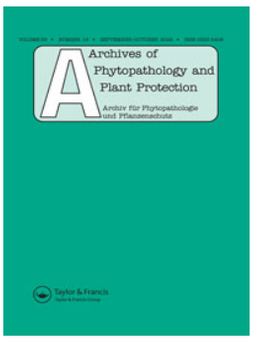

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RESEARCH ARTICLE

Fungi associated with rice sheath rot in Lampung, Indonesia

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ABSTRACT

Rice sheath rot caused by some pathogens. It occurs in the upper leaf sheath that wraps the rice panicle, its major features are rotting, discoloration, sometimes affecting rice grain production. Lampung is an important rice-producing area in Indonesia. Currently, rice sheath rot in the area is reportedly caused by *Fusarium sulawesiense* and *Fusarium hainanense*. This study aimed to identify the rice sheath rot pathogen accurately by sampling locations at varying altitudes, plant ages, and varieties in Lampung Sampling was conducted in Lampung, infected plants were collected and the pathogen isolates were molecularly characterized on the basis of DNA sequence data for the internal transcribed spacer and translation elongation factor 1-α. Pathogenicity test results showed that 16 fungal isolates caused rice sheath rot. These isolates were identified as *Sarocladium oryzae*, *Fusarium bubalinum*, *F. hainanense*, *Setophoma poaceicola*, *Curvularia geniculata*, and *Alternaria padwickii*. This study is the first to report that *S. poaceicola* is a pathogen of rice sheath rot.

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Alternaria padwickii; *Curvularia geniculata*; FIESC; *Sarocladium oryzae*; *Setophoma poaceicola*

Introduction

Rice (*Oryza sativa* L.) is an energy-giving food with high carbohydrate content and thus is a staple food in Indonesia and several other countries. Rice grains contain starch (75–80%), water (12%), and protein (7%) (Verma and Srivastav 2017). In the 2019/2020 harvest year, the total quantity of rice produced internationally reached 497.7 million tons. China produced the highest rice production in the world, which

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was around 146.7 million metric tons. Meanwhile, India was in second place with 118.9 million metric tons and Indonesia was in third place with 34.7 million metric tons (Statista 2021). The increase in human population is also followed by a high demand for rice. Rice sheath rot is a plant disease that can affect rice production, causing up to 85% yield losses (Bigirimana et al. 2015). Therefore, this disease needs serious attention.

Rice sheath rot has spread in various countries worldwide, the first confirmed in Japan in 1976 (Tanii et al. 1976), Burundi (Duveiller et al. 1988), Madagascar (Rott 1989), Latin America (Zeigler 1987), Australia (Cother et al. 2009), South Korea (Kim et al. 2015) and in Indonesia (Pramunadipta et al. 2020, Afifah et al. 2020). The major pathogens of this disease are *Pseudomonas fuscovaginae* (Tanii et al. 1976), *Sarocladium oryzae* (Bills et al. 2004), and *Fusarium fujikuroi* complex (Abbas et al. 1998). The symptoms of this disease are usually observed on leaf sheaths surrounding the panicles of rice plants. Infected leaf sheaths are rotting, red or gray-brown spots appear depending on the rice variety, and sometimes does not produce rice grain occurs in severe cases (Bigirimana et al. 2015; Mvuyekure et al. 2017). In Indonesia, *S. oryzae* is the major fungal pathogen of rice sheath rot (Pramunadipta et al. 2020). *Fusarium proliferatum*, *F. fujikuroi*, *F. sacchari,* and *F. pseudocircinatum* belonging to the *Fusarium fujikuroi* species complex (FFSC); *F. grosmichelii* belonging to the *Fusarium oxysporum* species complex; a species belonging to the *Fusarium solani* species complex; and *F. hainanense*, *F. sulawesiense*, *F. bubalinum*, and *F. tanahbumbuense* belonging to the *Fusarium incarnatum-equiseti* species complex (FIESC) were also confirmed as pathogens of rice sheath rot in Indonesia (Pramunadipta et al. 2022a, Pramunadipta et al. 2022b).

