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Research

e-Xtra*

Shoot Blight on Chinese Fir (*Cunninghamia lanceolata*) is Caused by *Bipolaris oryzae*

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Abstract

Chinese fir (*Cunninghamia lanceolata*) is a significant timber species that has been broadly cultivated in southern China. A shoot blight disease on Chinese fir seedlings was discovered in Fujian, China and a fungus was then consistently associated with the symptoms. This fungus was determined to be causing this disease, among others by fulfilling Koch's postulates. Based on morphological characteristics and multilocus phylogenetic analyses with the sequences of the internal transcribed spacer, partial glyceraldehyde-3-phosphate dehydrogenase gene, partial translation elongation factor 1-α gene, and partial 28S large subunit ribosomal RNA gene, the fungus was identified as *Bipolaris oryzae*. These characteristics and phylogenetic analyses clearly support that this pathogen is different from *B. sacchari*, which was, until now, considered to be the causal agent of a similar blight on Chinese fir in Guangdong, China. The fungus was also shown to be strongly pathogenic to rice, one of the most susceptible hosts to *B. oryzae*. Crop rotation involving rice is often carried out with Chinese fir in southern China, a practice that most likely increases the risk of shoot blight on *C. lanceolata*. To our knowledge, shoot blight caused by *B. oryzae* is reported for the first time in a gymnosperm species.

The ascomycete genus *Bipolaris* includes significant phytopathogens with a worldwide distribution. Species in this genus cause leaf spots, leaf blights, melting outs, root rots, foot rots, and other diseases, primarily in the Poaceae family, which includes maize, rice, sorghum, and wheat, and on other hosts (Berbee et al. 1999; Ellis 1971; Sivanesan 1987; Verma et al. 2002). In addition to hosts in the Poaceae family, species of *Bipolaris* have been reported on at least 60 other plant genera in nongrass families as either saprobes or pathogens (Ellis 1971; Manamgoda et al. 2011; Sanahuja et al. 2017; Sivanesan 1987). Like most fungi, species of *Bipolaris* are difficult to identify based on morphology alone (Sivanesan 1987). Considerable progress has been made in defining species in *Bipolaris*. Manamgoda et al. (2014) recognized 47 species in *Bipolaris* based on morphological features and phylogenetic analysis using combined alignment of internal transcribed spacer (ITS), partial glyceraldehyde-3-phosphate dehydrogenase gene (*GPDH*), and partial translation elongation factor 1-α gene (*TEF-1*α) sequences. However, lack of sequences from ex-type or authenticated cultures in public databases is a major factor hindering the correct identification of *Bipolaris* spp. using molecular methods (Cai et al. 2011; Manamgoda et al. 2012).

Rice brown spot caused by *Bipolaris oryzae* is one of the most damaging diseases on rice worldwide, which reduces the yield by around 16 to 43% (Jatoi et al. 2016). This disease has led to historical damage to rice crops, causing the starvation of large human populations (Manamgoda et al. 2014). Considerable variation in conidial morphology and genetic characteristics has been reported within this species (Cholil and de Hoog 1982; Subramanian and Bhat 1978). The variation is reflected by its broad host range. Hosts of *B. oryzae* include not only species of the Poaceae family such as *Oryza sativa*, *Panicum maximum*, and *Zizania latifolia* but also Boraginaceae trees such as *Cordia trichotoma* (Manamgoda et al. 2014).

Chinese fir (*Cunninghamia lanceolata* (Lamb.) Hook, family Cupressaceae) is an important conifer species for lumber production in China, where it has been cultivated for over 3,000 years (Shi et al. 2010). Chinese fir is cultivated in the areas between latitude 20 and 34°N and from longitude 100 to 120°E in China, and contributes about 40% of timber supply in southern China (Zheng et al. 2016). However, Chinese fir is frequently decimated by various pests (Lan et al. 2015). Shoot blight is one of the fungal diseases affecting Chinese fir. Wang et al. (1995) reported that *B. sacchari* (E. J. Butler) Shoemaker appears to be the main pathogen causing shoot blight disease on Chinese fir in Guangdong, China.

The objectives of this research are to better characterize the causal agent of shoot blight disease on *C. lanceolata* in Fujian, China, and to identify the pathogen using both morphological and multilocus phylogenetic approaches.

Materials and Methods

Plants materials. Eleven-month-old seedlings of *C. lanceolata* obtained from aseptic tissue culture were supplied by Yangkou State Forest Farm, Fujian Province, China, and used for pathogenicity tests. Seeds of susceptible rice variety CO39 and maize variety Denghai11 were sown in the soil matrix (Pindstrup, Denmark) and grown in an incubator at 25°C with a 12-h photoperiod. For pathogenicity tests, 13-day-old rice and 15-day-old maize plants were used.

