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Cultural, Morphological and Pathological Characterization of *Colletotrichum falcatum* Causing Red Rot Disease of Sugarcane in Thailand

Pakamart Sangdit[1], Chalida Leksomboon[1,*] and Rewat Lertsrutaiyotin [2,3]

ABSTRACT

Fifteen isolates of *Colletotrichum falcatum* were isolated from sugarcane in five different areas in Thailand. All isolates were identified by polymerase chain reaction (PCR) technique using primers its1 and its4 derived from internal transcribed spacer (ITS) sequences to amplify a unique DNA fragment of 590 bp from *C. falcatum*. Amplified DNA bands of each isolate were sequenced and determined their identity using the GenBank database. Nucleotide sequence analysis of the ITS sequences indicated that the 15 isolates showed 95.32–100% identity with each other and 96.30–97.74% related to *C. falcatum*. Variation in the cultural, morphological and pathological characteristics of the 15 isolates were investigated. The 15 isolates were differentiated into two distinct groups (light and dark types) based on the colony color character. Ten isolates (LB1-4, LB1-6, LB1-7, LB2-1, LB2-2, LB2-3, LB2-4, LB2-5, LB2-7 and NM1) in the light type produced white color colonies whereas isolates KB1, KBL1-1, KBL1-2, KBL1-3 and SBL1 in the dark type had gray color colonies. The growth rate among all isolates varied from 11.6 to 12.9 $mm.d^{-1}$ and was not significantly different. The 15 isolates produced setae and conidia that were hyaline, one-celled, falcate or sickle-shaped. The conidia of all isolates ranged between 21.42 and 28.56 µm in length and from 2.38 to 4.76 µm in width. Appressoria were terminal, rarely intercalary, aseptate and the overall shape was globose or clavate, with edges entire. The appressorial size ranged between 10.68 and 16.02 µm in length and from 8.01 to 13.35 µm in width. Pathogenicity of the 15 isolates on stalks of sugarcane varieties E-Heaw, K84-200, K88-92 and K93-236 by the plug method was used to characterize the pathogenic variability among the isolates of *C. falcatum*. Nine isolates (LB1-4, LB1-6, LB1-7, LB2-1, LB2-2, LB2-3, LB2-4, LB2-5, LB2-7) from localities affected with the red stalk rot epidemic possessed a degree of pathogenicity toward the susceptible varieties E-Heaw and K93-236, while those from localities without the red stalk rot epidemic (NM1, KB1, KBL1-1, KBL1-2, KBL1-3, SBL1) were nonpathogenic on both varieties. None of the isolates produced the typical red rot symptom on stalks of the resistant varieties K84-200 and K88-92. A principal finding in this study was that only *C. falcatum* samples originating from localities affected with the red stalk rot were capable of infecting stalks of sugarcane and they had a light type of colony character. The results presented in the

[1] Department of Plant Pathology, Faculty of Agriculture, Kasetsart University, Kamphaeng Saen Campus, Nakhon Pathom 73140, Thailand.

[2] Department of Agronomy, Faculty of Agriculture, Kasetsart University, Kamphaeng Saen Campus, Nakhon Pathom 73140, Thailand.

[3] Cane and Sugar Research and Development Center, Kasetsart University, Kamphaeng Saen Campus, Nakhon Pathom 73140, Thailand.

* Corresponding author, e-mail: agrchl@ku.ac.th

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pathogenicity test demonstrated that *C. falcatum* causing red rot disease of sugarcane in Thailand may be differentiated into two distinct races (pathogenic and nonpathogenic) based on their pathological character on the stalks of sugarcane.

Keywords: *Colletotrichum falcatum*, red rot disease, sugarcane

INTRODUCTION

Colletotrichum falcatum Went, causing red rot disease of sugarcane, belongs to the Glomerallaceae of the Ascomycota and its telemorph is *Glomeralla tucumanesis* (Rafay and Singh, 1957) but it is also named *Physalospora tucumanesis* (Bailey and Jeger, 1992). *C. falcatum* produces falcate or sickle-shaped conidia (Sutton, 1992). The appressoria are terminal, rarely intercalary, aseptate, smooth but thick-walled and cinnamon buff in color, clavate to ovate, edge entire (Sutton, 1968; Sutton, 1992). On medium, two distinct cultural races may be differentiated—a light one producing white to light gray, cottony, floccose mycelial growth, and a dark one with compact, velvety, dark-grey mycelia. Isolates have also been differentiated on the basis of the texture, amount and color of mycelia, growth rate, production and color of spore masses in culture (Abbott and Hughes, 1961). Red rot of sugarcane is distributed worldwide but is found mainly in subtropical and tropical regions (Kumar *et al*., 2011). Red rot may affect any of the vegetative parts of the sugarcane plant but it is of principal importance as a disease of the standing stalks and the planted seed pieces or cutting. It is often very obvious on the leaf midribs (Singh and Singh, 1989). Sugarcane is an economic crop in Thailand, which is the fourth largest producer in the world (OAE, 2013). Red rot is one of the most serious diseases of sugarcane in Thailand (Leksomboon and Lertsrutaiyotin, 2014). There are many reports of the characterization of *C. falcatum* in the major countries of cultivation (Kalaimani, 1995; Rohana Wijesekara and Agarwal, 2006; Mishra and Behera, 2009a; Abbas *et al*., 2010; Prihastuti *et al.* 2010), but very little information in Thailand. Characterization of the *C. falcatum* isolates would enable red rot management strategies and the development of a resistant cultivar. The objective of this study was to characterize the cultural, morphological and pathogenic variability among isolates of *C. falcatum* infecting sugarcane in some sugarcane-growing provinces of Thailand.