Lampung Province is one of the rice centers in Indonesia. Lampung's area and rice production are in the top seven nationally and the top three in Sumatera Island. In 2018, the rice harvested area and production in Lampung reached 397,435 ha and 1,900,987 tons, respectively, with a productivity of 47.83 quintal per hectare. Pramunadipta et al. (2022a) conducted a survey of this disease in two locations in Lampung Province and found two *Fusarium* species (*F. sulawesiense* and *F. hainanense*) associated with the disease. Geographically, Lampung has lowland areas close to the coast and highlands. Rice plants in this province can grow and thrive under these varied conditions. Surveys and sampling in the varied topographical conditions of the land allowed the discovery of other pathogens that cause sheath rot such as *Cochliobolus lunatus* (Gao et al. 2015), *Sclerotium oryzae* (Hu et al. 2008), *Burkholderia gladioli* (Paganin et al. 2011). In addition, information regarding the status and

incidence of this disease in Lampung is lacking. Therefore, these pathogens in Lampung Province should be identified by sampling locations at varying altitudes, plant ages, varieties, and cultivation systems to provide detailed information about the pathogen and develop basic control measures. Correct pathogen identification is needed to control pathogens effectively and efficiently (Luchi et al. 2020). The present study may serve as a reference to prevent the spread of these pathogens.

Materials and methods

Research site

Sampling and disease observation was carried out in Lampung Province (South Lampung, East Lampung, Central Lampung, West Lampung, Tanggamus, Pesawaran, and Pringsewu) Indonesia with a gradient altitude of 20–900 m. The research was conducted from March to October 2021.

Observation of disease intensity

In each regency, two rice planting locations were selected for survey and sample collection. Five sample points of observation each with a land area of 200 m^2 were systematically determined in the two locations. Twenty rice clumps were observed in each sample point.

Disease incidence and severity were observed at 65–90 days after rice planting and calculated using the formula provided by Vivekananthan et al. (2005) with slight modifications.

Disease incidence (*DI*) was calculated using the formula

$$DI = \frac{n}{N} \times 100,$$

where n is the number of diseased clumps, and N is the number of observed clumps (20 clumps).

Disease severity (*DS*) was calculated on the basis of the disease severity score (Table 1) using the formula

$$\mathrm{DS} = \frac{\sum(n \times v)}{N \times V} \times 100,$$

where n is the number of networks attacked in each category (score), v is the category (score) of attacks, N is the total number of networks observed, and Z is the highest attack category.

Table 1. Scale use for scoring disease severity of rice sheath rot (Vivekananthan et al. 2005).

Score	Description
0	No incidence
1	<1% sheath area affected
3	6–10% sheath area affected
5	11–25% sheath area affected
7	26–50% sheath area affected
9	51–100% sheath area affected

Sample collection and pathogen isolation

The pathogen was isolated from rice plant tissue showing reddish or grayish-brown spots on the sheath under the flag leaf and on the panicles. The isolation was accomplished by cutting the sheath tissue between asymptomatic and symptomatic (±5 mm^2) parts with a sterile scalpel. The surface of these pieces was sterilized with a 0.5% NaOCl (sodium hypochlorite) solution for 1 m, washed with Aqua Dest, and dried on a sterile tissue paper. The sheath pieces were placed on potato dextrose agar (PDA) plates and incubated at 26 °C for 5–7 days (Gnanamanickam and Mew 1991). For future studies, the grown fungi were transferred to a new PDA plate for purification using a single spore (Choi et al. 1999, Noman et al. 2018) and hyphal tip technique (Afanasiev 1937; Jensen et al. 2013).

Pathogenicity test

The fungal isolates tested were colonized in rice grains, sterilization of washed rice grain was performed at 121 °C under *1.5 atm pressure* for 10 minutes. The 5–7 days of the fungal isolate was put into sterilized rice grains and incubated at room temperature for 14 days accompanied by homogenizing after visible fungal growth. A pathogenicity test of the fungal isolates was carried out on 8-week-old rice plants by inoculating the colonized rice grains with fungi on the rice sheath (without injuries). Healthy grains (without fungus) served as the control. Then, the sheath was wrapped with cotton soaked in sterile water, and the cotton was opened after 24 h. The plant clumps were placed in the greenhouse, and then symptoms were observed daily. The diseased plant parts were re-isolated on PDA media to confirm the isolates obtained (Pramunadipta et al. 2020).

