Hirt, 2004; Kowaltowski *et al.*, 2009). In addition, H_2O_2 is relatively stable and able to pass freely through cell membranes (Branco *et al.*, 2004). It acts as a signalling molecule during cellular differentiation and development in eukaryotes (Takemoto *et al.*, 2007; Veal and Day, 2011; Tudzynski *et al.*, 2012). However, excessive H_2O_2 is harmful for cell components, so its rapid and efficient removal is essential for aerobic organisms (Zamocky *et al.*, 2008). Moreover, microorganisms must counteract the effects of environmental H_2O_2 due to redox reactions or the oxidative burst generated by the host during an invasion (Camejo *et al.*, 2016).

The antioxidant effect of catalase involves the decomposition of H_2O_2 to form water (H_2O) and oxygen (O_2) (Kwok *et al.*, 2004). This enzyme is produced by many prokaryotic and eukaryotic organisms (Zamocky *et al.*, 2008). Together with superoxide dismutase and glutathione peroxidase, catalase plays a vital role in the antioxidant systems that protect against the adverse effects of free radicals (Blackman and Hardham, 2008; Ighodaro and Akinloye, 2018). Many studies have confirmed that catalase is required for the growth, development, and pathogenesis of various organisms (Wysong *et al.*, 1998; Schriener *et al.*, 2005; Pan *et al.*, 2017; Wang *et al.*, 2019). Moreover, organisms may contain multiple catalase genes that are required for various biological processes. For example, in *Neurospora crassa*, *CAT-1* is involved in oxidative stress responses (Wang *et al.*, 2007), whereas *CAT-3* is required for growth and differentiation as well as for oxidative stress responses (Michán *et al.*, 2003). In *Beauveria bassiana*, *CatA* affects pathogenicity and the tolerance to abiotic stresses, whereas *CatB* and *CatC* only influence the resistance to oxidative stress (Wang *et al.*, 2013). However, there has been relatively little research on catalases in oomycetes, which form an important group of filamentous, multinucleate, and non-fungal eukaryotes.

Oomycetes belong to the kingdom Stramenopila (Baldauf *et al.*, 2000; Yoon *et al.*, 2002), which includes diverse microorganisms living in marine, freshwater, and terrestrial environments (Sparrow, 1960; Karling, 1981). Many oomycetes are important pathogens of plants and animals, and cause severe economic losses. Examples include *Phytophthora infestans*, the causal pathogen of potato late blight, which resulted in the death of millions of people during the Irish potato famine in the 19th century (Birch and Whisson, 2001), and *Pythium insidiosum*, a unique pathogen of human and animals (Mendoza *et al.*, 1993). The life cycle of oomycetes in the genus *Phytophthora* has been well studied. *Phytophthora* species reproduce asexually by producing sporangia. In response to cold shock conditions, sporangia cleave into zoospores, which swim, encyst, and germinate to form mycelia or a specialized infection structure (appressorium) on plants or on hydrophobic surfaces (Erwin and Ribeiro, 1996; Hardham, 2001). Sporangia can also germinate directly to produce mycelia or form an appressorium (Nowicki *et al.*,

2012). Both sporangia and zoospores are important for dissemination and host infection (Lacy and Hammerschmidt, 1984).

Many factors, such as nutrient availability, humidity, oxygen levels, and pH, affect the formation of sporangia and the production of zoospores in *Phytophthora* species (Erwin and Ribeiro, 1996). Previous studies revealed that many genes, including those encoding cell cycle regulators, PiCdc14 (Ah-Fong and Judelson, 2003) and PcSDA1 (Zhu *et al.*, 2016), G protein β and γ subunits (Latijnhouwers and Govers, 2003; van den Hoogen *et al.*, 2018), Myb and MADS-box transcription factors (Xiang and Judelson, 2014; Leesutthiphonchai and Judelson, 2018), and the loricrin-like protein PiLLP (Guo *et al.*, 2017), are associated with the sporulation and pathogenicity of *Phytophthora* species. However, whether catalase activity affects the sporulation and pathogenicity of *Phytophthora* species remains unclear.

P. infestans is a destructive pathogen and a model oomycete species. A genomic analysis revealed that *P. infestans* has two catalase genes, *PiCAT1* (PITG_15292) and *PiCAT2* (PITG_15248). Published RNA-sequencing data from Ah-Fong *et al.* (2017) and microarray data from Haas *et al.* (2009) indicated that the *PITG_15248* expression level is up-regulated during asexual reproduction and in the late infection stage. A previous study proved that the silencing of *PiLLP* in *P. infestans* inhibits the formation of sporangia and decreases virulence as well as catalase activity (Guo *et al.*, 2017). These findings suggest that catalase may be important for the asexual reproduction and pathogenesis of oomycetes. In this study, we functionally characterized *PiCAT2* by analysing the phenotypes of *PiCAT2*-silenced and -over-expressed transformants. The results of this study indicate that a proper *PiCAT2* level is important for the growth, sporulation, sporangium function, abiotic stress tolerance, and virulence of oomycetes. Moreover, altering *PiCAT2* activity affects the expression of genes related to sporulation, abiotic stress responses, and pathogenicity.

2 | RESULTS

2.1 | *PiCAT2* is the major catalase gene in sporulation stage of *P. infestans*

A sequence analysis revealed that oomycetes contain one to four catalase genes. A phylogenetic analysis indicated that all oomycete catalases are clustered in one clade and have conserved sequences, including a typical catalase domain with no signal peptide. The *P. infestans* genome contains two catalase genes, *PiCAT1* (PITG_15292; 1,539 bp) and *PiCAT2* (PITG_15248; 1,569 bp), which are designated according to a published article (Blackman and Hardham, 2008) (Figure S1a). These two genes are located on supercontig1.35 and the encoded proteins are similar (71% amino acid sequence identity). Both proteins contain a catalase proximal active site, a proximal heme-ligand signature, and residues thought to be important for the formation of tetramers (Gouet *et al.*, 1995) (Figure S1b, Table S3). However, *PiCAT2*, but not *PiCAT1*, also has two peroxisomal-targeting signals, SKL and PRL, both belonging to PTS1, at the C-terminus (Figure S1b).

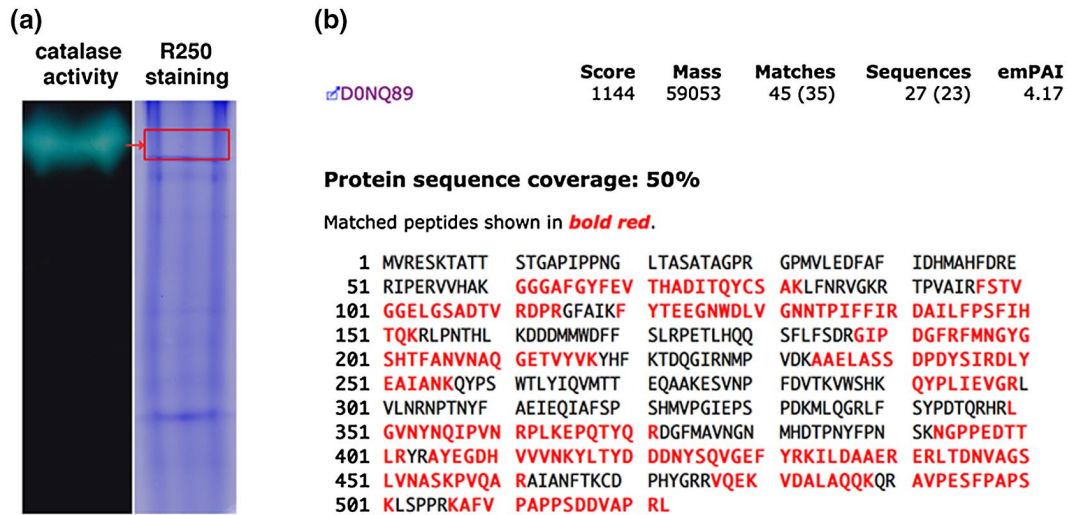


FIGURE 1 Catalase activity in sporulating hyphae of *Phytophthora infestans*. (a) In-gel assay for measuring catalase activity. The corresponding bands stained with Coomassie brilliant blue (CBB) R250 were used for mass spectrometry. (b) Results of mass spectrometry analysis of catalases

When the total proteins from sporulating *P. infestans* MX5-1 hyphae were analysed by native-PAGE, only one band was associated with catalase activity (Figure 1a). The results of a mass spectrometry analysis indicated that this band contained PiCAT2, but not PiCAT1 (Figure 1b), implying that PiCAT2 is the major catalase in the sporulation stage of *P. infestans*. Thus, we focused on PiCAT2 for the subsequent analyses.

2.2 | PiCAT2 was highly expressed during asexual reproduction and in the late infection stage

To functionally characterize PiCAT2, we analysed the PiCAT2 transcript level in *P. infestans* at various developmental and infection stages. The quantitative reverse transcription polymerase chain reaction (RT-qPCR) results revealed that PiCAT2 was highly expressed in sporangia, zoospores, cysts, and germinated cysts (Figure 2a). In infected potato leaf tissues, PiCAT2 was expressed at low levels during the early infection stage (1–2 days post-inoculation, dpi), but the expression level increased significantly in the late infection stage (3–5 dpi) (Figure 2a). These results imply that PiCAT2 may be important for *P. infestans* sporulation and the infection of susceptible hosts.

2.3 | PiCAT2 activity affected mycelial growth and sporulation

To determine whether PiCAT2 affects *P. infestans* asexual development, we generated PiCAT2-silenced transformants (STs) with a polyethylene glycol-mediated protoplast transformation method. Three STs (C2ST7, C2ST10, and C2ST33) and a control (CK) strain were obtained. They were analysed regarding the PiCAT2 expression levels (Figure 2b) and the in-gel catalase

activities of sporulating hyphae (Figure 2c,d). In addition, the ROS level increased in STs (Figure S2). An examination of the PiCAT1 expression level indicated it was slightly higher in STs than in the wild-type (WT) strain, but was much lower than the PiCAT2 expression level in the CK strain (Figure 2b). Moreover, the STs grew more slowly than the WT and CK strains ($p < .01$) and produced significantly fewer sporangia (Figure 3a–c). The ST sporangia were morphologically similar to the WT and CK sporangia (Figure 3a,d). However, the ST sporangia had a significantly lower indirect germination rate (through zoospore release) and direct germination rate (through germ-tube production) compared with the WT and CK strains (Figure 3e,f). We also assessed the expression levels of neighbouring genes *PITG_15247* and *PITG_15249* in the PiCAT2-silenced transformants. The expression levels of these two genes did not decrease consistently in the three STs compared with WT and CK strains (Figure S3). Therefore, the common characteristics of STs can be attributed to the silencing of the PiCAT2 gene. These results indicate that reducing the PiCAT2 level considerably affects the formation and the function of *P. infestans* sporangia.