Isolation of the pathogen. The shoot blight disease was discovered in 1-year-old seedlings of Chinese fir in October 2016 in Yangkou State Forest Farm (26°49′18″N, 117°53′30″E), Fujian, China. In order to isolate the fungus, infected shoots and needles were collected and surface sterilized using the method of Huang et al. (2016). Lesion margins were cut into pieces (0.2 by 0.2 cm) and placed on 2% potato dextrose agar (PDA) Petri plates. The plates were incubated at 25°C for 7 days. Fungal isolates were purified with the monosporic isolation method described by Li et al. (2007). Single-spore isolates were maintained on PDA medium plates.

Pathogenicity tests. To stimulate sporulation of fungal isolates, 7-day-old cultures were exposed to a fluorescent cycle of 12 h of light

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and 12 h of darkness at 25°C. Four days later, conidia were collected and suspended in sterile distilled water, and the final concentration was adjusted to 1×10^5 spores ml^{-1}. Unwounded seedlings each of Chinese fir, rice leaves, and maize leaves were inoculated by spraying with the conidial suspension until leaves were covered with fine droplets. At the same time, the shoots of Chinese fir were slightly wounded by a sterile needle and inoculated with 5 μl of the conidial suspension. Plants treated with the sterile distilled water were employed as controls. The inoculated plants were placed in the dark for 24 h. Then, these plants were kept in an incubator at 25°C under a 12-h photoperiod. The experiment was repeated twice and there were three replicates for each treatment.

In order to investigate the pathogenicity of the fungal isolates on Chinese fir in the field, new shoots were collected. A conidial suspension was prepared as described above. The conidial suspension (10 μl) was inoculated on the apical portion of the shoots of Chinese fir. After inoculation, the shoots were placed in 9-cm Petri dishes containing a piece of wet paper and placed in darkness for 24 h, then incubated under a 12-h photoperiod at 25°C. Shoots treated with sterile distilled water were used as controls. Three shoots were inoculated for each treatment, and the experiment was replicated three times.

In order to complete Koch's postulates, the fungus was reisolated from the lesion margins as described above. Colony, conidium, and conidiophore characteristics of the reisolated fungus were observed to confirm the identity of the fungus inoculated. The experiment was conducted three times, with three replicates per treatment for each host.

Morphological analysis. Three plates of either PDA, complete medium (CM), corn meal agar (CMA), or V8 vegetable juice medium (V8) were used to assess the colony growth rates of fungal isolates. Mycelial blocks (5 mm in diameter) of the fungus taken from PDA were inoculated in the center of the plates that were placed in an inoculator at 25°C. Colony diameters were measured at 1, 3, 5, and 7 days postinoculation (dpi), and the average growth rates were calculated.

Colony characteristics and pigment production on media plates at 25°C were examined at 7 dpi. Conidiophores and conidia were measured following the procedure of Manamgoda et al. (2014). In order to observe conidial germination, 20 μl of the conidial suspensions at the concentration of 1×10^5 spores ml^{-1} were added onto the glass slides and incubated at 25°C. Conidial germination and the length of germ tubes were observed and measured at 1, 2, 4, and 8 h. At least 30 measurements were made for each fungal structure with a ZEISS Axio Imager A2m microscope (ZEISS) using differential interference contrast. In order to observe fungal structures on the surface of seedlings, shoots and leaves were collected and prepared according to Zhou et al. (2016). Photomicrographs were taken under a Quanta 200 environmental scanning electron microscope (FEI).

Molecular identification and phylogenetic analysis. Genomic DNA was extracted following the method of Damm et al. (2008). ITS, *GPDH*, *TEF-1*α, and partial 28S large subunit ribosomal RNA gene (*LSU*) were amplified and sequenced with the primer pairs ITS-1/ITS-4 (White et al. 1990), gpd1/gpd2 (Manamgoda et al. 2012), EF983/2218R

Table 1. GenBank accession numbers of strains used for the phylogenetic analysis in this study