Molecular identification

DNA extraction

Molecular identification was performed on fungi isolates aged 1–2 weeks on PDA media. Sterile water (10 mL) was added into the fungal culture

to collect the fungi and then placed in a centrifuge tube. The suspension was centrifuged at 14,000 rpm for 10 m. The pellet was added with 500 µL of 70% alcohol and then centrifuged at 14,000 rpm for 10 m. Subsequently, it was added with 1,000 µL of buffer extraction (0.5 mL Tris HCl, 1 mL sodium dodecyl sulfate 1% + 2.8 mL NaCl, 0.2 mL mercapthoethanol, 2 mL EDTA, and 3.5 mL sterile water), homogenized using a rotamixer, placed into a mortar, and then incubated at −38 °C for 24 h. DNA extraction was pulverized or ground until smooth for 15 min, and then 500 µL of the results were placed in a 1.5 mL tube. Then, 400 µL of 2% CTAB was added and then incubated in a water bath at 65 °C for 1 h (Brookfield TC 550 MX-230, USA). After that, 500 µL of phenol, chloroform, isoamyl alcohol was added and then centrifuged at 14,000 rpm for 10 m. Then, 500 µL of the supernatant was taken, and 500 µL of chloroform, isoamyl alcohol was added and centrifuged at 14,000 rpm for 10 m. A 300–500 µL aliquot of the supernatant added with isopropanol in a ratio of 1:1 and then shaken. The solution was incubated at −38 °C for 20 m and then centrifuged at 14,000 rpm for 10 m. The pellet was added with 500 µL of 70% alcohol and then centrifuged at 14,000 rpm for 5 m. The pellet was air-dried for 24 h and then added with the last 20 µL of TE buffer (Swibawa et al. 2020).

PCR amplification

DNA was PCR amplified. The master mix for PCR amplification was composed of 12.5 µL My Taq™ red mix (Bioline, UK), 1 µL reverse primer, 1 µL forward primer, 1 µL DNA, and 9.5 µL sterile water. The primers were designed using transcription elongation factor (TEF) 1α gene for isolates with *Fusarium* morphology (Prabhukarthikeyan et al. 2020) and internal transcribed spacer (ITS) region rDNA gene for isolates with *Sarocladium* (Giraldo et al. 2015) and other fungi morphology (White et al. 1990).

The ITS region was amplified using fungal rDNA ITS primers ITS1 (5′-CCGTAGGTGAACCTGCGG-3′) and ITS4 (5′-TCCTCCGCTT ATTGATATGC-3′) (White et al. 1990). PCR was performed under the following conditions: initial denaturation at 95 °C for 5 min; 30 cycles of denaturation at 95 °C for 1 min, annealing at 48 °C for 1 min, and extension at 72 °C for 5 min; and a final extension at 72 °C for 5 min. The PCR products were stored at 25 °C. Meanwhile, the TEF 1-α gene region of *Fusarium* spp. was amplified using primer forward (5′-CGACTCTGGCAAGTCGACCA-3′) and reverse (5′-ACGRTGRC CGGGRGCRTYTG-3′). PCR was performed under the following conditions: initial denaturation at 95 °C for 5 min, 30 cycles of denaturation

at 95 °C for 1 min, annealing at 58 °C for 1 min, and extension at 72 °C for 5 min; and a final extension at 72 °C for 5 min. The PCR products were stored at 25 °C. All reactions were performed in the Sens Quest Lab cycler. The electrophoresis of PCR results was performed using 0.5% agarose gel in 20 mL 1x buffer Tris-Boric Acid-EDTA (TBE) (Invitrogen) and 1 µL ethidium bromide (EtBr 10 mg/mL) (Swibawa et al. 2020). The electrophoresis was approved using 1x TBE buffer at 50 V for 50 m and imaged using a DigiDoc UV transilluminator (UVP, USA).