We also generated PiCAT2-overexpressed transformants (OTs) via a polyethylene glycol-mediated protoplast transformation method. Three OTs (C2OT11, C2OT12, and C2OT41) were obtained, and they were verified based on the PiCAT2 expression levels (Figure 4a) and the in-gel catalase activities of sporulating hyphae (Figure 4b,c). Moreover, the ROS levels decreased in the OTs (Figure S2). The PiCAT1 transcript levels in the OTs were similar to that in the WT strain (Figure 4a). Furthermore, the OT colonies were denser than the WT and CK colonies, but there were no differences in the mycelial growth rates (Figure 5a,b). Interestingly, compared with the results for the WT, CK, and ST strains, the length:width ratio of the OT sporangia was smaller (Figure 5a,c), although no significant differences were observed in the width of sporangia. Similar to the STs, the OTs exhibited significantly inhibited sporangia production,

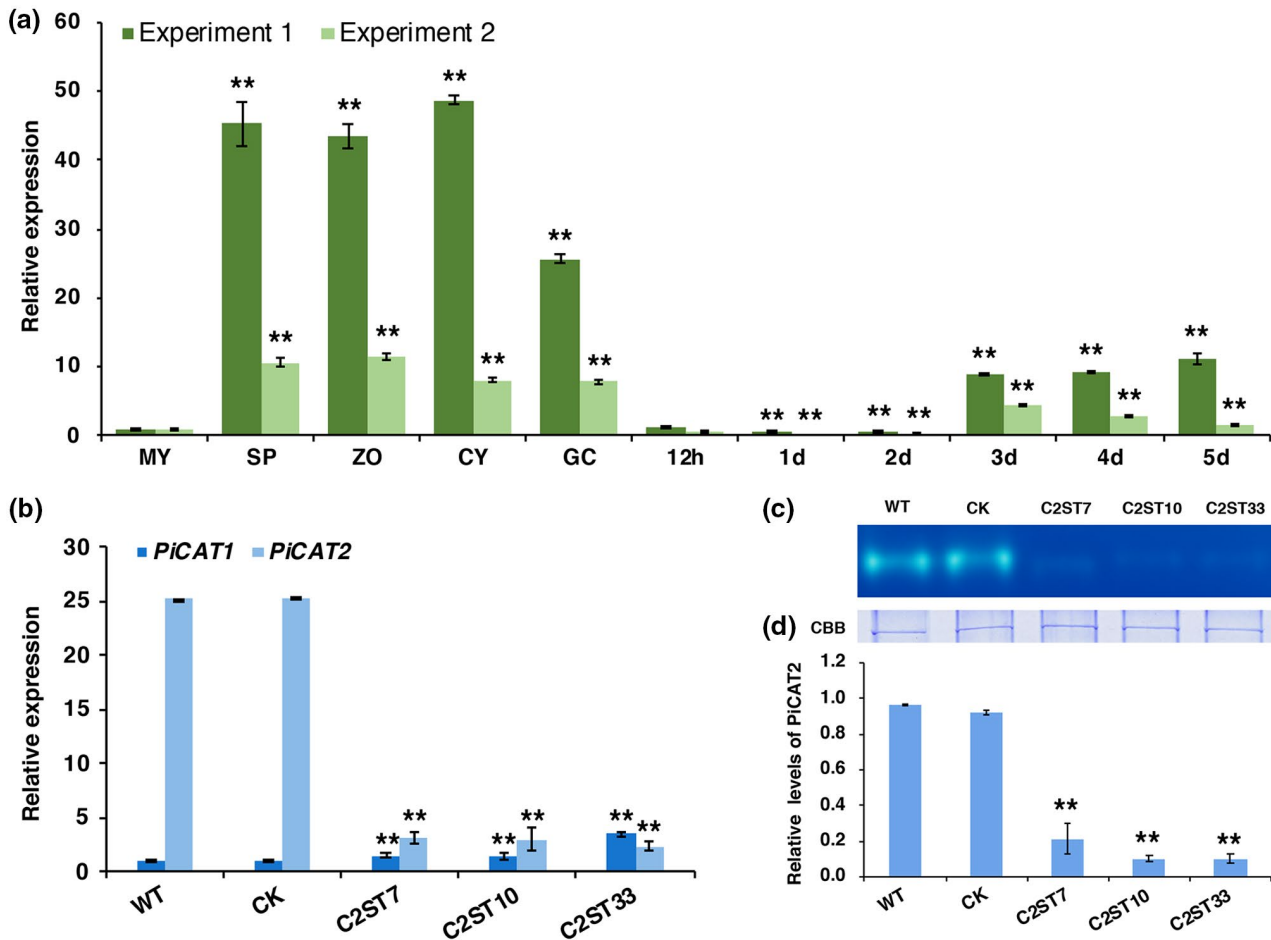


FIGURE 2 The expression pattern of mRNA and protein levels of *PiCAT2* in *Phytophthora infestans*. (a) The expression pattern of *PiCAT2* in various developmental stages. MY, mycelia; SP, sporangia; ZO, zoospores; CY, cysts; GC, germinated cysts. Leaflet of susceptible cv. Impala at 12 hr as well as at 1, 2, 3, 4, and 5 days after inoculation, in comparison to MY and at 0 hr (value 1.0). The ubiquitin-conjugating enzyme (*ubc*) gene was used as an internal control. There were significant differences between the two biological replicates, but the expression of *PiCAT2* showed the same trend in the two biological replicates. (b) Transcript levels of *PiCAT1* and *PiCAT2* in mycelia of wild-type (WT), control (CK) and *PiCAT2*-silenced transformants. The *ubc* gene was used as an internal control and the transcript level of *PiCAT1* in WT was used as a reference (value 1.0). Three replicates were used for each treatment and the whole experiment was repeated with a different set of biological samples. The data are averages (and standard errors) of values from two biological replicates. Statistical significance was analysed using Student's *t* test between WT and each transformant (***p* < .01). (c) In-gel assay of catalase activity of sporulating hyphae. (d) Quantification of the *PiCAT2* activities. Error bars show standard errors of two biological replicates

with decreased zoospore release and sporangia germination rates ($p < .01$) (Figure 5a,d,e,f). These results suggested that high *PiCAT2* levels are detrimental to *P. infestans* asexual development.

2.4 | *PiCAT2* is important for *P. infestans* pathogenicity

To determine whether *PiCAT2* is required for *P. infestans* pathogenicity, equivalent amounts of sporangia (based on the germination rate) were used to inoculate potato leaves (cv. Impala). An analysis of the infected leaves indicated the pathogenicity of the STs and OTs decreased significantly (Figure 6a,b). To determine the reasons for the decreased pathogenicity, inoculated leaf tissues were stained with 3,3'-diaminobenzidine (DAB). No differences in ROS accumulation

were detected between the leaves inoculated with the ST or OT strains and the leaves inoculated with the WT or CK strains (Figure 6a). However, the trypan blue staining results indicated there was less mycelia in the leaf tissues inoculated with STs or OTs than in the leaf tissues inoculated with the WT or CK strains. Because the appressorium produced by *P. infestans* sporangia is critical for the infection of susceptible hosts, we compared the appressorium formation of WT, CK, ST, and OT strains (Figure S4a). Appressorium formation was significantly lower for the STs and OTs ($p < .01$) than for the WT and CK strains (Figure S4b), implying that excessive and inadequate amounts of catalase adversely affect appressorium formation in *P. infestans*.

To bypass the appressorium formation stage, we used mycelial plugs as inocula. At 5 days post-inoculation, typical disease symptoms (i.e., water-soaked lesions) were observed on the leaves inoculated with WT, CK, and OT strains, but not on leaves inoculated with