Species	Strain number	GenBank accession number[a]			
		ITS	*GPDH*	*EF1-α*	*LSU*
Bipolaris bicolor	CBS 690.96	KJ909762	KM042893	KM093776	KM243287
B. chloridis	CBS 242.77	JN192372	JN600961	…	…
B. clavate	BRIP 12530	KJ415524	KJ415422	KJ415471	KJ415477
B. coffeana	BRIP 14845	KJ415525	KJ415421	KJ415470	KJ415478
B. crotonis	BRIP 14838	KJ415526	KJ415420	KJ415469	KJ415479
B. cynodontis	CBS 109894	KJ909767	KM034838	KM093782	KM243288
B. drechsleri	FIP 373	KF500531	KF500534	KM093759	…
B. eleusines	CBS 274.91	KJ909768	KM034820	KM093758	KM243289
B. gossypina	BRIP 14840	KJ415528	KJ415418	KJ415467	KJ415481
B. heveae	CBS 241.92	KJ909763	AY004811	KM093791	KM243294
B. heliconiae	BRIP 17189	KJ415530	KJ415417	KJ415465	KJ415483
B. maydis	AR 5182	KM230388	KM034844	KM093792	…
B. microlaenae	BRIP 15613	JN192378	JN600973	JN601017	JN600995
B. microstegii	AR 4840	JX089579	JX089575	KM093756	JX100808
	AR 5192	KM230391	KM034819	KM093757	…
B. multiformis	CBS 480.74	KJ909771	KM034827	KM093768	KM243282
B. oryzae	SMYK1	MF185132	MF431722	MF431724	MF431723
	AR 5204	KM230393	KM042895	KM093787	KM243277
	MAFF 235499	KJ922383	KM042897	KM093789	…
	MFLUCC 10-0694	JX256413	JX276428	JX266582	…
	MFLUCC 10-0715	JX256416	JX276430	JX266585	JX256384
	MFLUCC 10-0733	JX256417	JX276431	JX266586	JX256385
	MFLUCC 13-0511	KF688965	KF688971	KF688977	…
	MFLUCC 13-0512	KF688966	KF688972	KF688978	…
	MFLUCC 13-0513	KF688967	KF688973	KF688979	…
	MFLUCC 13-0514	KF688968	KF688974	KF688980	…
B. panici-miliacei	CBS 199.29	KJ909773	KM042896	KM093788	KM243281
B. peregianensis	BRIP 12790	JN601034	JN600977	JN601022	JN601000
B. pluriseptata	BRIP 14839	KJ415532	KJ415414	KJ415461	KJ415486
B. sacchari	ICMP 6227	KJ922386	KM034842	KM093785	…
B. secalis	BRIP 14453	KJ415537	KJ415409	KJ415455	KJ415492
B. sorokiniana	CBS 120.24	KJ909776	KM034821	KM093762	KM243278
	MAFF 235500	KJ909789	KM034823	KM093764	…
B. victoriae	CBS 327.64	KJ909778	KM034811	KM093748	KM243271
B. zeae	AR 5181	KM230394	KM034817	KM093754	…
B. zeicola	AR 5166	KJ909788	KM034813	KM093750	…
Curvularia lunata	CBS 730-96	JX256429	JX276441	JX266596	JX256396

[a] Internal transcribed spacer (ITS), partial glyceraldehyde-3-phosphate dehydrogenase gene (*GPDH*), partial translation elongation factor 1-α gene (*TEF-1*α), and partial 28S large subunit ribosomal RNA gene (*LSU*).

(Schoch et al. 2009), and LR5/LROR (Schoch et al. 2009). Polymerase chain reaction (PCR) was carried out in an Eppendorf Nexus Thermal Cycler (Eppendorf) in a total volume of 50 μl using the method of Huang et al. (2016). Amplicons were sequenced by the Sangni Biotechnology Company. DNA sequences obtained were aligned and edited using the Molecular Evolutionary Genetic Analysis (MEGA 7.0) with Clustal W (Thompson et al. 1994). All sequences generated in the present study have been blasted in GenBank. Sequences with high similarities were selected for phylogenetic analysis (Table 1).

The phylogenetic analysis was conducted with each gene or region and the concatenated sequences of ITS, *GPDH*, *TEF1*-α, and *LSU* using MEGA 7.0 software (Kumar et al. 2016). Other *Bipolaris* sequences were obtained from GenBank for the analysis. *Curvularia lunata* was employed as an out-group. The multilocus phylogenetic tree was built using neighbor-joining analysis (the Tamura three-parameter model) with the gaps pairwise deletion option. The tree was drawn with branch lengths measured in the number of substitutions per site. The interior-branch test was performed using 1,000 bootstrap replications to evaluate the relative stability of the branches.

Results

Symptoms of shoot blight disease on *Cunninghamia lanceolata* in nature. The disease mainly infected the shoots of Chinese fir and the infection rate of the seedlings reached 82% (Fig. 1A). Some white resin was visible on infected shoots (Fig. 1B). These shoots, and the leaves in particular, appeared brown to brownish red (Fig. 1C). On dead shoots, abundant mycelium was observed (Fig. 1D). A large number of conidia and conidiophores developed on the surface of the leaves (Fig. 1E). Scanning electron micrographs also confirmed the presence of mycelium, conidia, and conidiophores on the surface of infected shoots (Fig. 1F).

Pathogenicity tests using fungal isolate SMYK1 on Chinese fir. Seven fungal isolates were obtained from the infected shoots. Because these isolates shared nearly identical colony characteristics and conidia morphological features, an isolate was randomly selected, and named SMYK1, for the pathogenicity assessment and morphological study.