Sequencing and phylogenetic tree construction

Reactions were performed in the Sens Quest Lab cycler. The PCR product sequences were obtained using the genetic analyzer ABI 3100 (Applied

Biosystems, USA) and sequenced using BioEdit software. Furthermore, the DNA sequences obtained were compared with the GenBank data (https://blast.ncbi.nlm.nih.gov/) to find areas that had similarities between biological sequences (Table 2). A phylogenetic tree was constructed using the Maximum-Likelihood (ML) method and implemented in Mega XI software with 100 bootstrap replications (Tamura et al. 2021).

Results

Distribution of rice sheath rot in Lampung province

The intensity of rice sheath rot in seven districts in Lampung Province was observed, and results showed that this disease had spread throughout the observed rice fields with disease intensity ranging from 10.5% to 36.2% (Figure 1). The intensity of the disease in East Lampung, Central Lampung, Tanggamus, and South Lampung districts reached more than 30%. The lowest disease intensity was obtained in Pringsewu District.

In this study, sheath rot infected rice plants at 70–93 days after planting (DAP) with different varieties (Figure 2). At 90 DAP, the IR 64 variety had the highest disease intensity among the varieties (Figure 3), whereas red rice had the lowest one. These results suggest that disease intensity is not only influenced by the variety but also by the age of the plant. The higher the plant age, the higher the disease intensity. However, the disease intensity at various altitudes (57.9–896 masl) of rice planting locations showed that altitude did not affect the intensity of rice sheath rot (Figure 4).

Table 2. Isolates used in this study and their GenBank accession numbers.

Species	Strain (original identification)	Host	Origin	GenBank acc. no.		Reference
				ITS	TEF 1 α	
Fusarium bubalinum	CBS 161.25	Unknown	Australia		MN170448	Xia et al. 2019
	SMB 1	*Oryza sativa*	Indonesia		MT138461	Pramunadipta et al. 2022a
	LSE6*	*Oryza sativa*	Indonesia		OL552355	This study
F. equiseti	CBS 185.34	Soil	Netherlands		MN170466	Xia et al. 2019
	CBS 414.86	Potato peel	Denmark		MN170467	Xia et al. 2019
	CBS 35123	Maize husk	Switzerland		MN170468	Xia et al. 2019