MATERIALS AND METHODS

Fungal isolates

C. falcatum was isolated from sugarcane leaves and stalks from different areas in Thailand. The infected tissues were cut (5 × 5 mm) and surface sterilized by dipping in 10% household chlorine bleach for 5 min and rinsing three times with sterilized distilled water before drying on sterilized tissue paper. The samples were placed on potato dextrose agar (PDA) and incubated at room temperature (28–30 °C) under 12 hr light (two cool daylight lamps 1,200 W; Philips Electronics Ltd,. Bangkok, Thailand). After 5 d of incubation, the growing edges of the fungal hyphae developing from the tissues were transferred to PDA. When the fungus showed sporulation, spore masses were picked off with a sterilized wire loop and streaked on the surface of water agar. The hyphal tips of single germinated spores were transferred to PDA and maintained on PDA to keep the cultures viable. When isolations of pure cultures from infected plant tissues had been achieved, pathogenicity tests were conducted to fulfill the requirements of Koch's postulates (Agrios, 2005). Pathogenicity tests were conducted using the detached leaf method. The midribs of detached leaves were pricked 1 mm deep using a needle and replaced with mycelial plugs (5.5 mm in diameter) of each isolate. The ability of each isolate to cause red

rot was determined on the midribs of sugarcane variety K93-236. After inoculation, the leaves were incubated in a humid chamber. Control leaves were inoculated with agar plugs onto the wound.

Identification of *C. falcatum* by polymerase chain reaction technique

All isolates were identified by a polymerase chain reaction (PCR) technique using universal primers its1 (GCCGTAGGTGAACCTGCGG) and its4 (GCCTCCGCTTATTGATATGC) (White *et al*., 1990; Kumar *et al.*, 2011) to amplify a unique DNA fragment of 590 bp. Mycelial discs from pure culture were taken from actively growing areas near the growing edge of culture aged 7 d and transferred to potato dextrose broth and agitated at 150 rpm at room temperature (28–30 °C) for 10 d. Total genomic DNA was extracted from mycelia according to Kumar *et al.* (2011). PCR reactions were performed in 50 µL as the total volume, containing 100 ng of DNA, 0.2 mM dNTPs, 2 mM $MgCl_2$, 1 µM of each primer, and 1.25 U *Taq* polymerase (Fermentas®; Vilnius, Lithuania). The reaction mixtures were incubated in a Thermal cycler (Model TC-25/H; Bioer Technology Co., Ltd.; Hangzhou, China). The PCR temperature profiles were: initial DNA denaturation at 95 °C for 2 min, followed by 30 cycles at 95 °C for 30 s, 52 °C for 30 s, 72 °C for 45 s and a final step at 72 °C for 10 min. All the amplified PCR products were resolved using electrophoresis on 1% agarose for 25 min in 1× Tris-acetate buffer at 100 V. DNA sequencing was performed at First Base Laboratories Sdn Bhd, Seri Kembangan, Malaysia.

The alignments of the internal transcribed spacer (ITS) sequences were performed using the Clustal X software (version 2.0; The Conway Institute of Biomolecular and Biomedical Research; Dublin, Ireland), and the ITS1-5.8S-ITS2 sequences were used to construct a phylogenetic tree using the maximum parsimony method (Tamura *et al.*, 2011) and MEGA version 5 (http://www.megasoftware.net). Bootstrapping was performed with 1,000 replications. The ITS sequence information was used to match the fungal isolates with the NCBI BLAST program from the GenBank database (http://www.ncbi.nlm.nih.gov).

Cultural and morphological characterization

The growth rate and colony characteristics were recorded from cultures grown on PDA. The cultures were incubated as previously described and the diameter of the colony was recorded daily for 7 d and the growth rate was assessed as the 7 d average of mean daily growth. The colony characters, texture, density, color and presence of conidial masses were recorded on the tenth day from culture. Three replicate cultures of each isolate were investigated. For each isolate, the length and width of 20 spores were measured and the shaped recorded. Appressoria were determined using the slide culture technique (Dhingra and Sinclair, 1995), in which 10 mm^2 samples of PDA were placed on a sterile microscopic slide. The edge of the agar block was inoculated with spores taken from a sporulating culture and a sterile cover slip was placed on top of the agar block. The slide was placed on glass rods in a Petri dish lined with moistened, sterile filter paper which was kept damp. After incubation for 3–5 d, the shape and size of the appressoria were studied. Morphological data were analyzed using analysis of variance ($P < 0.05$) with Duncan's multiple range test using the statistical programs R (R Core Team, 2014) and Minitab (2010).