In order to fulfill Koch's postulates, conidia were sprayed on healthy, aseptic tissue culture of seedlings of *Cunninghamia lanceolata*. Seven days after inoculation, blight symptoms occurred on the shoots (Fig. 2A). At the early stage of symptom development, there were irregular, brown spots on the surface of the leaves; later, white to gray centers were formed on the brown lesions (Fig. 2B). When the wounded shoots were inoculated with conidial suspension, similar symptoms were observed at 7 dpi (Fig. 2C). At the same time, abundant mycelium appeared on the infected leaves and stems (Fig. 2D). After exposing the diseased tissues to a 12-h fluorescent photoperiod for 3 days, conidiophores and conidia were observed (Fig. 2E). Control seedlings remained healthy and did not yield any microorganisms. When shoots of Chinese fir collected from the field were inoculated by the fungus, typical symptoms were observed by 9 dpi whereas no symptoms developed on shoots treated with water (Fig. 3). These symptoms were similar to the typical symptoms of this disease in nature. In addition to morphological features of colonies, aerial mycelia

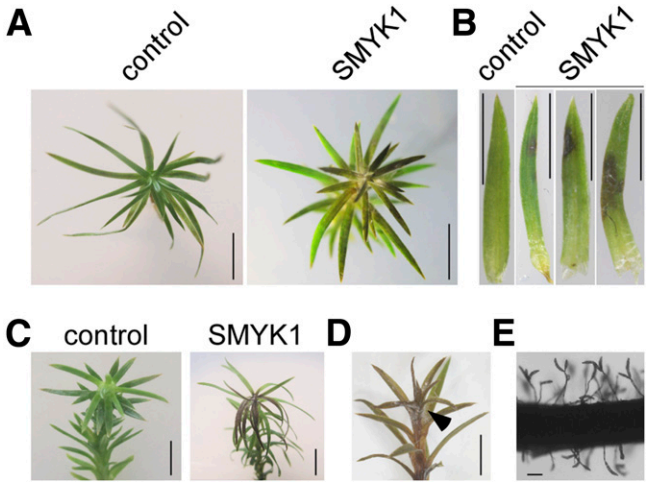

Fig. 2. Pathogenicity of fungal isolate SMYK1 on seedlings of Chinese fir obtained by tissue culture. **A,** Unwounded seedlings were inoculated with conidia of SMYK1. **B,** Disease spots formed at an early stage of symptom development. **C,** Wounded seedlings were inoculated with conidia of SMYK1. **D,** Diseased shoot of *Cunninghamia lanceolata*. Arrowhead indicates mycelium on the diseased shoot. Bars (A to D) = 0.5 cm. **E,** Conidia and conidiophores formed on the diseased leaves. Bar (E) = 100 μm.

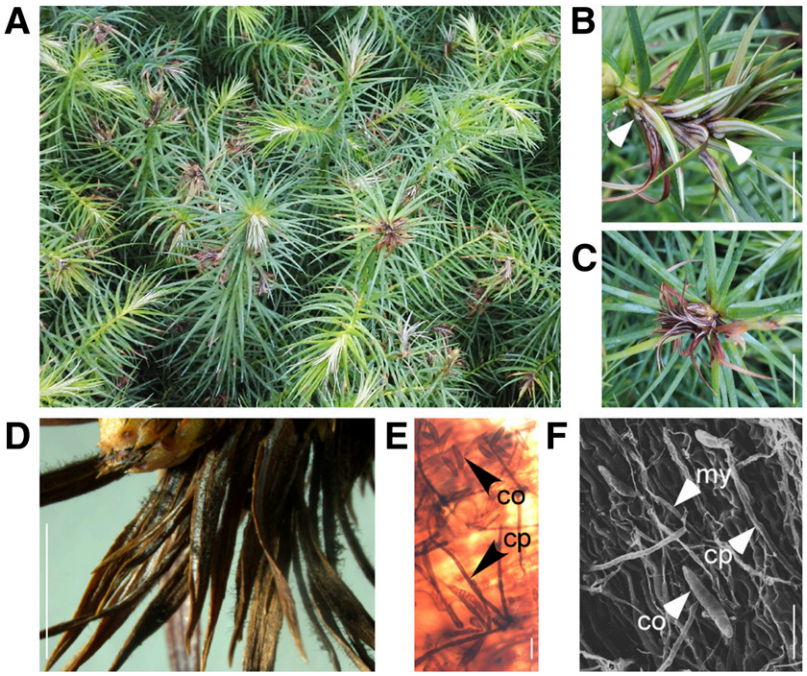

Fig. 1. Symptoms of shoot blight disease on *Cunninghamia lanceolata*. **A,** Diseased shoots in the field. **B** and **C,** Diseased shoots of *C. lanceolata*. Arrows indicate the white oozed resin on the shoot. **D,** Mycelium on the infected shoot. Bars (A to D) = 2 cm. **E,** Conidia and conidiophores on the infected shoot. **F,** Scanning electron photomicrograph of fungal mycelium, conidia and conidiophores on the infected shoot; my, co, and cp indicate mycelium, conidia, and conidiophores, respectively. Bars (E and F) = 100 μm.

and conidia of the fungus reisolated from the margin of necrotic areas after the appearance of symptoms were similar to the initial inoculated strain SMYK1. No fungal isolate was reisolated from the controls. These results indicated that SMYK1 was the pathogen of shoot blight on *C. lanceolata*. The living culture of SMYK1 has been deposited in the China Center for Type Culture Collection (CCTCC AF 2017004) at Wuhan University, Wuhan City, Hubei Province, China.