F. guilinense	NRRL 13335	Alfalfa	Australia		GQ505590	O'Donnell et al. 2009
	NRRL 32865	*Human endocarditis*	Brazil		GQ505614	O'Donnell et al. 2009
F. hainanense	CBS 131386	*Oryza australiensis*	Australia		MN170510	Xia et al. 2019
	NRRL 28714	*Acacia* sp.	Costa Rica		GQ505604	O'Donnell et al. 2009
	CBS 544.96	Leaf litter	Cuba		GQ505598	O'Donnell et al. 2009
	LTM 8*	*Oryza sativa*	Indonesia		OL552356	This study
	LTE O 2*	*Oryza sativa*	Indonesia		OL552357	This study
	TGM 1*	*Oryza sativa*	Indonesia		OL552358	This study
F. incarnatum	CBS 132.73	*Trichosanthes dioica*	Malawi		MN170476	Xia et al. 2019
	NRRL 13379	*Triticum* sp.	Iran		MN170477	O'Donnell et al. 2009
	NRRL 32866	*Oryza sativa*	India		GQ505591	O'Donnell et al. 2009
F. tanahbumbuense	CBS 145.44	Unknown	Unknown		MN170505	Xia et al. 2019
	CBS 131009	*Triticum* sp.	Iran		MN170506	Xia et al. 2019
	InaCC F965 ^{T}	*Musa* sp.	Indonesia		LS479448	Maryani et al. 2019
F. sulawesiense	CBS 131.73	*Musa sampientum* var. *robusta*	Bahamas		MN170500	Xia et al. 2019
	CBS 163.57	*Sorghum vulgare*	Trinidad and Tobago		MN170501	Xia et al. 2019
	InaCC F940^{T}	*Musa acuminata*	Indonesia		LS479443	Maryani et al. 2019
Sarocladium bacillisporum	CBS 425.67 ^{T}	Soil	Ontario, Canada	HE608639		Giraldo et al. 2015
	CBS 212.79	Insect	Romania	HG965002		Giraldo et al. 2015
S. bifurcatum	UTHSC 05-3311^{T}	Bronchoalveolar lavage fluid	USA	HG965009		Giraldo et al. 2015
	CBS 383.73	Dead stem of bamboo	India	HG965008		Giraldo et al. 2015
S. bactrocephalum	CBS 749.69^{T}	*Ustilago* sp.	Canada	HG965006		Giraldo et al. 2015
	UTHSC 09-384	Eye	USA	HG965007		Giraldo et al. 2015
S. gamsii	CBS 707.73 ^{T}	Dead stem of *Pandanus lerum*	Sri Langka	HG965015		Giraldo et al. 2015
	CBS 425.73	Dead petiole of *Pandanus lerum*	Sri Langka	HG965014		Giraldo et al. 2015
S. glaucum	CBS 796.69 ^{T}	Woolen overcoat	Solomon Islands	FN691454		Giraldo et al. 2015
	UTHSC 07-1181	Sputum	USA	FN691445		Giraldo et al. 2015
S. hominis	UTHSC04-1034 ^{T}	Right calf tissue	USA	HG965012		Giraldo et al. 2015
	UTHSC 02-2564	Leg	USA	HG965011		Giraldo et al. 2015
S. implicatum	CBS 959.72 ^{NT}	Desert soil	Egypt	HG965023		Giraldo et al. 2015
	CBS 825.73	*Saccharum officinarum*	India	HG965022		Giraldo et al. 2015
S. kiliense	CBS 122.29 ^{T}	Skin	Germany	FN691446		Giraldo et al. 2015