Pathological characterization

The pathogenic variability of the 15 isolates was determined on 60 cm stalks of sugarcane varieties E-Heaw, K84-200, K88-92 and K93-236 from the Cane-Agricultural Centre in Kanchanaburi province, Office of the Cane and Sugar Board. Mature stalks were cut and the leaf sheaths were stripped. The stalks were surface disinfected with 10% household chlorine bleach. Inoculation was carried out using the

standard plug method (Srinivasan and Bhat, 1961) in the laboratory. The median internode of the experimental stalks was punctured with a 7 mm diameter cork borer instrument and a mycelial disc of *C. falcatum* was introduced into the stalks. The inoculated stalks were wrapped with wrapping film (M Wrap®; MMP Corporation Ltd.; Bangkok, Thailand). Control stalks were inoculated with agar plugs. Eight stalks of each sugarcane variety were inoculated with each isolate. After 28 d of incubation, the inoculated stalks were split longitudinally and the amount of disease in the inoculated internodes was recorded for each stalk as 1, 2 and 3 for red discoloration at the inoculated internode, red discoloration throughout the length of the inoculated internode and red discoloration that had crossed the inoculated internode, respectively. The punctured controls showed red discoloration at the point of puncture and thus received ratings of 0.

RESULTS

Fungal isolates

A total of 15 isolates *C. falcatum* were received from five different sites in different provinces except for Lopburi province where two samples were sourced. Summary information of the *C. falcatum* samples isolated in this study is provided in Table 1. The results of the pathogenicity tests revealed that all isolates were pathogenic on the midrib of sugarcane leaves and this was considered as the cause of the disease. Midrib lesions on sugarcane leaves began to appear 3 d after inoculation. Lesions formed bright red at first and later became a straw color in the center with dark red margins. No symptoms were observed in the control treatment.

Identification of *C. falcatum* by polymerase chain reaction technique

In total, 15 isolates were analyzed by PCR. The size of all amplified PCR products was estimated to be 590 bp (Figure 2). The ITS sequences

Table 1 Isolation of *C. falcatum* from sugarcane in this study.

Isolate	Infected material	Host	DS	Location / site
KB1	Stalk	-	0	Kanchanaburi, central Thailand, site 1
KBL1-1	Leaf midrib	-	0	Kanchanaburi, central Thailand, site 1
KBL1-2	Leaf midrib	-	0	Kanchanaburi, central Thailand, site 1
KBL1-3	Leaf midrib	-	0	Kanchanaburi, central Thailand, site 1
LB1-4	Stalk	K93-236	1	Lop Buri, central Thailand, site 2
LB1-6	Stalk	K93-236	1	Lop Buri, central Thailand, site 2
LB1-7	Stalk	K93-236	1	Lop Buri, central Thailand, site 2
LB2-1	Stalk	K93-236	1	Lop Buri, central Thailand, site 3
LB2-2	Stalk	K93-236	1	Lop Buri, central Thailand, site 3
LB2-3	Stalk	K93-236	1	Lop Buri, central Thailand, site 3
LB2-4	Stalk	K93-236	1	Lop Buri, central Thailand, site 3
LB2-5	Stalk	K93-236	1	Lop Buri, central Thailand, site 3
LB2-7	Stalk	K93-236	1	Lop Buri, central Thailand, site 3
NM1	Stalk	-	0	Nakhon Ratchasima, northeastern Thailand, site 4
SBL1	Leaf midrib	-	0	Sing Buri, central Thailand, site 5

DS = Disease severity: 0 = No external symptoms on stalks and no plants dead; 1 = Stalk rot and plant death.
- = Not known.

of the 15 isolates were aligned using Clustal X, and a phylogenetic tree was generated using the maximum parsimony method and the MEGA version 5 software. The phylogenetic tree was rooted with *C. falcatum* (AJ536231), *Glomerella tucumanensis* (HM592294), *C. graminicola* (AF059676), *C. coccodes* (GU935878) and *C. gloeosporioides* (HQ645076). Phylogenetic analysis divided the 15 isolates into two distinct groups designated A and B (Figure 3). Group A (68% bootstrap) was represented by eight isolates (LB1-6, LB1-7, LB2-1, LB2-2, LB2-3, LB2-4, LB2-5, LB2-7) and included the *C. falcatum* isolate KPS which was collected from Nakhon Pathom province (provided by Dr. Chalida Leksomboon, Kasetsart University, Nakhon Pathom, Thailand), *C. falcatum* (AJ536231) and *G. tucumanensis* (HM592294), where *G. tucumanensis* is an anamorph of *C. falcatum*. Group B (98% bootstrap) contained seven isolates (NM1, KB1, SBL1, LB1-4, KBL1-3, KBL1-1 and KBL1-2). A comparison of the ITS sequence data from sequences deposited in the GenBank database found that the 15 isolates showed 95.32–100% identity with each other and the 15 isolates shared 96.30–97.74 and 94.85–98.40% ITS sequence identities with *C. falcatum* (AJ536231) and *G. tucumanensis* (HM592294), respectively. The other species—*C. graminicola* (AF059676), *C. coccodes* (GU935878) and *C. gloeosporioides* (HQ645076)—did not group with any isolates of *C. falcatum*.