Morphological characteristics of SMYK1. Colonies grown on PDA were irregularly round, and mycelial growth rate was 1.11 cm/day, on average (Fig. 4A; Supplementary Fig. S1). Aerial mycelium was dense, felted, and pale gray at an early stage (Fig. 4A). With age, the color darkened and finally became grayish green. The colonies produced black pigments (Fig. 4A and B). On CM, CMA, and V8 plates, the colonies showed similar color changes. The colonies showed slower growth rates on CMA.

When the colonies growing on PDA, CM, CMA, and V8 plates were exposed to a 12-h fluorescent photoperiod for 3 days, abundant conidia and conidiophores were observed (Fig. 4B and C). Conidiophores were solitary or in groups, brown, less branched, multiseptate, and flexuous, with upper parts geniculate (Figs. 1E, 4C, and 5A). The average length of conidiophores was 190.9 ± 38.5 μm and the average width was 8.1 ± 1.2 μm. Conidia were usually curved, rarely straight, fusiform, navicular, obclavate or nearly cylindrical, colorless when immature, turning brown when mature, oblong, spindle-shaped, cylindrical, rarely straight, and usually bent to one side (Fig. 5B). Most conidia were 7- to 9-distoseptate (Fig. 5C). Conidial size varied from 76.8 to 117.8 by 13.4 to 18.4 μm, with a mean ± standard deviation of 97.3 ± 20.5 by 15.9 ± 2.5 μm (n = 30).

Conidia germinated from both ends. Most conidia germinated within 1 h in double-distilled H_2O (Supplementary Fig. S2). At 4 h, conidium germination rate was up to 97.2%. At 1, 2, 3, and 4 h, the average length of the germ tubes was 43.4 ± 36.1, 110.7 ± 56.3, 169.7 ± 90.8, and 180.6 ± 131.7 μm, respectively (n > 20). The primary germ tubes were colorless. These morphological characteristics matched the epitype culture selected for *B. oryzae* by Manamgoda et al. (2014).

Molecular characterizations of the fungal pathogen. The ITS, *GPDH*, *EF1*-α, and *LSU* sequences obtained from *B. oryzae* SMYK1 were deposited in GenBank (accession numbers MF185132, MF431722, MF431724, and MF431723, respectively). The ITS sequence showed 99% identity, with 100% Query Cover, to many *B. oryzae* strains deposited in GenBank (e.g., KU499535). The *GPDH* sequence matched 99% with the *B. oryzae* strain MFLUCC 13-0511 (KF688971.1). The *TEF1*-α sequence showed 100% identity to the *B. oryzae* strain MFLUCC 13-0511 (KF688977.1). The *LSU* sequence showed 99% similarity with *B. oryzae* strain B34 (KM111240.1).

A phylogenetic tree calculated from *GPDH* sequences showed that SMYK1 is most closely related to *B. oryzae*, and grouped together with *B. oryzae* isolate MFLUCC 10-0733 with a 64% bootstrap value support (Fig. 6). This group clustered with other five *B. oryzae* isolates with a 75% bootstrap support (Fig. 6). A phylogenetic tree calculated from concatenated sequences of the ITS and *GPDH* gene also showed that SMYK1 was clustered with six *B. oryzae* strains with 74% bootstrap support (Supplementary Fig. S3). Phylogenetic relationships analyzed with concatenated sequences of ITS, *GPDH*, and *TEF1*-α also supported the finding that SMYK1 was clustered with seven *B. oryzae* strains with 82% bootstrap support (Supplementary Fig. S4). Phylogenetic analysis using the concatenated sequences of ITS, *GPDH*, *TEF1*-α, and *LSU* also illustrated that SMYK1 and authentic strains of *B. oryzae* were monophyletic, supported with a significantly higher bootstrap value (Fig. 7). All these results showed that SMYK1 is most likely *B. oryzae*.

Pathogenicity of SMYK1 to rice and maize. According to the morphological and molecular characteristics, SMYK1 was identified as *B. oryzae*. Pathogenicity tests of SMYK1 on rice showed that brown spots occurred at 3 dpi (Fig. 8A). These spots enlarged and amalgamated to form larger chlorotic to necrotic spots at 4 to 5 dpi. At 6 dpi, these spots further developed and formed lesions. There were no symptoms on the negative controls (Fig. 8A). Conidia were produced on the spots when the diseased leaves were exposed to a 12-h fluorescent photoperiod (Fig. 8B). When conidia were sprayed on the maize leaves, gray spots were formed. These spots failed to enlarge to produce the typical lesions. No spots occurred on the leaves of the controls (Fig. 8C). After the appearance of symptoms on rice and maize leaves, the fungus was reisolated from the transitional region between infected and healthy areas. Morphological characteristics of colonies, aerial mycelia, and conidia were similar to the initial inoculated strain SMYK1. No fungi were isolated from the healthy leaves of rice and maize. These results strongly suggested that SMYK1 is the sole cause of shoot blight observed on Chinese fir in the present study, and this strain also appeared strongly pathogenic on rice and weakly pathogenic on maize.