Table 2. Isolates used in this study and their GenBank accession numbers.

Species	Strain (original identification)	Host	Origin	GenBank acc. no.		Reference
				ITS	TEF 1-α	
S. ochraceum	CBS 428.67 [T]	*Zea mays*	Kenya	HG965025		Giraldo et al. 2015
S. oryzae	CBS 180.74 [ET]	*Oryza sativa*	India	HG965026		Giraldo et al. 2015
	PSW 3*	*Oryza sativa*	Indonesia	OL519131		This study
	TGM 3*	*Oryza sativa*	Indonesia	OL519132		This study
	LSE 1*	*Oryza sativa*	Indonesia	OL519133		This study
	LTM 1*	*Oryza sativa*	Indonesia	OL519134		This study
	LTE 1*	*Oryza sativa*	Indonesia	OL519135		This study
S. pseudostrictum	UTHSC 02-1892 [T]	Sputum	USA	HG965029		Giraldo et al. 2015
S. subulatum	MUCL 9939 [T]	Soil	Egypt	HG965031		Giraldo et al. 2015
S. summerbellii	CBS 430.70 [T]	Soil from greenhouse,	The Netherlands	HG965034		Giraldo et al. 2015
S. strictum	CBS 346.70 [T]	*Triticum aestivum*	Germany	FN691453		Giraldo et al. 2015
	CBS 640.75	Decaying wood	The Netherlands	HG965030		Giraldo et al. 2015
S. terricola	CBS 243.59 [T]	Forest soil	USA	FN706553		Giraldo et al. 2015
	MUCL 12011	Decaying leaf of *Milleta laurentii*	Democratic Republic of Congo	HG965039		Giraldo et al. 2015
	UTHSC 03-2933	Bronchial wash fluid	USA	HG965041		Giraldo et al. 2015
	UTHSC 07-110	Bone	USA	HG965032		Giraldo et al. 2015
	CBS 200.84	Water in air moistener	The Netherlands	HG965033		Giraldo et al. 2015
S. zeae	CBS 800.69 [T]	*Zea mays* stalk	USA	FN691451		Giraldo et al. 2015
	CBS 414.81	*Oryza sativa*	Nigeria	HG965028		Giraldo et al. 2015
Acremonium curvulum	CBS 430.66 [T]	Wheatfield soil	Germany	HE608638		Giraldo et al. 2015
Setophoma chromolaenae	CBS 135105 [T]	*Chromolaena odorata*	Brazil	KF251244		Quaedvlieg et al. 2013
S. cyperi	CBS 141450 [T]	Unknown	Unknown	KX228286		Crous et al. 2014
S. poaceicola	MFLUCC 16-0880 [T]	Unknown	Unknown	KY568988		Thambugala et al. 2017
	TGM *	*Oryza sativa*	Indonesia	OL700034		This study
	LSE 4*	*Oryza sativa*	Indonesia	OL519137		This study
	LTM 7*	*Oryza sativa*	Indonesia	OL519138		This study
S. sacchari	MFLUCC 12-0241	Sugarcane	Thailand	KJ476145		Phookamsak et al. 2014
	CBS 333.39 [T]	*Saccharum officinarum*	Brazil	KF251245		Quaedvlieg et al. 2013
S. terrestris	CBS 335.87 [T]	*Allium cepa*	Sinegal	KF251247		Quaedvlieg et al. 2013
	CBS 335.29	*Allium sativum*	USA	KF251246		Quaedvlieg et al. 2013
	CBS 135470	*Zea Mays*	South Africa	KF251236		Quaedvlieg et al. 2013
S. vernoniae	CBS 137988 [T]	Unknown	Unknown	KJ869141		Crous et al. 2014
Bipolaris maydis	CBS 137271	*Zea mays*	USA	AF071325		Manamgoda et al. 2014
Curvularia australiensis	CBS 172.57	*Oryza sativa*	Vietnam	JN601026		Manamgoda et al. 2015
C. geniculata	CBS 187.50	*Andropogon sorghum*	Indonesia	KJ909781		Manamgoda et al. 2015
	LTM 6*	*Oryza sativa*	Indonesia	OL700035		This study
C. oryzae	CBS 169.53 [T]	*Oryza sativa*	Vietnam	KP400650		Manamgoda et al. 2015
C. lunata	CBS 730.96 [T]	Lung biopsy	USA	JX256429		Manamgoda et al. 2012b
C. alcornii	MFLUCC 10-0703 [T]	Zea	Thailand	JX256420		Manamgoda et al. 2012a
C. borreirae	AR 5175	*Sorghum bicolor*	South Africa	KP400635		Manamgoda et al. 2015