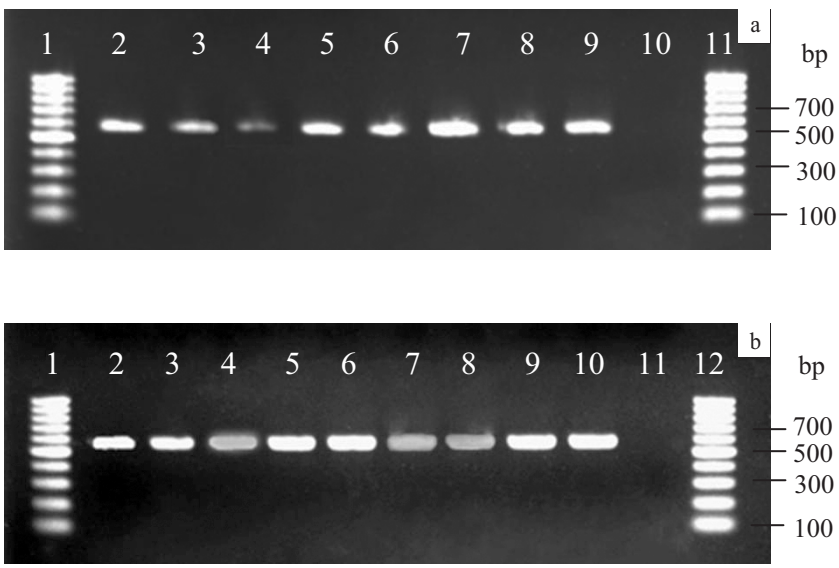

Figure 2 Agarose gel electrophoresis of polymerase chain reaction products from DNA of *Colletotrichum falcatum* isolates from sugarcane using universal primers its1 and its4: (a) Lanes 1 and 11 = 100 bp molecular weight marker (Fermentas®; Vilnius, Lithuania). Lanes 2-10 = *C. falcatum* isolates; Lane 2 = isolate LB1-4; Lane 3 = isolate LB1-6; Lane 4 = isolate LB1-7; Lane 5 = isolate KBL1-1; Lane 6 = isolate KBL1-2; Lane 7 = isolate KBL1-3; Lane 8 = isolate SBL1; Lane 9 = isolate KB1; Lane 10 = no template control; (b) Lanes 1 and 12 = 100 bp molecular weight marker (Fermentas). Lane 2-11 = *C. falcatum* isolates; Lane 2 = isolate LB2-1; Lane 3 = isolate LB2-2; Lane 4 = isolate LB2-3; Lane 5 = isolate LB2-4; Lane 6 = isolate LB2-5; Lane 7 = isolate LB2-7; Lane 8 = isolate NM1; Lane 9 = isolate KB1; Lane 10 = isolate LB1-4; Lane 11 = no template control.

Cultural and morphological characterization

The 15 *C. falcatum* isolates were examined for cultural and morphological characterization. The fungal growth was initiated with white mycelia. Colony color was variable, white or gray, aerial mycelia white, cottony with salmon conidial masses. Differences in the colony color character among isolates allowed them to be separated into two distinct groups. Group 1 (the light type) comprised 10 isolates consisting of LB1-4, LB1-6, LB1-7, LB2-1, LB2-2, LB2-3, LB2-4, LB2-5, LB2-7, NM1 and group 2 (the dark type) consisted of the other five isolates—KB1, KBL1-1, KBL1-2, KBL1-3 and SBL1 (Table 2 and Figure 4). Growth of all isolates varied from 11.6 to 12.9 mm.d^{-1}, averaging 12.1 mm d^{-1}. The growth rate among isolates was not significant (Table 2). All isolates produced setae and conidia were hyaline, one-celled, falcate or sickle-shaped (Figure 5). The conidial size of all the isolates ranged between 21.42 and 28.56 µm in length by 2.38 and 4.76 µm in width, averaging 25.01 × 4.46 µm. There were statistically significant differences in the mean lengths of conidia but the mean width of the conidia of all isolates was not significant (Table 2). Appressoria were terminal, rarely intercalary, aseptate and the overall shape was globose or clavate, edges entire (Figure 5), 10.68 to 16.02 µm in length by 8.01 to 13.35 µm in width, averaging 12.92 × 9.44 µm. The mean length and width of appressoria were significantly different (Table 3).