Discussion

In this study, a shoot blight was discovered on young seedlings of *C. lanceolata* in Fujian, China and determined to be caused by *B. oryzae*. Wang et al. (1995) reported that *B. sacchari* was the causal agent of a similar blight on *C. lanceolata* in Guangdong, China. However, the morphological characteristics of *B. sacchari* are different from those of *B. oryzae*. For example, the average conidial size of *B. sacchari* is 74 ± 19 by 13 ± 1 μm (Manamgoda et al. 2014), which is significantly smaller than that reported in the present study for SMYK1. Leaf spot symptoms of *B. sacchari* were initially small, red, elongating parallel to the midvein, and then producing eye spots with a light yellow center and red halo. This is also different from the symptoms induced by SMYK1, which formed brown spots on leaves, having later white to gray centers. Because the specimen, culture, and sequence accession number of the

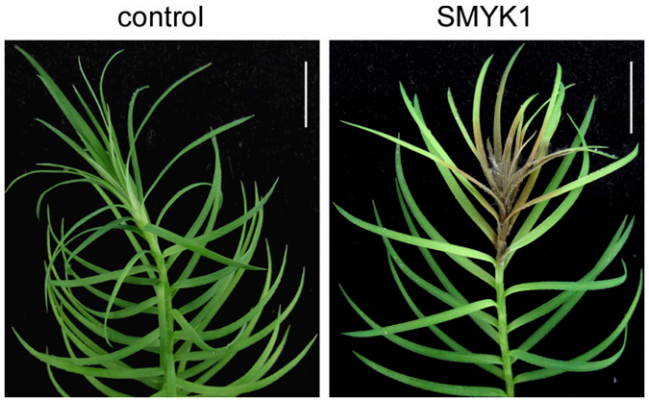

Fig. 3. Pathogenicity of fungal isolate SMYK1 on detached shoots of Chinese fir. Bars = 2 cm.

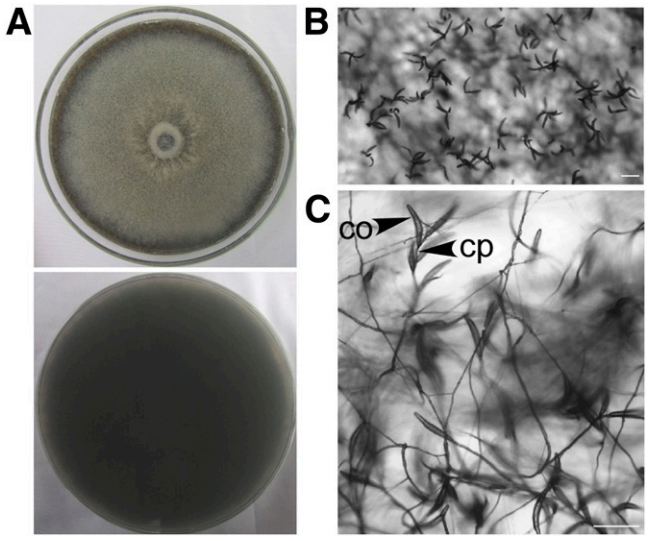

Fig. 4. Colony morphology and conidia development of SMYK1 on the potato dextrose agar medium plates. **A,** Top and reverse view of a colony. **B,** Numerous dark conidia were obvious and **C,** at higher magnification, conidia (co) were seen attached to their conidiophores (cp). Bars = 100 μm.

fungus of Wang et al. (1995) is not available, the similarity between their pathogen and SMYK1 cannot be verified or determined. However, our phylogenetic analysis using sequences of *GPDH* (Fig. 6); the concatenated sequences of ITS and *GPDH*; and ITS, *GPDH*, and TEF-1α showed that *B. sacchari* and *B. oryzae*, including strain SMYK1, fall into two different clades. Thus, these results indicate for the first time that this shoot blight is actually caused by *B. oryzae* on Chinese fir.

In terms of molecular characterization of eukaryotic microbes, the ITS sequence is considered an important region often employed for species identification (Johannesson and Stenlid 1999; Malan et al. 2011). For several fungi, the ITS region has been proposed as a universal DNA marker (Schoch et al. 2012); however, for some large genera of fungi such as *Alternaria*, *Bipolaris*, and *Colletotrichum*, their species cannot be efficiently distinguished using solely ITS sequence data (Brun et al. 2013; Manamgoda et al. 2014; Weir et al. 2012). Consequently, other genes such as *GPDH* must be used to be able to differentiate at the species level (Cannon et al. 2012; Manamgoda et al. 2014). Manamgoda et al. (2014) opined that the *GPDH* gene is the best single marker for species of *Bipolaris*. In order to differentiate SMYK1 from closely related species, its phylogenetic relationships with allied taxa were analyzed using concatenated sequences of ITS, *GPDH*, *TEF-1α*, and *LSU*, and it was determined to be *B. oryzae*.