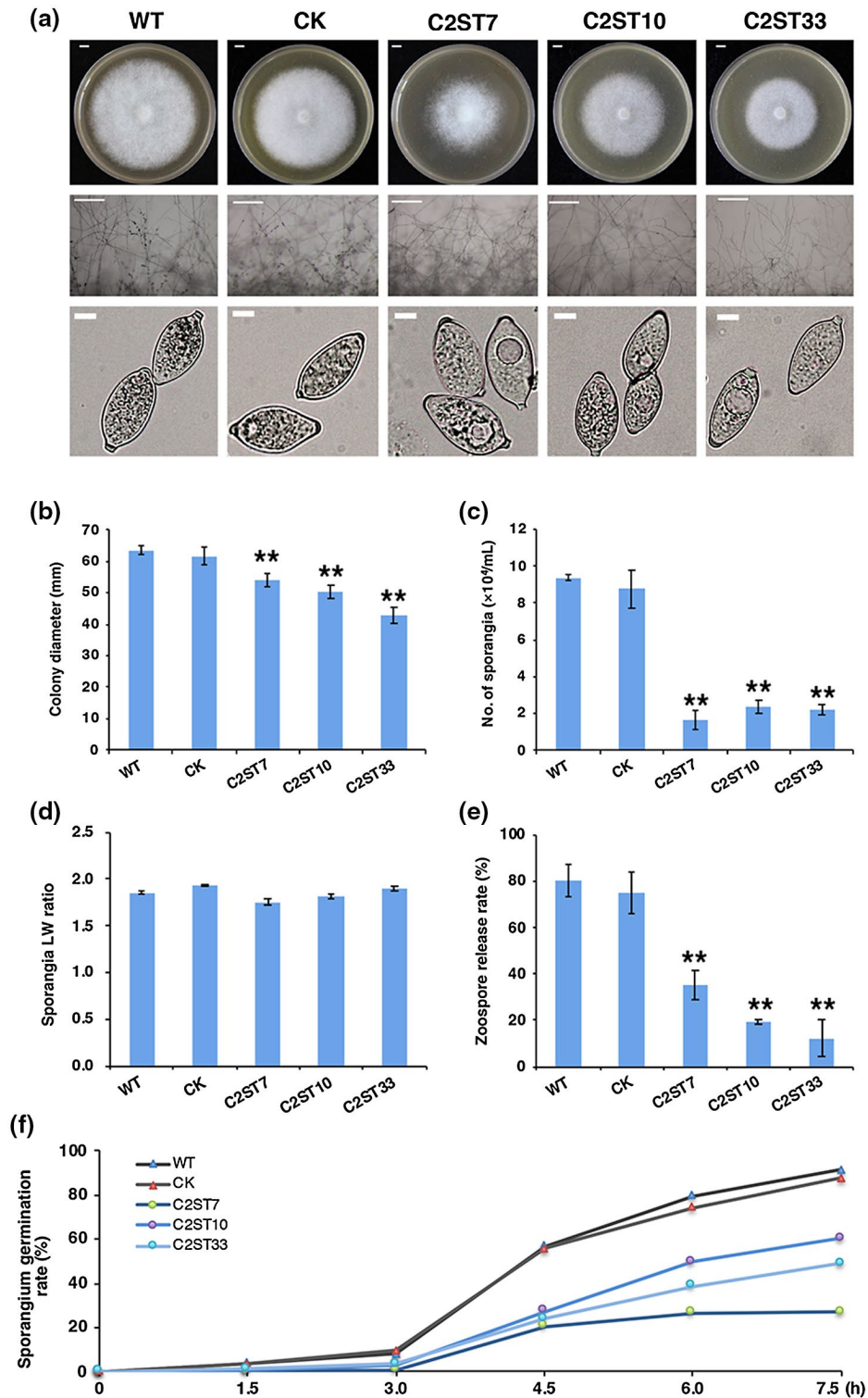


FIGURE 3 Asexual phenotypic characterization of *PiCAT2*-silenced transformants (STs). (a) Mycelial growth, sporangia production and morphology of the wild-type (WT), control (CK), and STs. Images were taken at 7 days after incubation on rye V8 agar. Bars indicate 5 mm, 0.5 mm, and 10 μm , respectively. (b) Statistics of colony diameters of WT, CK, and STs were based on 7 days of growth on rye V8 agar. (c) Number of sporangia of the WT, CK, and STs. (d) Length:width (LW) ratios from 50 sporangia per strain. (e) Sporangial suspension was incubated in a plate for 2 hr at 10 $^{\circ}\text{C}$. The ratios of the number of empty sporangia to the total number of sporangia were calculated for each strain. (f) Sporangial suspension was incubated in 200 g/L pea broth at 18 $^{\circ}\text{C}$. The numbers of germinated and ungerminated sporangia were counted under a microscope at time points (0, 1.5, 3, 4.5, 6, and 7.5 hr), and the percentages of the germinated sporangia to the total number of sporangia were calculated for each strain. Three replicates were used for each treatment and the whole experiment was repeated once. The data are averages (and standard errors) of values from two biological replicates. Statistical significance was analysed with Student's *t* test between WT and each transformant (** $p < .01$)

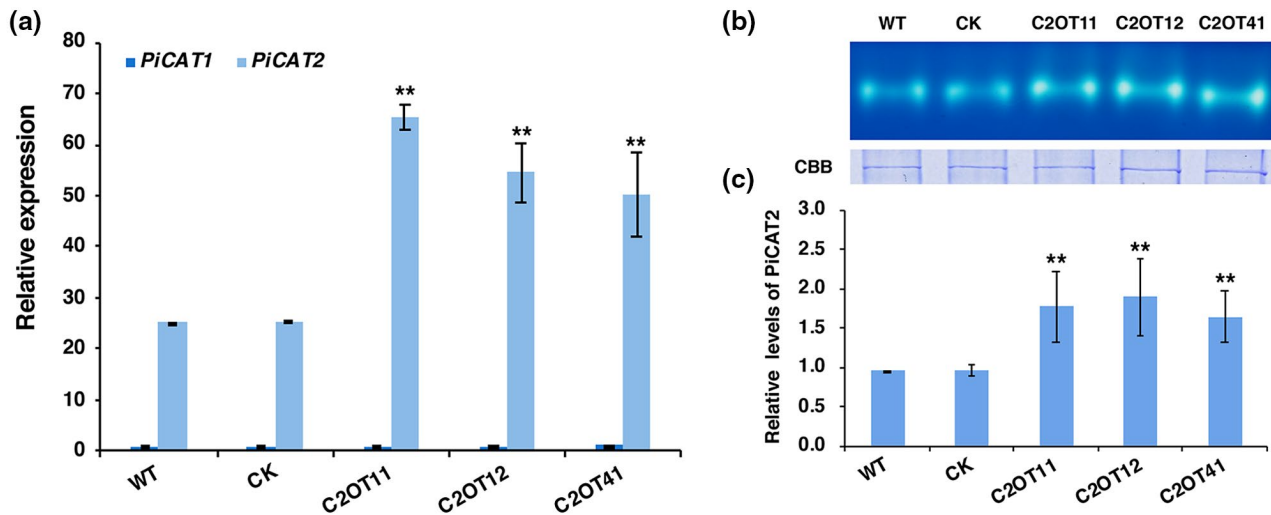


FIGURE 4 Detection of the mRNA and protein expression levels of PiCAT2 in wild-type (WT), control (CK), and PiCAT2-overexpressed transformants (OTs). (a) Transcript levels of PiCAT1 and PiCAT2 in mycelia of WT, CK and OTs. The *ubc* gene was used as an internal control and the transcript level of PiCAT1 in WT was used as a reference (value 1.0). Three replicates were used for each treatment and the whole experiment was repeated with a different set of biological samples. The data are averages (and standard errors) of values from two biological replicates. Statistical significance was analysed using Student's *t* test between WT and each transformant (** $p < .01$). (b) In-gel assay of catalase activity of sporulating hyphae. (c) Quantification of the PiCAT2 activities. Error bars show standard errors of two biological replicates

the STs (Figure 6a,c). The DAB staining results revealed significantly more ROS accumulated in the leaves inoculated with STs than in the leaves infected with the WT, CK, or OT strains. Trypan blue staining also confirmed that there were few invading hyphae in leaf tissues inoculated with STs, whereas substantial amounts of hyphae were detected in leaf tissues inoculated with the WT, CK, or OT strains (Figure 6a). This finding indicates that ST mycelia did not scavenge plant-derived ROS at the infection site.

To determine whether the lack of infection of STs is simply due to the slower growth of the STs, we added a slow-growing transformant C2-24, which grew significantly slower than STs but the PiCAT2 was not silenced (Figure S5a), in the pathogenicity test using mycelium as inoculum. At 3 dpi, the wounded and unwounded potato leaves were infected by WT, CK, and C2-24, but not the STs (Figure S5b,c). Moreover, ROS accumulation on leaves inoculated by C2-24 was not significantly different from that of WT (Figure S5d). These results show that lack of infection on leaves inoculated with the STs is not simply due to the slower growth of the silenced lines; the decreased catalase activity does play an important role.

2.5 | PiCAT2 activity affected the expression of genes related to sporulation and pathogenicity

Previous investigations proved that the cell cycle-related genes *PiCdc14* and *PcSDA1* are associated with oomycete sporulation (Ah-Fong and Judelson, 2003; Zhu *et al.*, 2016). Consequently, we analysed the expression levels of *PiCdc14* (PITG_18578) and *PiSDA1* (PITG_18755; homolog of *PcSDA1* in *P. capsici*) in the STs and OTs. The *PiCdc14* expression level was significantly lower in the STs and

OTs than in the WT and CK strains, whereas the opposite pattern was observed for the *PiSDA1* expression level. These results suggest that reducing and elevating PiCAT2 activity influences the expression of sporulation-related genes (Figure 7a).

Previous studies determined that *PiMPK7*, *PiVPS1*, and *PiGPG1* are pathogenicity-related genes in oomycetes (Li *et al.*, 2013; Gao *et al.*, 2015; van den Hoogen *et al.*, 2018). An analysis of the expression levels of these genes in the STs and OTs indicated that reducing the PiCAT2 activity significantly decreased the expression levels of *PiMPK7* (PITG_07274), *PiVPS1* (PITG_00183), and *PiGPG1* (PITG_16898) ($p < .01$). However, elevating the PiCAT2 activity did not affect the expression of *PiMPK7*, *PiVPS1*, and *PiGPG1*. Therefore, reducing the PiCAT2 activity appears to affect the expression of pathogenicity-related genes, including *PiMPK7*, *PiVPS1*, and *PiGPG1* (Figure 7b).

2.6 | PiCAT2 activity was important for oxidative and osmotic stress tolerance

In this study, *PiMPK7* and *PiCAT2* expression levels exhibited similar trends in STs. Considering that *PiMPK7* is an abiotic stress response-related gene in *Phytophthora sojae* (Gao *et al.*, 2015), we tested the abiotic stress resistance of the STs and OTs by growing the strains on rye V8 agar (RVA) supplemented with H₂O₂, NaCl, or sorbitol (Figure 8a). Compared with the WT and CK strains, the STs and OTs were more and slightly less sensitive to abiotic stress, respectively (Figure 8a,b). These results imply that PiCAT2 activity is important for the resistance of *P. infestans* to abiotic stresses, including oxidative and osmotic stresses.