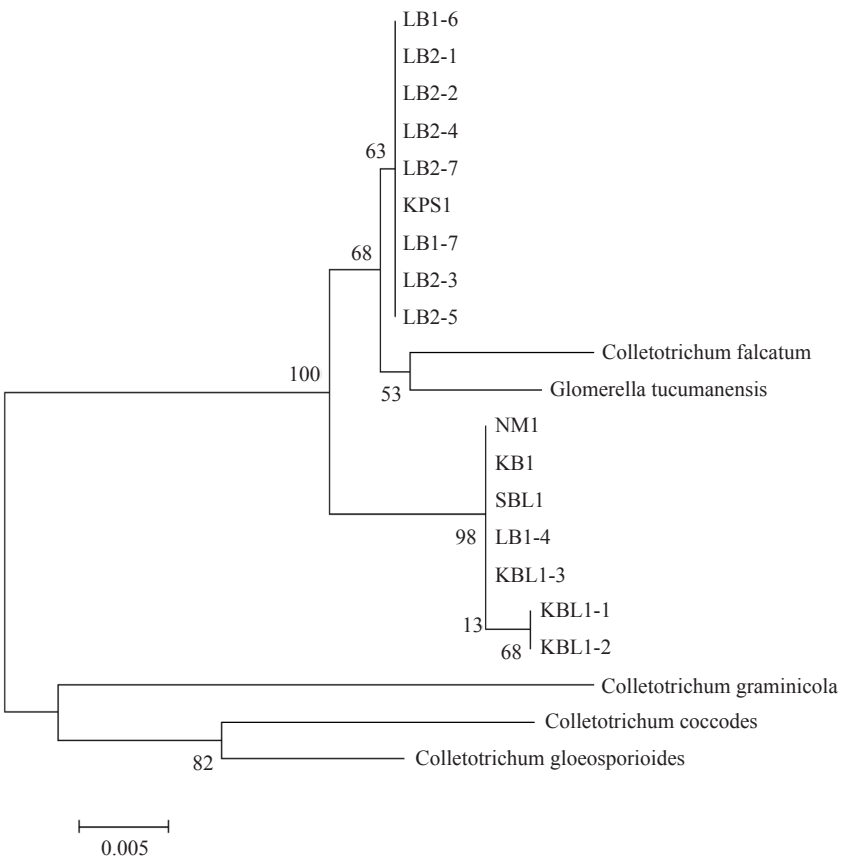

Figure 3 Neighbour-joining tree depicting relationships among *Colletotrichum falcatum* isolates from sugarcane and other hosts, as well as *C. graminicola*, *C. coccodes* and *C. gloeosporioides* based on internal transcribed spacer sequences. The numbers on the nodes are the frequency (in percent) with which a cluster appears in a bootstrap test of 1,000 runs.

Pathological characterization

Analysis of the 15 isolates on the stalks of sugarcane varieties E-Heaw, K84-200, K88-92 and K93-236 by the plug method revealed variable pathogenicity. There were two distinct pathogenic groups based on their pathogenicity to the tested stalks of susceptible varieties E-Heaw and K93-236. Group 1 was pathogenic on E-Heaw and K93-236 and was represented by nine isolates (LB1-4, LB1-6, LB1-7, LB2-1, LB2-2, LB2-3, LB2-4, LB2-5, LB2-7). All isolates in group 1 were highly pathogenic on K93-236. However, there were marked differences among the isolates in group 1 on E-Heaw. Isolates LB1-7, LB2-2, LB2-3, LB2-5 and LB2-7 were highly pathogenic to E-Heaw, whereas isolates LB2-1 and LB2-4 produced moderate disease and isolates LB1-4 and LB1-6 were slightly pathogenic. Group 2 was nonpathogenic on E-Heaw and K93-236 and consisted of another six isolates (KB1, KBL1-1,

Table 2 Cultural characteristics and conidial dimensions of *C. falcatum* isolates.

Isolates	Colony Type	Growth rate ($mm.d^{-1}$)	Conidia			
			Length (μm)		Width(μm)	
			Range	Mean	Range	Mean
KB1	Dark type	11.9	21.42–26.18	24.51[b]	2.38–4.76	3.81
KBL1-1	Dark type	11.7	21.42–28.56	24.51[b]	2.38–4.76	4.05
KBL1-2	Dark type	12.0	23.80–28.56	25.47[ab]	2.38–4.76	4.52
KBL1-3	Dark type	11.6	23.80–28.56	25.70[ab]	2.38–4.76	4.28
LB1-4	Light type	11.8	21.42–26.18	24.04[b]	4.76–4.76	4.76
LB1-6	Light type	12.9	21.42–26.18	24.75[b]	2.38–4.76	4.52
LB1-7	Light type	12.1	21.42–26.18	24.28[b]	4.76–4.76	4.76
LB2-1	Light type	12.3	21.42–28.56	24.99[b]	2.38–4.76	3.81
LB2-2	Light type	11.7	21.42–26.18	23.80[b]	2.38–4.76	4.52
LB2-3	Light type	12.1	23.80–28.56	25.23[ab]	2.38–4.76	4.52
LB2-4	Light type	12.5	21.42–28.56	24.99[b]	2.38–4.76	4.52
LB2-5	Light type	12.5	23.80–26.18	24.28[b]	2.38–4.76	4.52
LB2-7	Light type	12.3	23.80–26.18	25.94[ab]	4.76–4.76	4.76
NM1	Light type	12.1	23.80–28.56	25.23[ab]	4.76–4.76	4.76
SBL1	Dark type	12.0	26.18–28.56	27.37[a]	4.76–4.76	4.76

Mean of 20 spores.