B. oryzae has a broad host range (Manamgoda et al. 2014). Its hosts include not only gramineous and nongramineous crops but also some trees (Dela Paz et al. 2006; Manamgoda et al. 2014; Sanahuja et al. 2017). In this study, *B. oryzae* was shown to be the cause of shoot blight of Chinese fir, and was also determined to be pathogenic to rice and maize. In southern China, rotation of rice and *C. lanceolata* is usually used to improve the productivity of Chinese fir in nurseries and the quality of the seedlings (Qiu 2007). In Fujian, rice plants are sown in May to June and are harvested in November. In the following spring, from April to May, young Chinese fir seedlings are cultivated in the same field by sowing or cutting, and the seedlings are removed from the nurseries for afforestation in December. After rice harvesting, the remaining rice roots, stalks, and leaves are directly used as the matrix to cultivate Chinese fir. The Fujian location under study is part of the distribution area of rice brown spot disease caused by *B. oryzae*. To complicate matters, due to long-term artificial selection mainly based on the growth, only a few Chinese fir varieties or clones are used and, thus, they are most likely to be

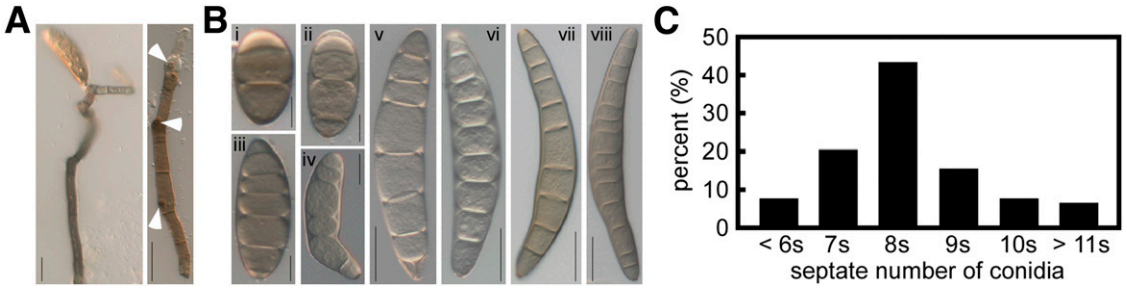

Fig. 5. Conidiophore and conidial morphological characteristics of SMYK1. **A,** Conidiophores with septations. Arrowheads indicate sporulation pores. **B,** Conidia: i to iii show straight conidia whereas iv to viii indicate curved conidia. **C,** Percentage of conidia with different septate numbers. Bars = 20 μm.

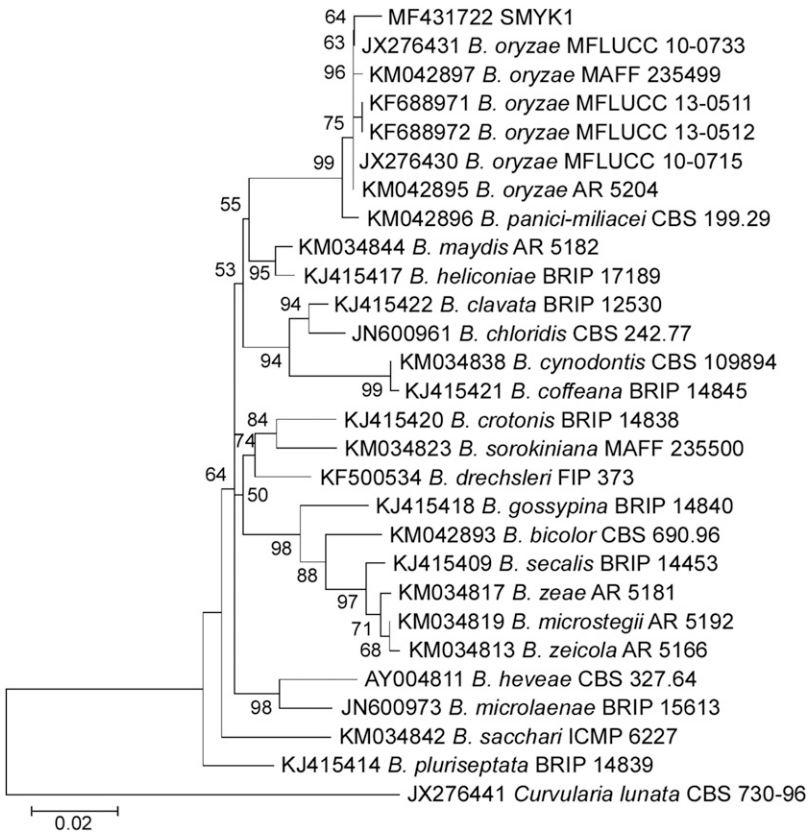

Fig. 6. Phylogenetic tree of SMYK1 with allied taxa calculated from the partial glyceraldehyde-3-phosphate dehydrogenase gene using neighbor-joining method. Bootstrap values >50% (1,000 replications) are given at the nodes. *Curvularia lunata* CBS 730-96 is used as an outgroup. Bar = 0.02 substitution per nucleotide position.