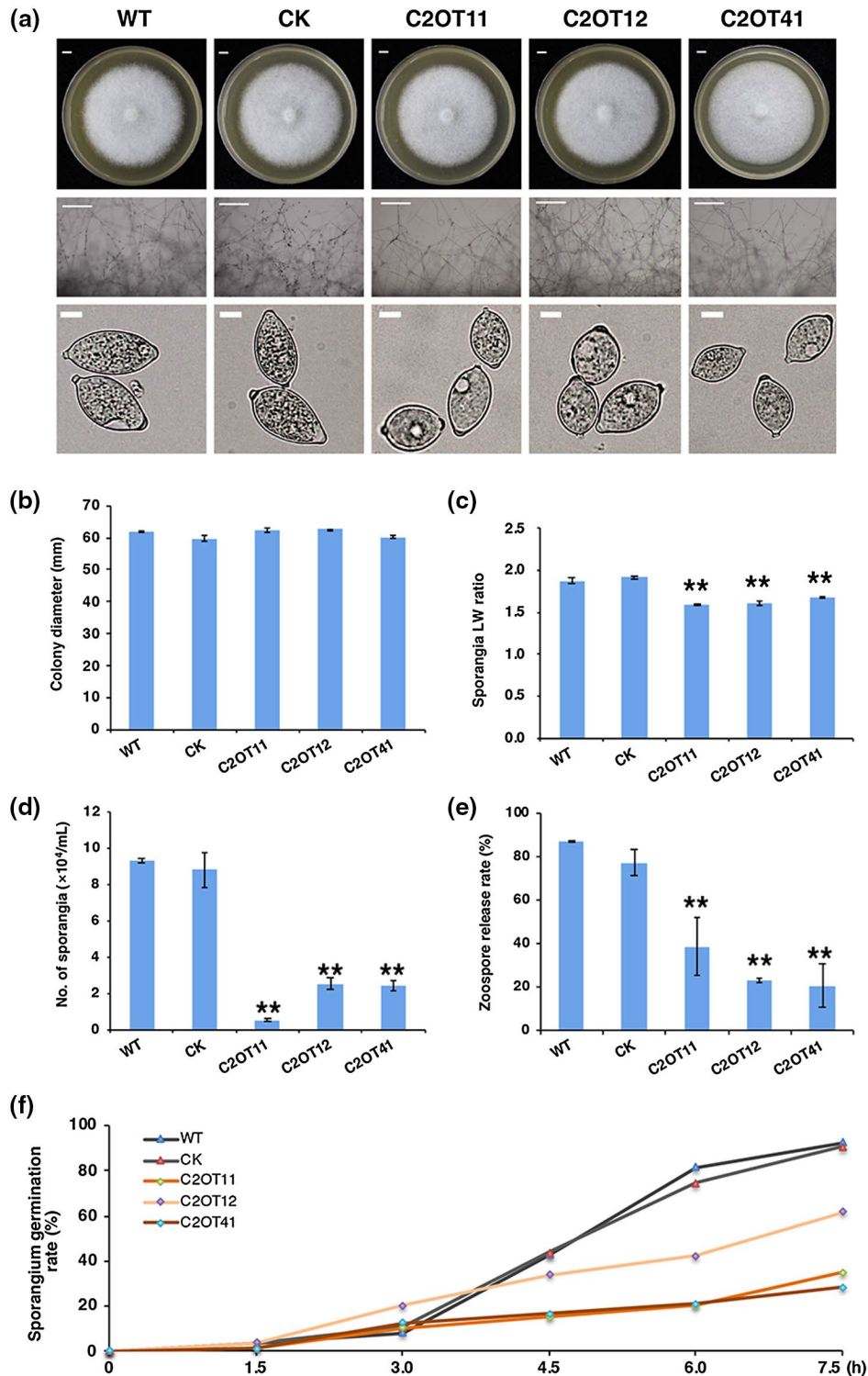


FIGURE 5 Asexual phenotypic characterization of *PicAT2*-overexpressed transformants (OTs). (a) Mycelial growth, sporangia production, and morphology of the wild-type (WT), control (CK), and OTs. Images were taken at 7 days after incubation on rye V8 agar. Bars indicate 5 mm, 0.5 mm and 10 μm , respectively. (b) Statistics of colony diameters of WT, CK, and OTs were based on 7 days of growth on rye V8 agar. (c) Length:width (LW) ratios from 50 sporangia per strain. (d) Number of sporangia of the WT, CK, and OTs. (e) Sporangial suspension was incubated in a plate for 2 hr at 10 $^{\circ}\text{C}$. The ratios of the number of empty sporangia to the total number of sporangia were calculated for each strain. (f) Sporangial suspension was incubated in 200 g/L pea broth at 18 $^{\circ}\text{C}$. The numbers of germinated and ungerminated sporangia were counted under a microscope at different time points (0, 1.5, 3, 4.5, 6, and 7.5 hr), and the percentages of the germinated sporangia to the total number of sporangia were calculated for each strain. Three replicates were used for each treatment and the whole experiment was repeated once. The data are averages (and standard errors) of values from two biological replicates. Statistical significance was analysed with Student's *t* test between WT and each transformant (** $p < .01$)

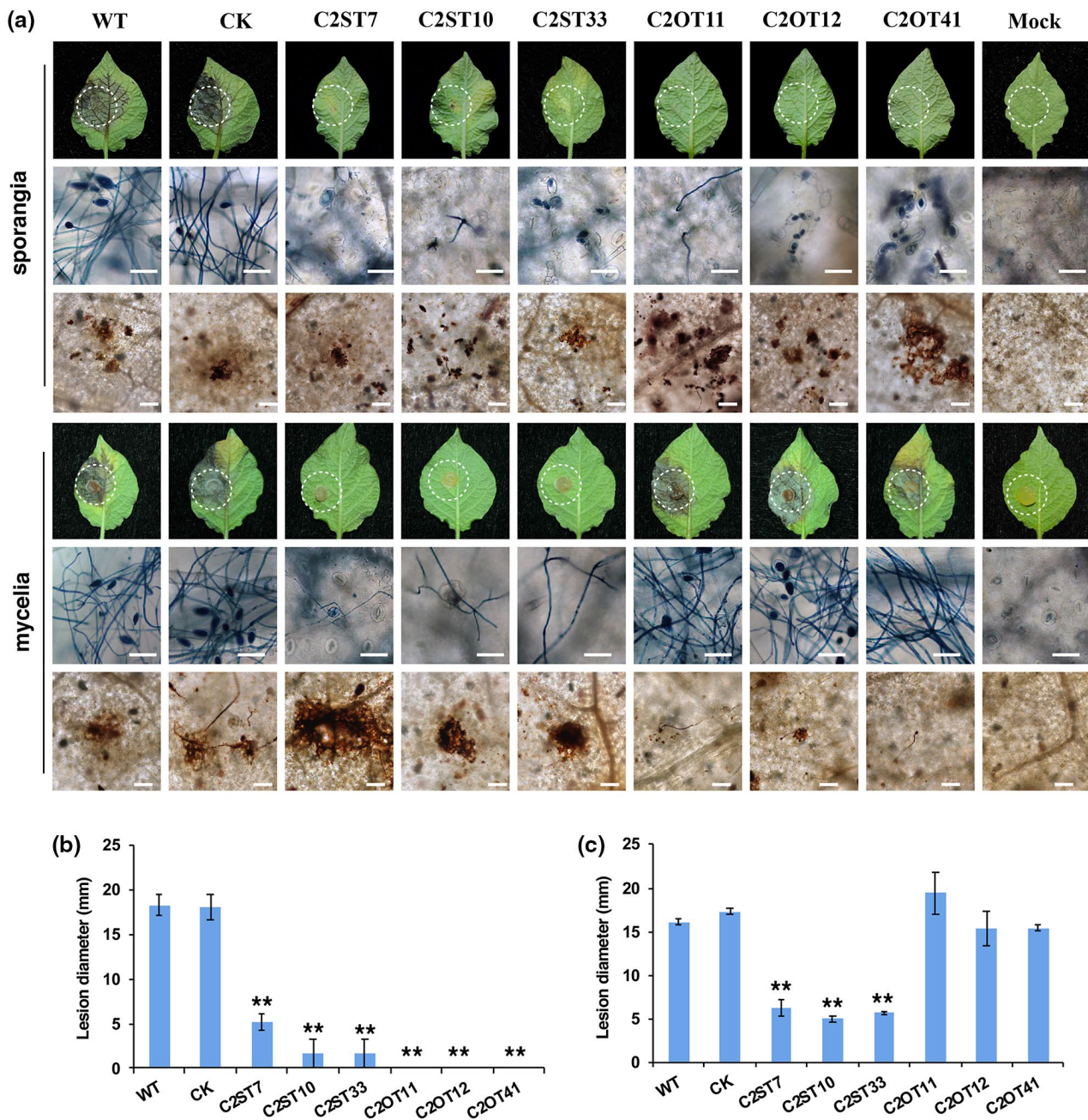


FIGURE 6 Infection assay on detached potato leaflet of susceptible cv. Impala. (a) Each leaflet was inoculated with a mycelial plug or equivalent amounts of sporangia (based on the germination rate), then incubated at 21 °C with a 12-hr light/12-hr dark cycle. At 12 hr after the inoculation, the leaves were incubated in 1 mg/mL 3,3'-diaminobenzidine (DAB) solution (pH 3.8) at room temperature for 8 hr and destained with ethanol. Five days after inoculation, leaves were boiled for c. 2 min in lactophenol-trypan blue and decolourized in 1 g/mL chloral hydrate for 30–40 min, then observed under a light microscope. Bars indicate 50 μ m. (b) Lesions induced by sporangia. (c) Lesions induced by mycelial plug. The diameter of each lesion was measured at 5 days after inoculation. Three replicates were used for each treatment in these tests and the whole experiment was repeated once. WT, wild-type; CK, control; ST, *PiCAT2*-silenced transformants; OT, *PiCAT2*-overexpressed transformants. The data are averages (and standard errors) of values from two biological replicates. Statistical significance was analysed using Student's *t* test between wild-type (WT) and each transformant (***p* < .01)

3 | DISCUSSION

Catalase is crucial for maintaining the intracellular redox balance and is a required antioxidant that influences fungal growth, development, and pathogenesis (Aguirre *et al.*, 2005; Skamnioti *et al.*, 2007). In this study, we identified two *P. infestans* catalase

genes that encode proteins with 71% amino acid sequence identity. A bioinformatics analysis proved that both catalases contain a typical catalase domain, but lack a signal peptide. However, only *PiCAT2* peptides were identified in sporulating mycelia by mass spectrometry, probably due to the low expression of *PiCAT1* and the sensitivity of mass spectrometry, indicating that

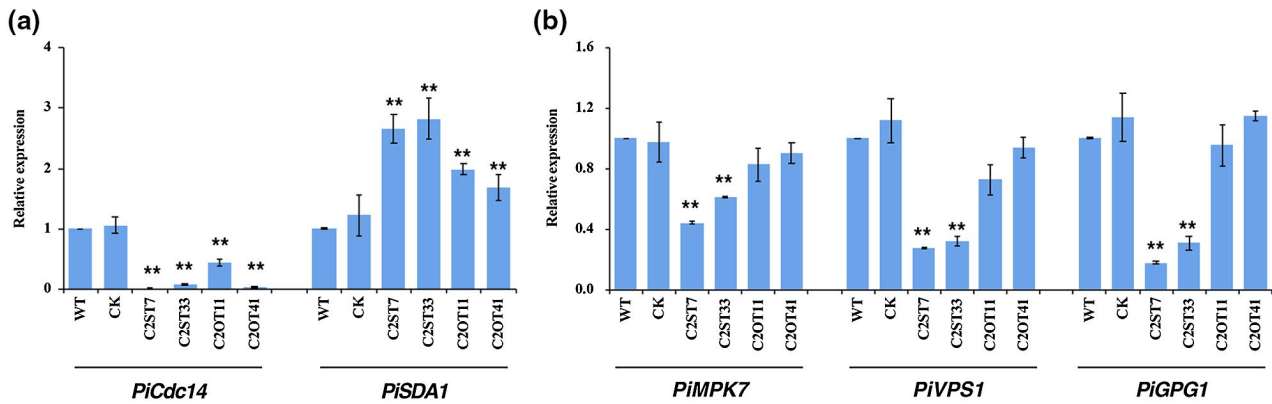


FIGURE 7 Expression levels of genes in mycelia of wild-type (WT), control (CK), *PiCAT2*-silenced transformants (STs), and *PiCAT2*-overexpressed transformants (OTs) by quantitative reverse transcription PCR. (a) Genes related to sporulation (*PiCdc14* and *PiSDA1*). (b) Genes related to pathogenicity (*PiMPK7*, *PiVPS1*, and *PiGPG1*). Expression level in transformants was determined relative to that of *ubc*, as the internal reference, and WT was used as a reference (value 1.0). The data are averages (and standard errors) of values from two biological replicates. Statistical significance was analysed using Student's *t* test between CK and each transformant (***p* < .01)

PiCAT2 exhibits mainly catalase activity. Compared with the WT and CK strains, the STs grew more slowly and were more sensitive to abiotic stress, whereas the OTs were slightly less sensitive to abiotic stress. In addition, down-regulating or up-regulating *PiCAT2* expression is inhibitory toward sporulation and the germination of sporangia. The data presented herein imply that *PiCAT2* is required for *P. infestans* mycelial growth, asexual reproduction, and abiotic stress tolerance. Moreover, a proper *PiCAT2* level is critical for the formation and normal function of sporangia.