Values in each column followed by the same lower case superscript letters are not significantly different at $P < 0.05$.

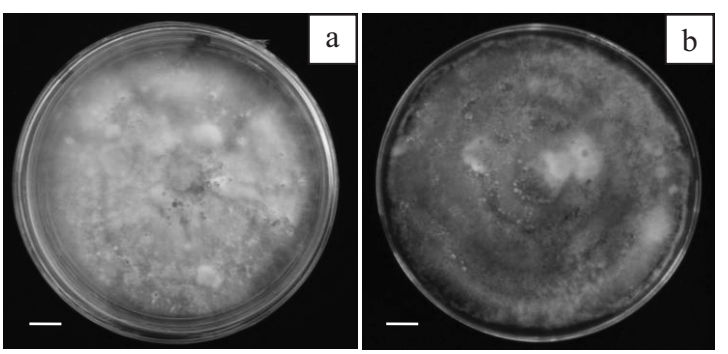

Figure 4 Cultural characters of *C. falcatum* types: (a) Light; (b) Dark. (Scale bar = 10 mm).

KBL1-2, KBL1-3, NM1, SBL1) (Table 4). None of these isolates were pathogenic on the resistant varieties K84-200 and K88-92. The varieties K84-200 and K88-92 showed high resistance to red stalk rot which was similar to observations in the field under conditions of natural infection (data not shown). The response of the varieties E-Heaw and K93-236 to the nine isolates in group 1, (the

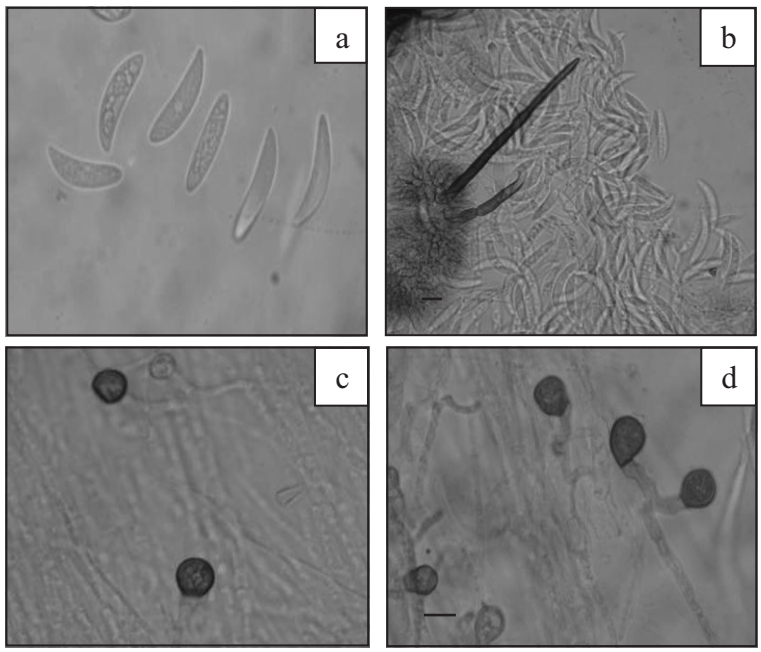

Figure 5 Morphology of *C. falcatum*: (a) Conidia; (b) Setae; (c) Globose appressoria; (d) Clavate appressoria (d). (Scale bar = 10 μm).

Table 3 Appressoria dimensions of *C. falcatum* isolates.

Isolate	Length (μm)		Width (μm)	
	Range	Mean	Range	Mean
KB1	10.68–13.35	11.21^{bc}	8.01–10.68	9.61^{ab}
KBL1-1	13.35–13.35	13.35^{abc}	8.01–8.01	8.01^{c}
KBL1-2	10.68–13.35	12.82^{abc}	8.01–10.68	9.08^{bc}
KBL1-3	13.35–13.35	13.35^{abc}	8.01–10.68	8.54^{c}
LB1-4	10.68–16.02	13.88^{abc}	8.01–10.68	8.54^{c}
LB1-6	13.35–16.02	14.42^{ab}	10.68–13.35	11.21^{ab}
LB1-7	10.68–13.35	12.28^{abc}	8.01–10.68	9.61^{ab}
LB2-1	10.68–16.02	13.35^{abc}	8.01–8.01	8.01^{c}
LB2-2	13.35–16.02	14.95^{a}	8.01–10.68	10.15^{ab}
LB2-3	10.68–16.02	13.88^{abc}	10.68–8.01	11.75^{a}
LB2-4	10.68–13.35	11.75^{bc}	8.01–10.68	10.14^{ab}
LB2-5	13.35–16.02	13.88^{abc}	10.68–13.35	11.28^{ab}
LB2-7	10.68–13.35	11.75^{bc}	8.01–8.01	8.01^{c}
NM1	10.68–13.35	11.75^{bc}	8.01–10.68	9.08^{bc}
SBL1	10.68–13.35	11.21^{c}	8.01–10.68	8.54^{c}

Mean of 20 spores.