vulnerable to the disease. These factors, and particularly crop rotation with rice and Chinese fir, substantially increase the risk of shoot blight on *C. lanceolata*. Another possibility is that crop rotation may favor the appearance of populations of the pathogen more virulent on both hosts. In conclusion, we can say with a great level of confidence that the gymnosperm Chinese fir is a new host for *B. oryzae*, which causes a shoot blight previously reported as being induced by *B. sacchari*. To our knowledge, this is the first time that *B. oryzae* is reported on a conifer species.

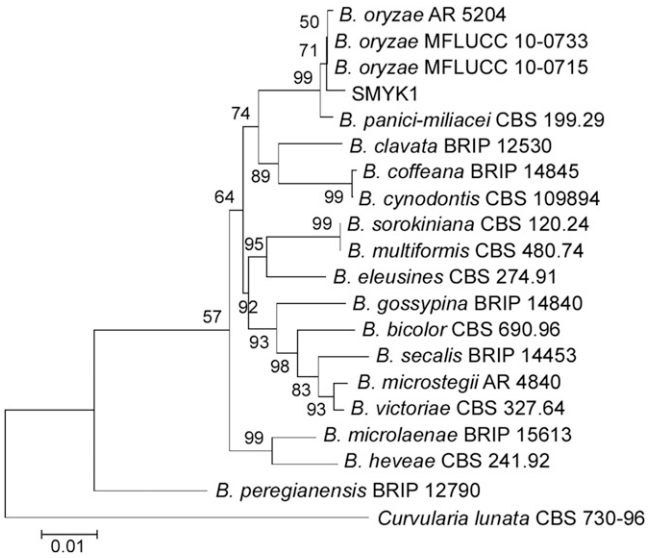

Fig. 7. Phylogenetic tree of SMYK1 with allied taxa calculated from the alignment of concatenated sequences of the internal transcribed spacer, partial glyceraldehyde-3-phosphate dehydrogenase gene, partial translation elongation factor 1-α gene, and partial 28S large subunit ribosomal RNA gene using the neighbor-joining method. Bootstrap values >50% (1,000 replications) are given at the nodes. *Curvularia lunata* CBS 730-96 is used as an outgroup. Bar = 0.01 substitution per nucleotide position.

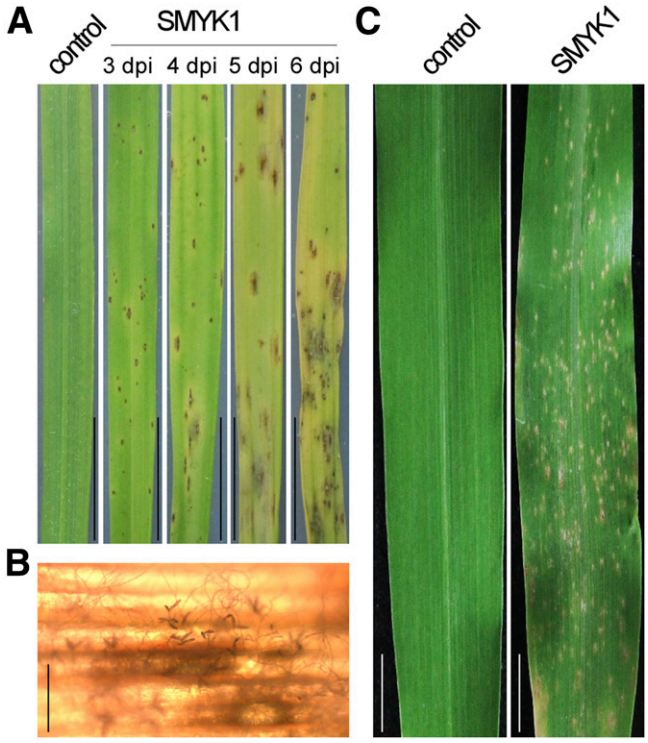

Fig. 8. Pathogenicity of SMYK1 on rice and maize leaves. **A,** Lesions on rice leaves at different days postinoculation (dpi). Bars = 1 cm. **B,** Conidia formed on rice leave lesions (6 dpi). Bar = 0.5 cm. **C,** Lesions on maize leaves (10 dpi). Bars = 1 cm.

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