Previous studies confirmed that catalase is required for the pathogenicity of fungi (Wysong *et al.*, 1998; Huang *et al.*, 2012; Yarden *et al.*, 2014). In this study, we detected a significant decrease in the pathogenicity of the ST mycelia and sporangia, suggesting that catalase is also required for oomycete pathogenicity. The accumulation of plant-derived ROS at the infection site is an early defence response in plants (Mellersh *et al.*, 2002; Torres and Dangl, 2005). To successfully invade plant cells, plant pathogens must avoid being damaged by ROS through self-protection and/or the scavenging of ROS. In this study, DAB staining revealed a significant increase in ROS accumulation in the leaves inoculated with ST mycelia, but not in the leaves infected with WT, CK, or OT mycelia. Hydrogen peroxide is a ROS that is converted to H₂O and O₂ via a reaction catalysed by catalase (Kwok *et al.*, 2004). An earlier study demonstrated that in *Magnaporthe grisea*, *CATB* is required for appressorium formation, conidial penetration, and virulence (Skamnioti *et al.*, 2007). Considered together, these results suggest that silencing *PiCAT2* affects the pathogenicity of *P. infestans* by adversely affecting self-protection and the scavenging of plant ROS.

Interestingly, the pathogenicity of the OT sporangia decreased, but not the mycelia. This inoculum-related difference in pathogenicity is probably due to the differences in the infection processes of sporangia and mycelium. When sporangia land on a susceptible plant, they must form an appressorium and then an infection peg

that penetrates the host epidermis to initiate a successful infection (Nowicki *et al.*, 2012). However, mycelia can grow and enter plants through stomata, and do not need to form an appressorium (Judelson and Blanco, 2005). A further analysis of appressorium formation revealed that silencing and overexpressing *PiCAT2* significantly inhibit appressorium formation. Trypan blue staining results indicated that considerable amounts of hyphae were present in leaf tissues inoculated with OT mycelia, but not in leaf tissues inoculated with OT sporangia. Accordingly, changes of *PiCAT2* activity appear to inhibit appressorium formation, which then affects the ability of sporangia to infect plants. In oomycetes, sporangia are important for dissemination and the infection of hosts (Lacy and Hammerschmidt, 1984). Collectively, these results reveal that a proper *PiCAT2* level is critical for *P. infestans* pathogenicity.

In multinucleate filamentous oomycetes, nuclear division and movement are essential for sporulation, zoospore release, and appressorium formation. Moreover, *Cdc14* and *SDA1* are conserved cell cycle regulators in eukaryotes. Specifically, *Cdc14* is involved in mitosis and DNA damage repair (Mocciaro and Schiebel, 2010), whereas *SDA1* is an important regulator of the actin cytoskeleton (Buscemi *et al.*, 2000). A previous study proved that the *MoCdc14* phosphatase is required for appressorium formation in *Magnaporthe oryzae* (Li *et al.*, 2018). In oomycetes, *Cdc14*-silenced transformants are reportedly defective regarding sporangia production and development (Ah-Fong and Judelson, 2003). Moreover, silencing and overexpressing *PcSDA1* inhibited sporangiophore formation, sporangial development, zoospore release, cyst germination, and decreased pathogenicity (Zhu *et al.*, 2016). In this study, the production of sporangia as well as the zoospore release rate and the sporangial germination rate were adversely affected in the STs and OTs, in which *PiCdc14* expression levels decreased and *PiSDA1* expression levels increased. These results suggest that reducing and elevating *PiCAT2* activity affects sporulation and the function of sporangia by altering *PiCdc14* and *PiSDA1* expression levels.

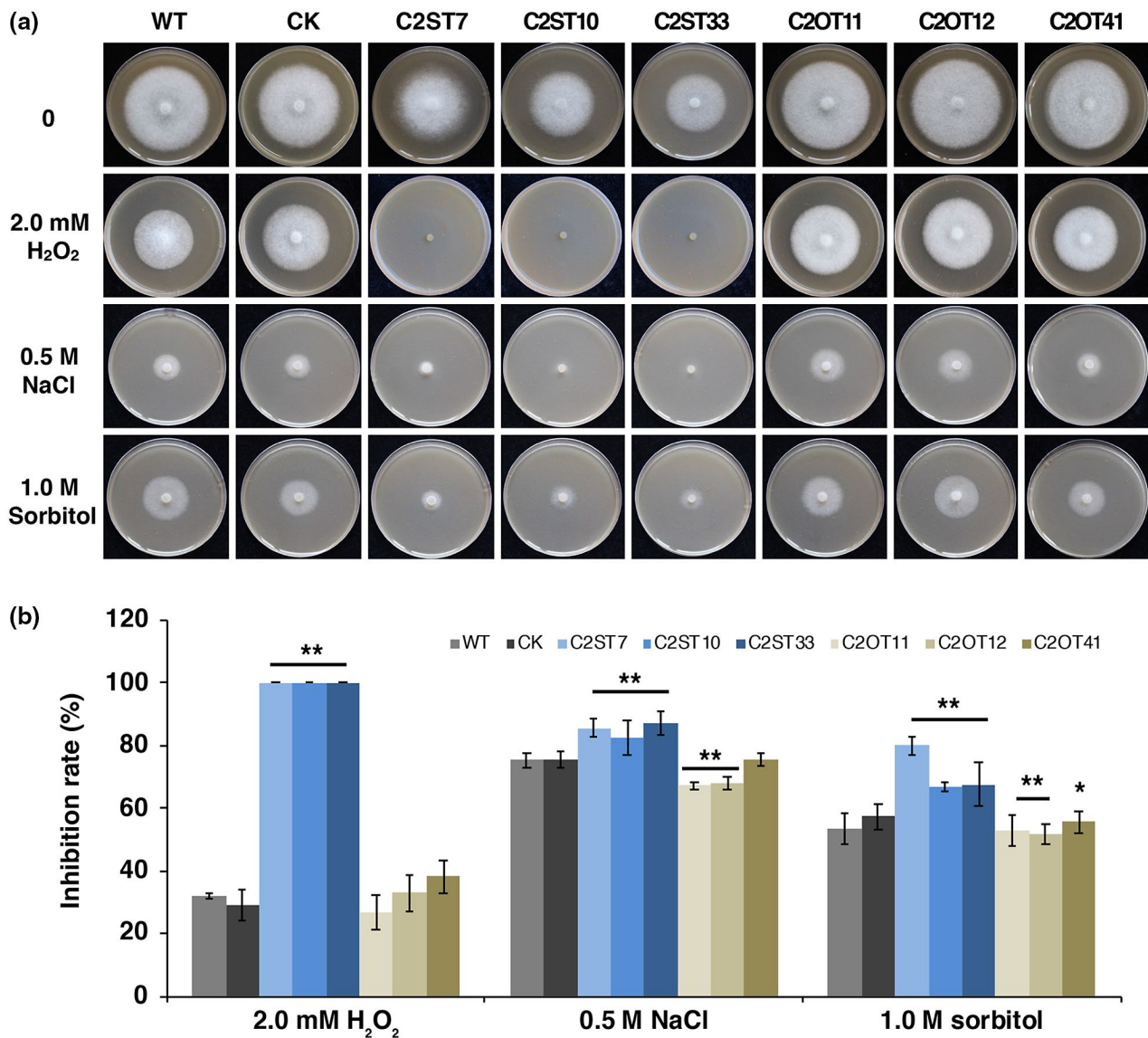


FIGURE 8 *PiCAT2* participates in the response to abiotic stress. (a) Colony expansion of wild-type (WT), control (CK), *PiCAT2*-silenced transformants (STs), and *PiCAT2*-overexpressed transformants (OTs) on rye V8 agar supplemented with 0, 2 mM H₂O₂, 0.5 M NaCl, 1 M sorbitol, and cultured at 18 °C. (b) Colony diameters were measured after 7 days of growth. Relative growth inhibition rates were calculated based on colony diameters. Three replicates were used for each treatment in these tests and the whole experiment was repeated once. The data are averages (and standard errors) of values from two biological replicates. Statistical significance was analysed using Student's *t* test between CK and each transformant (**p* < .05, ***p* < .01)

In eukaryotic organisms, mitogen-activated protein kinases (MAPKs) help mediate the transduction of diverse extracellular signals that regulate growth, development, and infection processes (Chen and Thorner, 2007). In addition, the MAPK signalling network has many essential components, including MAPKs, vacuolar protein sorting proteins (VPSs), and G protein-coupled receptors (Chen and Thorner, 2007; Zhao *et al.*, 2007). In oomycetes, *PsMPK7*, *PsVPS1*, and *PiGPG1* are pathogenicity-related genes (Li *et al.*, 2013; Gao *et al.*, 2015; van den Hoogen *et al.*, 2018). In this study, we observed that reducing *PiCAT2* activity significantly down-regulated the expression of these three genes, whereas elevating *PiCAT2* activity did not affect *PiMPK7*, *PiVPS1*, and *PiGPG1* expression. An earlier investigation indicated that *PsMPK7* is also an abiotic stress response-related

gene in *Phytophthora* species (Gao *et al.*, 2015). These results suggest that *PiCAT2* affects the pathogenicity and abiotic stress tolerance of *P. infestans* by regulating the expression of MAPK pathway genes.

Hydrogen peroxide also functions as a signalling molecule and is required for many differentiation processes in eukaryotes (Veal *et al.*, 2007; Veal and Day, 2011). Thus, our data support a proposed model regarding how *PiCAT2* regulates *P. infestans* asexual reproduction, abiotic stress resistance, and pathogenicity (Figure 9). Briefly, during growth and development, *P. infestans* encounters endogenous and exogenous ROS, including H₂O₂, and needs catalase to maintain the intracellular redox balance. In the WT strain, ROS is maintained at a normal level, which is favourable for sporulation and the germination of sporangia (through zoospore release or germ-tube production).