Values in each column followed by the same lower case superscript letters are not significantly different at $P < 0.05$.

pathogenic group) indicated that K93-236 was more highly susceptible than E-Heaw.

DISCUSSION

Red rot disease caused by *C. falcatum* is one of the most serious diseases of sugarcane. In Thailand, it attracted special attention during 1991–1992 when it appeared in sugarcane plantations in Sing Buri and Nakhon Sawan provinces. Red rot resulted in significant yield losses in these provinces because the planted E-Haew variety is susceptible to stalk infection and subsequently, E-Haew has been discarded in commercial areas. A severe localized outbreak of red rot was reported again during 2004–2005 in Suphan Buri and Nakhon Pathom provinces and amongst the affected variety was K93-236 (Leksomboon and Jumpee, 2007). In the current study, red rot infected sugarcane was surveyed in central and northeastern Thailand. In the survey period (2011–2012), a severe red rot epidemic appeared only in Lopburi province where variety K93-236 was grown. New outbreaks have also caused concern in commercial fields where susceptible varieties were grown, but the incidence is now low following replacement of those varieties. There are now two sugarcane varieties, LK92-11 and Khon Kaen3 that are popular with planters. These two varieties represent about 80% of total sugarcane planted areas in Thailand (unpublished data) and fortunately, these widely planted commercial varieties are resistant to red rot disease. Thus, outbreaks of the disease have been controlled by replacing susceptible varieties with resistant ones and an effective method for screening genotype reactions to red rot pathogen is an important priority. A better understanding

Table 4 Mean pathogenicity of 15 isolates of *C. falcatum* on stalks of sugarcane varieties E-Heaw and K93-236 after inoculation using the plug method.

Isolate	Pathogenicity rating		Virulence	
	E-Heaw	K93-236	E-Heaw	K93-236
KB1	0	0	N	N
KBL1-1	0	0	N	N
KBL1-2	0	0	N	N
KBL1-3	0	0	N	N
LB1-4	0.75	2.12	L	H
LB1-6	0.88	2.22	L	H
LB1-7	2.75	3.00	H	H
LB2-1	1.50	2.75	M	H
LB2-2	2.50	2.50	H	H
LB2-3	2.88	3.00	H	H
LB2-4	1.38	2.75	M	H
LB2-5	2.75	2.38	H	H
LB2-7	2.75	2.75	H	H
NM1	0	0	N	N
SBL1	0	0	N	N
Control	0	0	N	N

Pathogenicity rating: 0–3; 0 = Red discoloration involving the point of puncture; 1 = Red discoloration at the inoculated internode; 2 = Red discoloration throughout the length of the inoculated internode; 3 = Red discoloration has crossed the inoculated internode.

Virulence: H = High virulence (2.00-3.00); M = moderate virulence (1.00-1.99); L = low virulence (0.1-0.99); N = No virulence (0).

of the pathogen is needed to develop genetically resistant varieties. In the present study, *C. falcatum* was isolated from Lopburi province as a part of the red stalk rot epidemic. In addition, mid-rib lesions and stalks of sugarcane were collected and isolated from other areas. All isolates were identified by PCR and investigated for their cultural, morphological and pathological characteristics.

On the basis of the cultural characters of *C. falcatum*, Abbott (1938) distinguished two races—a light one producing white to light-grey, cottony mycelia, and a dark one with compact, velvety, dark-grey mycelia. Chona and Srivastava (1960) subdivided each of these two races into groups and subgroups based on the texture of the mycelium and the degree of sporulation. They found that isolations made from diseased canes from localities affected with the red rot epidemic invariably yielded light, highly sporulating strains whether isolated from the diseased stalk or midrib lesions and the dark sparsely sporulating isolates were only rarely encountered in epidemic areas. In the present study, two types of colony morphology were classified in which the light type was observed more frequently than the dark type. The light type was isolated from the stalks of sugarcane in both localities affected and not affected with the red rot epidemic. However, the dark type could be isolated from the mid-rib lesions and stalks of sugarcane and was found only in localities without the red rot epidemic. All 15 isolates produced spore masses and high levels of sporulation, except for the dark type isolates KBL1-2 and KBL1-3 which exhibited sparse sporulation, while the dark type isolates KBL1-1 and SBL1 showed high levels of sporulation.

In morphological characterization, the 15 isolates reported in the current study had conidial size differences from previous reports. Sutton (1992) reported that the conidial size of *C. falcatum* ranged between 15.5 and 26.5 µm in length and from 4 to 5 µm in width. Kalaimani (1995) examined six isolates of *C. falcatum* and found variation in the length and width between 30.62 to 37.65 µm and 6.69 to 8.46 µm, respectively. Mishra and Behera (2009a) revealed significant variation in the size of conidia of *C. falcatum* from India where the dimensions varied between 23.94 and 30.83 µm in length and from 3.28 to 3.69 µm in width. The current study found the length of the conidia of the 15 isolates varied between 21.42 and 28.56 µm which was less than in the earlier findings of Kalaimani (1995) and Mishra and Behera (2009a). In addition, the width of the conidia of the 15 isolates varied between 2.38 and 4.76 µm which was shorter than in the previous reports by Sutton (1992), Kalaimani (1995) and Mishra and Behera (2009a). All 15 isolates were examined for the shape and size of appressoria. Sutton (1968) reported the appressorial size of an isolate of *C. falcatum* ranged between 6 to 21 µm in length and from 6 to 17 µm in width. Crouch *et al.* (2009) described the appressorial size ranging between 10.25 and 14.0 µm in length and from 7.25 to 10.5 µm in width. Prihastuti *et al.* (2010) reported differences in the size of conidia (16–35 µm long, 4–5 µm wide) and appressoria (7–14.5 µm long, 6.5–11.5 µm wide). The appressorial size of the 15 isolates reported in the current study ranged between 10.68 and 16.02 µm in length and from 8.01 to 13.35 µm in width; these dimensions were within the range of appressorial size recorded by Sutton (1968). Although the 15 isolates had conidial and appressorial sizes that differed from previous reports, the 15 isolates showed only slight differences in the size of conidia and appressoria. Furthermore, the 15 isolates of *C. falcatum* were identified using PCR. The ITS sequence analyses showed a low level of genetic divergence in the 15 isolates. The 15 isolates of *C. falcatum* in Thailand seemed to compose a homogenous population that did not differ in morphological characters and phylogenetic analyses. However, the 15 isolates were clearly separated into two distinct groups by colony color. Chona and Hingorani (1950) studied mutation in *C. falcatum* and found that the dark-colored mutants were less virulent than their light-colored parents. Understanding the virulence and

pathological behavior of *C. falcatum* is essential for the management of red rot in sugarcane. There are two aspects to the disease produced by *C. falcatum* on sugarcane—mid-rib lesions and red rot of the stalk. The results of the pathogenicity tests on leaves revealed that the 15 isolates were pathogenic on the midrib of sugarcane. However, red rot of the stalk is the most important phase of the disease and it was with respect to this phase of the disease that the pathogenicity of the 15 isolates was studied. The differences in pathogenic reactions of the 15 isolates on the stalks of sugarcane were determined, based on 10 isolates of the light type and 5 isolates of the dark type. The results indicated that the 15 isolates were differentiated into two distinct groups (pathogenic and nonpathogenic) based on the pathogenicity test on the stalks of the susceptible varieties. Group 1 (the pathogenic group) was represented by nine isolates (LB1-4, LB1-6, LB1-7, LB2-1, LB2-2, LB2-3, LB2-4, LB2-5, LB2-7) which were isolated from localities affected with the red stalk rot epidemic. Group 2 (the nonpathogenic group) consisted of the other six isolates (NM1, KB1, KBL1-1, KBL1-2, KBL1-3, SBL1) which were isolated from localities without the red stalk rot epidemic. Only isolates from localities affected with the red stalk rot epidemic produced red rot of stalks. The results indicated that all the dark type isolates were nonpathogenic on stalks and all the light type isolates were pathogenic on stalks, except for the light type isolate NM1 which was isolated from localities without the red stalk rot epidemic. The pathogenicity tests on stalks divided the pathogenic potential of the 15 isolates of *C. falcatum* into two groups which did not show complete congruency with the two groups based on the cultural and pathological characters of the tested isolates. Therefore, cultural characters could be used for pathogenic differentiation of the isolates, but not in absolute terms. In the virulence study of *C. falcaum*, the red discoloration in the detached stalks also was used for evaluation. The 15 isolates had variable virulence on the stalks of sugarcane and only the 9 isolates collected from localities affected with the red stalk rot epidemic had pathogenic potential. The results on pathogenic behavior of the nine isolates from the same variety at each site and the same site in each province were also presented as low, moderate and high virulence, indicating the high genetic diversity of *C. falcaum* in Thailand. The pathogen shows a great diversity in virulence as a number of pathotypes are known to occur in nature (Suman *et al.*, 2005). The pathogenic variability of different isolates of *C. falcaum* collected from different varieties and localities in India were differentiated into three (Beniwal *et al.*, 1989) and four distinct pathotypes (Mishra and Behera, 2009b). The high level of pathogenic variability of *C. falcaum* makes it difficult to breed for red rot resistance. It is necessary to take great care in the selection of *C. falcaum* isolates for varietal resistance tests. The present study used only the plug method of inoculation for pathogenic differentiation of the isolates. However, Beniwal *et al.* (1989) revealed that 10 isolates of *C. falcaum* were differentiated by the plug inoculation method and the same differential reactions against different isolates were also confirmed by nodal, nodal injury and whorl methods of inoculation. Based on the available literature, the current data are the first to study the cultural, morphological and pathological characterization of *C. falcatum* affecting sugarcane in Thailand. The use of this information will lead to a better understanding of the variability present in *C. falcatum* and will be used in the breeding program on red rot resistance in Thailand.

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