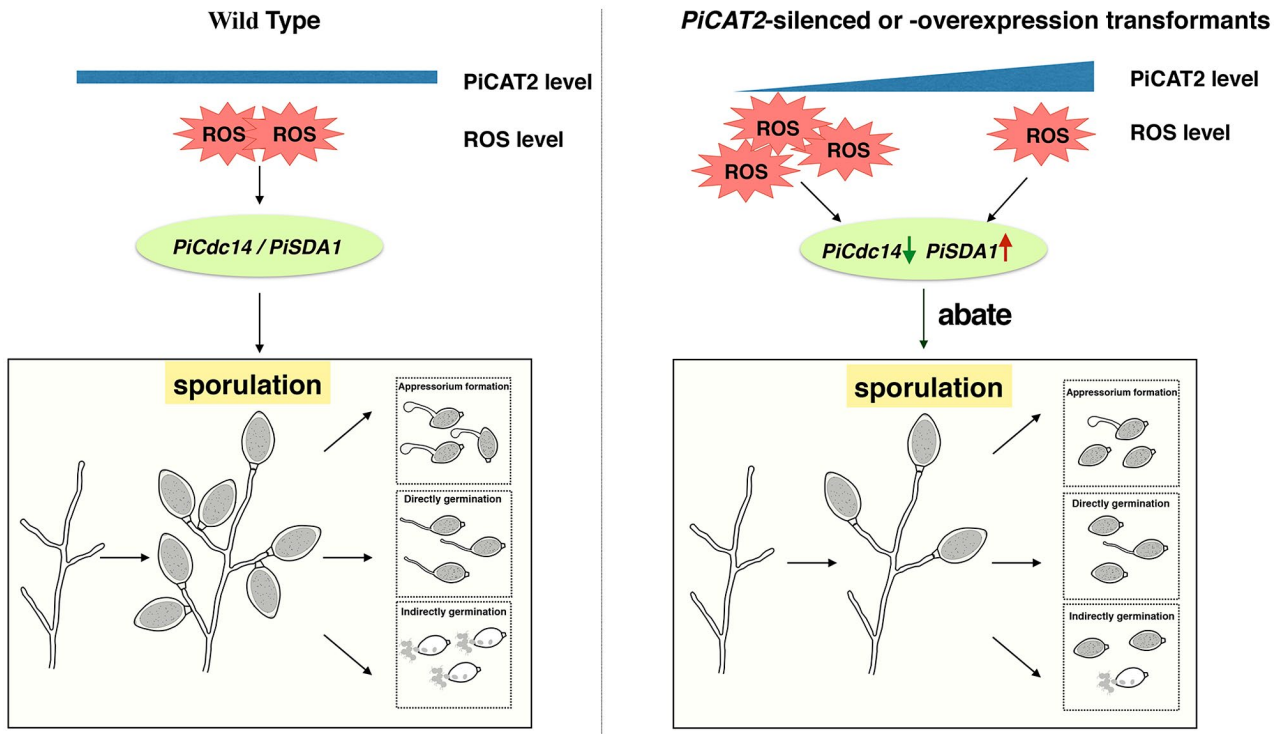


FIGURE 9 A model of PiCAT2-regulated sporulation of *Phytophthora infestans*. During growth and development, *P. infestans* encounters endogenous and exogenous reactive oxygen species (ROS) and needs catalase to maintain the intracellular redox balance. In the wild-type strain, ROS is maintained at a normal level, which is favourable for sporulation and the germination of sporangia. In PiCAT2-silenced or -overexpressed transformants, more or less catalase activity affects ROS accumulation, and induces the down-regulation of *PiCdc14* expression and the up-regulation of *PiSDA1* expression, ultimately suppressing sporangia formation and germination as well as appressorium formation

Reducing and elevating catalase activity affects ROS accumulation and induces the down-regulation of *PiCdc14* expression and the up-regulation of *PiSDA1* expression, ultimately suppressing sporangia formation and germination as well as appressorium formation. The inhibition of appressorium formation restricts the ability of sporangia to infect susceptible hosts. Interestingly, the length:width ratio of the OT sporangia was smaller, but the underlying mechanism is unclear. Reducing catalase activity results in increased ROS levels, decreased pathogenicity, and the expression of abiotic stress-related genes, including *PiVPS1*, *PiGPG1*, and *PiMPK7*. Elevating catalase activity decreases the ROS level, but does not affect the expression of *PiMPK7*, *PiVPS1*, and *PiGPG1*. Thus, OT mycelia exhibit increased abiotic stress resistance and remain pathogenic. However, the mechanism underlying the associated signal transduction and cross-talk between genes remains to be investigated.

In previous studies related to catalases, gene knockout and complementation techniques were commonly used, and the results suggested that catalase is required for the growth and development of pathogens (Skamnioti *et al.*, 2007; Wang *et al.*, 2013). In our gene silencing and overexpression study, we revealed that a proper catalase level is critical for *P. infestans* growth and development, especially in sporangia. To the best of our knowledge, this is the first study to demonstrate that elevating catalase contents is detrimental to sporulation and the function of sporangia.

So far, localization of PiCAT2 is still unknown. Sequence analysis revealed that PiCAT2 has two peroxisomal-targeting signals, SKL and PRL, both belonging to PTS1, at the C-terminus (Figure S1b). Prediction using the online program pSORT (<http://www.genscript.com/wolf-psort.html>; Horton *et al.*, 2007) and Euk-mPloc 2.0 (<http://www.csbio.sjtu.edu.cn/bioinf/euk-multi-2/>; Chou and Shen, 2010) suggested that PiCAT2 localized in mitochondria and peroxisomes. Because the GFP is poorly expressed in all the transformants of *P. infestans*, we detected the localization of PiCAT2-GFP (GFP fused at C-terminal) and GFP-PiCAT2 (GFP fused at N-terminal) in hyphae from regenerated protoplast of *P. sojae* strain P6497 (Figure S6). Both fusions displayed similar characteristics in transformants. Fluorescence localized to spherical to slightly elongated structures of various sizes in the cytoplasm, which matched the description of mitochondria and peroxisomes of *Phytophthora* (Ah-Fong and Judelson, 2011). This result is consistent with the predicted localization. However, further investigation is needed for the precise location of PiCAT2. In conclusion, PiCAT2 is required for *P. infestans* asexual reproduction, abiotic stress tolerance, and pathogenicity. Elevating catalase production may enhance abiotic stress tolerance, but a proper PiCAT2 level is required for the formation and function of sporangia as well as pathogenicity. The data presented herein provide new insights into catalase functions in eukaryotic pathogens.

4 | EXPERIMENTAL PROCEDURES

4.1 | *P. infestans* strains and culture conditions

The *P. infestans* MX5-1 isolate (Han *et al.*, 2012) was used in this study. The WT and transformant strains were maintained on 10% RVA (Chang and Ko, 1991) at 18 °C in darkness. To test the abiotic stress sensitivities of each strain, culture blocks (5 mm in diameter) were placed on fresh RVA supplemented with 2 mM H₂O₂, 0.5 M NaCl, or 1 M sorbitol. Colony diameters were measured after incubating the cultures at 18 °C in darkness for 7 days. All experiments were performed twice, and each treatment was completed in triplicate.

4.2 | Catalase gene analysis

Oomycete catalase gene sequences were retrieved from the DOE Joint Genome Institute (<http://www.jgi.doe.gov/>) and Ensembl Genomes (<http://www.ensemblgenomes.org/>) databases. Additional catalase genes were retrieved from the NCBI and KEGG databases for the following species: diatom (*Thalassiosira pseudonana*), protists (*Tetrahymena thermophila* and *Paramecium tetraurelia*), animals (*Homo sapiens* and *Mus musculus*), plant (*Arabidopsis thaliana*), and fungi (*M. oryzae* and *Saccharomyces cerevisiae*) (Table S1). The amino acid sequences encoded by these genes were aligned with ClustalW2 (<http://www.ebi.ac.uk/Tools/msa/clustalw2/>; Larkin *et al.*, 2007). In addition, phylogenetic trees were constructed with the maximum likelihood evolution algorithm in MEGA 7.0 (Kumar *et al.*, 2016). A Poisson correction was used for multiple substitution models and a pairwise deletion was used for handling missing data. Statistical accuracy was assessed with 1,000 bootstrap replicates.

4.3 | RNA extraction and gene expression analysis

To determine the *PiCAT2* transcript profiles during various *P. infestans* developmental stages, mycelium, sporangium, zoospore, cyst, and germinated cyst samples were collected (Wang *et al.*, 2016b). To measure the *PiCAT2* transcript levels during a *P. infestans* infection, potato (cv. Impala) leaves were inoculated with the *P. infestans* MX5-1 sporangia. Each leaflet was spot-inoculated with a 20- μ l sporangial suspension (10⁴ sporangia/ml) and then incubated at 21 °C with a 12-hr light/12-hr dark cycle for 5 days (Guo *et al.*, 2017). Lesion samples were collected at 0 and 12 hr as well as at 1, 2, 3, 4, and 5 days, and immediately placed in liquid nitrogen. Total RNA was extracted and gene expression was analysed as previously described (Wang *et al.*, 2016b). The gene of ubiquitin-conjugating enzyme (*ubc*, *PITG_00505*) in *P. infestans* was used as an internal control (Yan and Liou, 2006). Three replicates were used for each treatment and the whole experiment was repeated with two different sets of biological samples.

4.4 | Plasmid construction and transformation of *P. infestans*

We used the pGFPN vector to construct transformation plasmids (Ah-Fong and Judelson, 2011). The fragment of 5' untranslated region (UTR, 147 bp) and 3' UTR (137 bp) with highly specific sequence (less than 15 bp matched to other sequences in the genome of *P. infestans* based on sequence similarity search) and the open reading frame (ORF) of *PiCAT2* were amplified by a PCR with specific primers (Table S2) and cDNA derived from *P. infestans* MX5-1 as the template. The amplified 5' UTR and 3' UTR fragments were inserted in the anti-sense orientation into the pGFPN vector digested with the restriction enzymes *Sac*II and *Nhe*I to produce the gene silencing construct. The *PiCAT2* ORF was inserted in the sense orientation into the pGFPN vector digested with the restriction enzyme *Nhe*I to produce the gene overexpression construct. The accuracy of the plasmid constructs was verified by DNA sequencing, after which the plasmids were used to transform *P. infestans* MX5-1 as previously described (McLeod *et al.*, 2008). The G418 resistance was used to select for transformants. To validate the transformants, the *PiCAT2* transcript level was measured by RT-qPCR.

4.5 | In-gel assay for measuring catalase activity

The catalase activities of WT, control (CK, i.e., harbouring an empty pGFPN vector), and transformant strains were assayed as described by Guo *et al.* (2017). Briefly, mycelia cultured in pea broth (200 g/L) for 5 days were collected. Each mycelial sample was ground in liquid nitrogen and then mixed with ice-cold extraction buffer (50 mM HEPES pH 7.4, 137 mM NaCl, 10% glycerol, and protease inhibitors pepstatin A [1 μ g/ml], leupeptin [1 μ g/ml], and PMSF [1 mM]). Mixtures were centrifuged at 10,000 \times g for 10 min at 4 °C, after which the supernatants were collected and used as crude extracts (Wang *et al.*, 2016a). Protein concentrations were quantified with a protein assay dye (Bio-Rad) (absorbance at 595 nm). An in-gel assay was performed as described by Lledías *et al.* (1998). Equal amounts of total protein (20 μ g) from the different strains (WT, CK, STs, and OTs) were loaded into a 7.5% native polyacrylamide slab gel. The gel was immersed in 7 mM H₂O₂ for 10 min after the electrophoresis, and then incubated in a 1:1 mixture of freshly prepared 1% potassium hexacyanoferrate (III) and 1% iron (III) chloride hexahydrate. The gel was stained blue except in zones where H₂O₂ was degraded by catalases (Harris and Hopkinson, 1987).

4.6 | Phenotypic analysis of sporangia

The sporangia of each strain were collected by washing 12-day-old cultures with 200 g/L pea broth, then cultivated at 18 °C for a germination assay, or with 10 ml sterile water to count the sporangia

and analyse zoospore release. Zoospores were generated by incubating the sporangial suspension at 10 °C for 2 hr and then at room temperature.

The in vitro appressorium formation rates of sporangia on cellulose membranes were calculated as described by Jiang *et al.* (1996). Specifically, a 20- μ l sporangial suspension (containing approximately 1,000 sporangia) was placed on a cellulose membrane attached to the underside of a potato leaf. After a 36-hr incubation at 21 °C with a 12-hr light/12-hr dark cycle, the sporangia were washed and the appressorium formation rate (%) was calculated. All experiments were performed twice, and each treatment was completed in triplicate.

4.7 | Pathogenicity test and analysis of the infection process

Pathogenicity was tested as described by Guo *et al.* (2017). For plant inoculations, a 20- μ l droplet of sporangial suspension containing approximately 1,000 sporangia for WT and CK strains and approximately 2,000 sporangia for the ST and OT strains was used to inoculate detached potato (cv. Impala) leaves. Potato leaves were also inoculated with a mycelial plug of the WT, CK, ST, and OT strains. Two detached leaves placed on water-moistened filter paper in a Petri dish served as a replicate, and three plates were used for each treatment. Inoculated leaves were then incubated at 21 °C with a 12-hr light/12-hr dark cycle for 5 days. The size of each lesion was measured and analysed with Student's *t* test. Trypan blue staining was performed as described by Koch and Slusarenko (1990). The stained infected leaves were observed under a light microscope. At 12 hr after the inoculation, ROS were stained with the oxidant-sensitive probe DAB (Sigma Aldrich) as described by Liu and Friesen (2012). Samples were analysed with an Olympus microscope. All experiments were performed twice.

4.8 | Intracellular ROS assay

The sporulating mycelia of each strain grown on RVA for 5 days were collected and incubated with 10 μ M fluorogenic probe (2',7'-dichlorodihydrofluorescein diacetate; Sigma Aldrich). The treated mycelia were analysed with the SP8 confocal laser-scanning microscope (Leica Microsystems), with an excitation wavelength of 488 nm and an emission wavelength of 530 nm (Liu *et al.*, 2018).

ACKNOWLEDGMENTS

We sincerely thank Francine Govers (Wageningen University) for providing the pGFPN vector, and Brett M. Tyler (Oregon State University) and Zejian Guo (China Agricultural University) for helpful suggestions. This work was funded in part by the Special Fund for Agro-Scientific Research in the Public Interest (201303018) and the Chinese Universities Scientific Fund (2017ZB001).

COMPETING INTERESTS

The authors declare that no competing interests exist.

ORCID

Li-Yun Guo  <https://orcid.org/0000-0002-1688-5726>

REFERENCES

- Aguirre, J., Ríos-Momberg, M., Hewitt, D. and Hansberg, W. (2005) Reactive oxygen species and development in microbial eukaryotes. *Trends in Microbiology*, 13, 111–118.
- Ah-Fong, A.M.V. and Judelson, H.S. (2003) Cell cycle regulator Cdc14 is expressed during sporulation but not hyphal growth in the fungus-like oomycete *Phytophthora infestans*. *Molecular Microbiology*, 50, 487–494.
- Ah-Fong, A.M.V. and Judelson, H.S. (2011) Vectors for fluorescent protein tagging in *Phytophthora*: tools for functional genomics and cell biology. *Fungal Biology*, 115, 882–890.
- Ah-Fong, A.M.V., Kim, K.S. and Judelson, H.S. (2017) RNA-seq of life stages of the oomycete *Phytophthora infestans* reveals dynamic changes in metabolic, signal transduction, and pathogenesis genes and a major role for calcium signaling in development. *BMC Genomics*, 18, 198.
- Apel, K. and Hirt, H. (2004) Reactive oxygen species: metabolism, oxidative stress, and signal transduction. *Annual Review of Plant Biology*, 55, 373–399.
- Baldauf, S.L., Roger, A.J., Wenk-Siefert, I. and Doolittle, W.F. (2000) A kingdom-level phylogeny of eukaryotes based on combined protein data. *Science*, 290, 972–977.
- Birch, P.R. and Whisson, S.C. (2001) *Phytophthora infestans* enters the genomics era. *Molecular Plant Pathology*, 2, 257–263.
- Blackman, L.M. and Hardham, A.R. (2008) Regulation of catalase activity and gene expression during *Phytophthora nicotianae* development and infection of tobacco. *Molecular Plant Pathology*, 9, 495–510.
- Branco, M.R., Marinho, H.S., Cyrne, L. and Antunes, F. (2004) Decrease of H₂O₂ plasma membrane permeability during adaptation to H₂O₂ in *Saccharomyces cerevisiae*. *Journal of Biological Chemistry*, 279, 6501–6506.
- Buscemi, G., Saracino, F., Masnada, D. and Carbone, M.L. (2000) The *Saccharomyces cerevisiae* SDA1 gene is required for actin cytoskeleton organization and cell cycle progression. *Journal of Cell Science*, 113, 1199–1211.
- Camejo, D., Guzman-Cedeno, A. and Moreno, A. (2016) Reactive oxygen species, essential molecules, during plant-pathogen interactions. *Plant Physiology and Biochemistry*, 103, 10–23.
- Chang, T.T. and Ko, W.H. (1991) Factors affecting germination of oospores of *Phytophthora infestans*. *Journal of Phytopathology*, 133, 29–35.
- Chen, R.E. and Thorner, J. (2007) Function and regulation in MAPK signaling pathways: lessons learned from the yeast *Saccharomyces cerevisiae*. *Biochimica et Biophysica Acta*, 1773, 1311–1340.
- Chou, K.C. and Shen, H.B. (2010) A new method for predicting the subcellular localization of eukaryotic proteins with both single and multiple sites: Euk-mPLoc 2.0. *PLoS One*, 5, e9931.
- Erwin, D.C. and Ribeiro, O.K. (1996) *Phytophthora Diseases Worldwide*. St. Paul, MN: The American Phytopathological Society Press.
- Gao, J., Cao, M., Ye, W., Li, H., Kong, L., Zheng, X. *et al.* (2015) PsMPK7, a stress-associated mitogen-activated protein kinase (MAPK) in *Phytophthora sojae*, is required for stress tolerance, reactive oxygenated species detoxification, cyst germination, sexual reproduction and infection of soybean. *Molecular Plant Pathology*, 16, 61–70.
- Gouet, P., Jouve, H.M. and Dideberg, O. (1995) Crystal structure of *Proteus mirabilis* PR catalase with and without bound NADPH. *Journal of Molecular Biology*, 249, 933–954.

- Guo, T., Wang, X.W., Shan, K., Sun, W. and Guo, L.Y. (2017) The Loricrin-like protein (LLP) of *Phytophthora infestans* is required for oospore formation and plant infection. *Frontiers in Plant Science*, 8, 142.
- Haas, B.J., Kamoun, S., Zody, M.C., Jiang, R.H., Handsaker, R.E., Cano, L.M. et al. (2009) Genome sequence and analysis of the Irish potato famine pathogen *Phytophthora infestans*. *Nature*, 461, 393–398.
- Han, M., Liu, G., Li, J.P., Govers, F., Zhu, X.Q., Shen, C.Y. et al. (2012) *Phytophthora infestans* field isolates from Gansu province, China are genetically highly diverse and show a high frequency of self fertility. *Journal of Eukaryotic Microbiology*, 60, 79–88.
- Hardham, A.R. (2001) The cell biology behind *Phytophthora* pathogenicity. *Australasian Plant Pathology*, 30, 91–98.
- Harris, H. and Hopkinson, D.A. (1987) *Handbook of Enzyme Electrophoresis in Human Genetics*. Amsterdam: North-Holland Press.
- Horton, P., Park, K.J., Obayashi, T., Fujita, N., Harada, H., Adams-Collier, C. et al. (2007) WoLF PSORT: protein localization predictor. *Nucleic Acids Research*, 35, W585–587.
- Huang, Z., Ali, S. and Ren, S. (2012) Catalase production influences germination, stress tolerance and virulence of *Lecanicillium muscarium* conidia. *Biocontrol Science and Technology*, 22, 249–260.
- Ighodaro, O.M. and Akinloye, O.A. (2018) First line defence antioxidants-superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX): their fundamental role in the entire antioxidant defence grid. *Alexandria Journal of Medicine*, 54, 287–293.
- Jiang, J.Z., Dao, J.J.Z., Zheng, X.B. and Lu, J.Y. (1996) A new method for studying the interaction between *Phytophthora infestans* and plants before invasive use of cellulose membrane technology. *Acta Phytopathologica Sinica*, 26, 269–275.
- Judelson, H.S. and Blanco, F.A. (2005) The spores of *Phytophthora*: weapons of the plant destroyer. *Nature Reviews Microbiology*, 3, 47–58.
- Karling, J.S. (1981) *Predominantly Holocarpic and Eucarpic Simple Biflagellate Phycomycetes*. Germany: J. Cramer.
- Koch, E. and Slusarenko, A. (1990) *Arabidopsis* is susceptible to infection by a downy mildew fungus. *The Plant Cell*, 2, 437–445.
- Kowaltowski, A.J., De Souza-Pinto, N.C., Castilho, R.F. and Vercesi, A.E. (2009) Mitochondria and reactive oxygen species. *Free Radical Biology and Medicine*, 47, 333–343.
- Kumar, S., Stecher, G. and Tamura, K. (2016) MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Molecular Biology and Evolution*, 33, 1870–1874.
- Kwok, L.Y., Schlüter, D., Clayton, C. and Soldati, D. (2004) The antioxidant systems in *Toxoplasma gondii* and the role of cytosolic catalase in defence against oxidative injury. *Molecular Microbiology*, 51, 47–61.
- Lacy, M.L. and Hammerschmidt, R. (1984) *Diseases of Potato: Late Blight*. East Lansing, MI: Michigan State University, Cooperative Extension Service.
- Larkin, M.A., Blackshields, G., Brown, N.P., Chenna, R., McGettigan, P.A., McWilliam, H. et al. (2007) Clustal W and Clustal X version 2.0. *Bioinformatics*, 23, 2947–2948.
- Latijnhouwers, M. and Govers, F. (2003) A *Phytophthora infestans* G-Protein β subunit is involved in sporangium formation. *Eukaryotic Cell*, 2, 971–977.
- Leesutthiphonchai, W. and Judelson, H.S. (2018) A MADS-box transcription factor regulates a central step in sporulation of the oomycete *Phytophthora infestans*. *Molecular Microbiology*, 110, 562–575.
- Li, C., Cao, S., Zhang, C., Zhang, Y., Zhang, Q., Xu, J.R. et al. (2018) MoCDC14 is important for septation during conidiation and appressorium formation in *Magnaporthe oryzae*. *Molecular Plant Pathology*, 19, 328–340.
- Li, D., Zhao, Z., Huang, Y., Lu, Z., Yao, M., Hao, Y. et al. (2013) PsVPS1, a dynamin-related protein, is involved in cyst germination and soybean infection of *Phytophthora sojae*. *PLoS One*, 8, e58623.
- Liu, Z. and Friesen, T. (2012) DAB staining and visualization of hydrogen peroxide in wheat leaves. *Bioprotocols*, 2, e309.
- Liu, P., Wei, M., Zhang, J., Wang, R., Li, B., Chen, Q. et al. (2018) Changes in mycelia growth, sporulation, and virulence of *Phytophthora capsici* when challenged by heavy metals (Cu^{2+} , Cr^{2+} and Hg^{2+}) under acid pH stress. *Environmental Pollution*, 235, 372–380.
- Lledías, F., Rangel, P. and Hansberg, W. (1998) Oxidation of catalase by singlet oxygen. *Journal of Biological Chemistry*, 273, 10630–10637.
- McLeod, A., Fry, B.A., Zuluaga, A.P., Myers, K.L. and Fry, W.E. (2008) Toward improvements of oomycete transformation protocols. *Journal of Eukaryotic Microbiology*, 55, 103–109.
- Mellersh, D.G., Foulds, I.V., Higgins, V.J. and Heath, M.C. (2002) H_2O_2 plays different roles in determining penetration failure in three diverse plant–fungal interactions. *The Plant Journal*, 29, 257–268.
- Mendoza, L., Hernandez, F. and Ajello, L. (1993) Life cycle of the human and animal oomycete pathogen *Pythium insidiosum*. *Journal of Clinical Microbiology*, 31, 2967–2973.
- Michán, S., Lledías, F. and Hansberg, W. (2003) Asexual development is increased in *Neurospora crassa* cat-3-null mutant strains. *Eukaryotic Cell*, 2, 798–808.
- Mocciaro, A. and Schiebel, E. (2010) Cdc14: a highly conserved family of phosphatases with non-conserved functions? *Journal of Cell Science*, 123, 2867–2876.
- Nowicki, M., Lichocka, M., Nowakowska, M., Kłosińska, U., Golik, P. and Kozik, E.U. (2012) A simple dual stain for detailed investigations of plant–fungal pathogen interactions. *Vegetable Crops Research Bulletin*, 77, 61–74.
- Pan, X., Wu, J., Xu, S., Duan, Y. and Zhou, M. (2017) CatB is critical for total catalase activity and reduces bactericidal effects of phenazine-1-carboxylic acid on *Xanthomonas oryzae* pv. *oryzae* and *X. oryzae* pv. *oryzicola*. *Phytopathology*, 107, 163–172.
- Schriner, S.E., Linford, N.J., Martin, G.M., Treuting, P., Ogburn, C.E., Emond, M. et al. (2005) Extension of murine life span by over-expression of catalase targeted to mitochondria. *Science*, 308, 1909–1911.
- Skamnioti, P., Henderson, C., Zhang, Z., Robinson, Z. and Gurr, S.J. (2007) A novel role for catalase B in the maintenance of fungal cell-wall integrity during host invasion in the rice blast fungus *Magnaporthe grisea*. *Molecular Plant-Microbe Interactions*, 20, 568–580.
- Sparrow, F.K. (1960) *Aquatic Phycomycetes*. Ann Arbor, MI: University of Michigan Press.
- Takemoto, D., Tanaka, A. and Scott, B. (2007) NADPH oxidases in fungi: diverse roles of reactive oxygen species in fungal cellular differentiation. *Fungal Genetics and Biology*, 44, 1065–1076.
- Torres, M.A. and Dangel, J.L. (2005) Functions of the respiratory burst oxidase in biotic interactions, abiotic stress and development. *Current Opinion in Plant Biology*, 8, 397–403.
- Tudzynski, P., Heller, J. and Siegmund, U. (2012) Reactive oxygen species generation in fungal development and pathogenesis. *Current Opinion in Microbiology*, 15, 653–659.
- Van Den Hoogen, J., Verbeek-De Kruijff, N. and Govers, F. (2018) The G-protein γ subunit of *Phytophthora infestans* is involved in sporangial development. *Fungal Genetics and Biology*, 116, 73–82.
- Veal, E. and Day, A. (2011) Hydrogen peroxide as a signaling molecule. *Antioxidants & Redox Signaling*, 15, 147–151.
- Veal, E.A., Day, A.M. and Morgan, B.A. (2007) Hydrogen peroxide sensing and signaling. *Molecular Cell*, 26, 1–14.
- Wang, W., Cheng, Y., Chen, D., Liu, D., Hu, M., Dong, J. et al. (2019) The catalase gene family in cotton: genome-wide characterization and bioinformatics analysis. *Cells*, 8, 86.
- Wang, Y., Dong, Q., Ding, Z., Gai, K., Han, X., Kaleri, F.N. et al. (2016a) Regulation of *Neurospora* Catalase-3 by global heterochromatin formation and its proximal heterochromatin region. *Free Radical Biology and Medicine*, 99, 139–152.
- Wang, X.W., Guo, L.Y., Han, M. and Shan, K. (2016b) Diversity, evolution and expression profiles of histone acetyltransferases and deacetylases in oomycetes. *BMC Genomics*, 17, 927.

- Wang, N., Yoshida, Y. and Hasunuma, K. (2007) Loss of Catalase-1 (Cat-1) results in decreased conidial viability enhanced by exposure to light in *Neurospora crassa*. *Molecular Genetics and Genomics*, 277, 13–22.
- Wang, Z.L., Zhang, L.B., Ying, S.H. and Feng, M.G. (2013) Catalases play differentiated roles in the adaptation of a fungal entomopathogen to environmental stresses. *Environmental Microbiology*, 15, 409–418.
- Wysong, D.R., Christin, L., Sugar, A.M., Robbins, P.W. and Diamond, R.D. (1998) Cloning and sequencing of a *Candida albicans* catalase gene and effects of disruption of this gene. *Infection and Immunity*, 66, 1953–1961.
- Xiang, Q. and Judelson, H.S. (2014) Myb transcription factors and light regulate sporulation in the oomycete *Phytophthora infestans*. *PLoS One*, 9, e92086.
- Yan, H.Z. and Liou, R.F. (2006) Selection of internal control genes for real-time quantitative RT-PCR assays in the oomycete plant pathogen *Phytophthora parasitica*. *Fungal Genetics and Biology*, 43, 430–438.
- Yarden, O., Veluchamy, S., Dickman, M.B. and Kabbage, M. (2014) *Sclerotinia sclerotiorum* catalase SCAT1 affects oxidative stress tolerance, regulates ergosterol levels and controls pathogenic development. *Physiological and Molecular Plant Pathology*, 85, 34–41.
- Yoon, H.S., Hackett, J.D. and Bhattacharya, D. (2002) A single origin of the peridinin- and fucoxanthin-containing plastids in dinoflagellates through tertiary endosymbiosis. *Proceedings of the National Academy of Sciences of the United States of America*, 99, 11724–11729.
- Zamocky, M., Furtmüller, P.G. and Obinger, C. (2008) Evolution of catalases from bacteria to humans. *Antioxidants & Redox Signaling*, 10, 1527–1548.
- Zhao, X., Mehrabi, R. and Xu, J.R. (2007) Mitogen-activated protein kinase pathways and fungal pathogenesis. *Eukaryotic Cell*, 6, 1701–1714.
- Zhu, C., Yang, X., Lv, R., Li, Z., Ding, X., Tyler, B.M. et al. (2016) *Phytophthora capsici* homologue of the cell cycle regulator *SDA1* is required for sporangial morphology, mycelial growth and plant infection. *Molecular Plant Pathology*, 17, 369–387.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

FIGURE S1 Information for catalase in *Phytophthora infestans*. (a) Phylogenetic analysis of catalase. The phylogenetic tree was constructed with the domain sequences of catalase using the maximum likelihood evolution algorithm. Statistical accuracy was assessed with 1,000 bootstrap replicates. Bootstrap values ≥ 50 are shown near the tree nodes. (b) Amino acid sequence of PiCAT1 (PITG_15292) and PiCAT2 (PITG_15248). Amino acids in shaded regions are important for the tetramer formation. The wavy-lined region indicates the catalase domain, the underlined region indicates the catalase proximal active site signature, while the boxed region indicates the catalase proximal heme-ligand signature motif. The PTS1 of the PiCAT2 protein is shown in bold type

FIGURE S2 Intracellular ROS assay. Sporulation stages of WT, CK, ST (C2ST33), and OT (C2OT11) strain were incubated with 10 μ M

fluorogenic probe (2',7'-dichlorodihydrofluorescein diacetate; DCFH-DA), followed by the fluorescent microscopic observation. Bars indicate 20 μ m

FIGURE S3 Expression levels of genes flanking the silenced target PiCAT2. Expression levels of PITG_15247 and PITG_15249 in STs were determined relative to that of *ubc*, as the internal reference, and WT was used as a reference (value 1.0). The data are averages (and standard errors) of values from two biological replicates. Statistical significance was analysed using Student's *t* test between CK and each transformant (* $p < .05$, ** $p < .01$)

FIGURE S4 Appressorium formation of WT, CK, STs, and OTs. (a) Appressorium of WT, CK, ST (C2ST10), and OT (C2OT11) strains at 16 hr. Bars indicate 20 μ m. (b) Comparison appressorium formation of WT, CK, STs, and OTs at 36 hr. The data are averages (and standard errors) of values from two biological replicates. Statistical significance was analysed using Student's *t* test between WT and each transformant (** $p < .01$)

FIGURE S5 Colony diameters and pathogenicity of transformants with slow growth rate. (a) Statistics of colony diameters of WT, CK, and C2-24 were based on 7 days of growth on RVA medium. (b) Each leaflet was inoculated with a mycelial plug, and then incubated at 21 °C with a 12-hr light/12-hr dark cycle. (c) Lesions induced by mycelia plug. The diameter of each lesion was measured at 3 days after inoculation. Three replicates were used for each treatment in these tests and the whole experiment was repeated once. The data are averages (and standard errors) of values from two biological replicates. Statistical significance was analysed using Student's *t* test between WT and each transformant (** $p < .01$). (d) At 12 hr after the inoculation, the leaves were incubated in 1 mg/ml DAB solution (pH 3.8), at room temperature for 8 hr and destained with ethanol. Bars indicate 50 μ m

FIGURE S6 Localization of PiCAT2 in *Phytophthora sojae*. Fluorescence from green fluorescent protein (GFP), PiCAT2-GFP protein (with GFP at C-terminal), and GFP-PiCAT2 protein (with GFP at N-terminal). Bars indicate 10 μ m

TABLE S1 The catalase genes of *Phytophthora infestans* and other species

TABLE S2 Primers used in this study

TABLE S3 The information of catalases in *Phytophthora infestans*

How to cite this article: Wang T-H, Wang X-W, Zhu X-Q, He Q, Guo L-Y. A proper PiCAT2 level is critical for sporulation, sporangium function, and pathogenicity of *Phytophthora infestans*. *Molecular Plant Pathology*. 2020;21: 460–474. <https://doi.org/10.1111/mpp.12907>