C. carica-papayae	CBS 135941 [T]	*Carica papaya*	India	HG778984	Madrid et al. 2014
C. graminicola	BRIP 23186 [T]	–	Australia	JN192376	Manamgoda et al. 2012b
C. hawaiiensis	BRIP 11987	*Oryza sativa*	USA	KJ415547	Tan et al. 2014
C. aeria	CBS 294.61 [T]	Air	Brazil	HE861850	Da Cunha et al. 2013
C. bannonii	BRIP 16732 [T]	*Jasquemontia tamnifolia*	USA	KJ415542	Tan et al. 2014
C. bothriochloae	BRIP 12522 [T]	*Bothriachloa bladhii*	Australia	KJ415543	Tan et al. 2014
C. dactyloctenii	BRIP 12846 [T]	*Dactyloctenum radulans*	Australla	KJ415545	Tan et al. 2014
C. heteropogonicola	BRIP 14579 [T]	*Heteropogon contortus*	India	KJ415548	Tan et al. 2014
C. miyakei	CBS 19.29 [T]	*Eragrostis pilosa*	Japan	KJ909770	Manamgoda et al. 2014
C. neergardii	BRIP 12919 [T]	*Oryza sativa*	Ghana	KJ415550	Tan et al. 2014
C. nicotiae	CBS 655.74 [T]	Desert soil	Algeria	KJ909772	Manamgoda et al. 2014
C. robusta	CBS 624.68 [T]	*Dichanthium annulatum*	USA	KJ909783	Manamgoda et al. 2014
C. sorghina	BRIP 15900 [T]	*Sorgum bicolor*	Australia	KJ415558	Tan et al. 2014
C. tropicalis	BRIP 14834 [T]	*Coffea arabica*	India	KJ415559	Tan et al. 2014
C. tuberculata	CBS 146.63 [T]	*Zea mays*	India	JX256433	Manamgoda et al. 2012b
C. ucinata	CBS 221.52 [T]	*Oryza sativa*	Vietnam	HG779024	Madrid et al. 2014
Alternaria alstroemeriae	MAFF 1219 [T]	*Alstroemeria* sp.	Australia	KP124297	Woudenberg et al. 2015
A. alternata	CBS 106.24 [T]	*Malus sylvestris*	USA	KP124298	Woudenberg et al. 2015
	CBS 267.77	*Citrus paradisi*	USA	KP124311	Woudenberg et al. 2015
A. burnsii	CBS 107.38 [T]	*Cuminum cyminum*	India	KP124420	Woudenberg et al. 2015
	CBS 110.50	*Gossypium* sp	Mozambique	KP124421	Woudenberg et al. 2015
A. Eichhorniae	IMI 121518 [T]	*Eichhornia crassipes*	India	KC146356	Woudenberg et al. 2015
	CBS 119778	*Eichhornia crassipes*	Indonesia,	KP124426	Woudenberg et al. 2015
A. gaisen	CBS 632.93	*Pyrus pyrifolia*	Japan	KC584197	Woudenberg et al. 2015
	CPC 25268	Unknown	Portugal	KP124428	Woudenberg et al. 2015
A. Gossypina	CBS 104.32 [T]	Zimbabwe	Zimbabwe	KP124430	Woudenberg et al. 2015
	CBS 102597	*Minneola tangelo*	USA	KP124432	Woudenberg et al. 2015
A. iridiaustralis	CBS 118486 [T]	*Iris* sp.	Australia	KP124435	Woudenberg et al. 2015
	CBS 118487	*Iris* sp.	Australia	KP124436	Woudenberg et al. 2015
A. jacinthicola	MUCL 53159 [T]	*Eichhornia crassipes*	Mali	KP124438	Woudenberg et al. 2015
	CBS 878.95	*Arachis hypogaea*	Mauritius	KP124437	Woudenberg et al. 2015
A. longipes	CBS 113.35	*Nicotiana tabacum*	Unknown	KP124440	Woudenberg et al. 2015
	CBS 917.96	*Nicotiana tabacum*	USA	KP124442	Woudenberg et al. 2015
A. padwickii	KUFA2003	Unknown	Unknown	MG914429	Unknown
	LTE O 1*	*Oryza sativa*	Indonesia	OL584342	This study
	LTM 10*	*Oryza sativa*	Indonesia	OL584343	This study
	PRW 1*	*Oryza sativa*	Indonesia	OL584344	This study
A. tomato	CBS 103.30	*Solanum lycopersicum*	Unknown	KP124445	Woudenberg et al. 2015
	CBS 114.35	*Solanum lycopersicum*	Unknown	KP124446	Woudenberg et al. 2015
A. tenuissima	CBS 918.96	*Dianthus* sp.	UK	AF347032	Pryor and Michailides 2002

CBS: CBS-KNAW Fungal Biodiversity Center, Utrecht, The Netherlands; MUCL: Mycothèque de l'Université Catholique de Louvain, Louvain-la-Neuve, Belgium; UTHSC: Fungus Testing Laboratory, University of Texas Health Science Center, San Antonio TX; IMI: International Mycological Institute, CABI-Bioscience, Egham, Bakeham Lane, UK; InaCC: Indonesian Culture Collection, Research Center for Biology, Indonesian Institute of Science (LIPI), Cibinong, Indonesia; NRRL: Agricultural Research Service Culture Collection, USA; MFLUCC: Mae Fah Luang University Culture Collection, Center of Excellence in Fungal Research, Chiang Rai, Thailand; BRIP: The Queensland Plant Pathology Herbarium, Queensland Government, Australia; MAFF: Ministry of Agriculture, Forestry and Fisheries, Tsukuba, Ibaraki, Japan.

ET: Epitype strain; NT: Neotype strain; T: Type strain; *: Isolate found in this study.

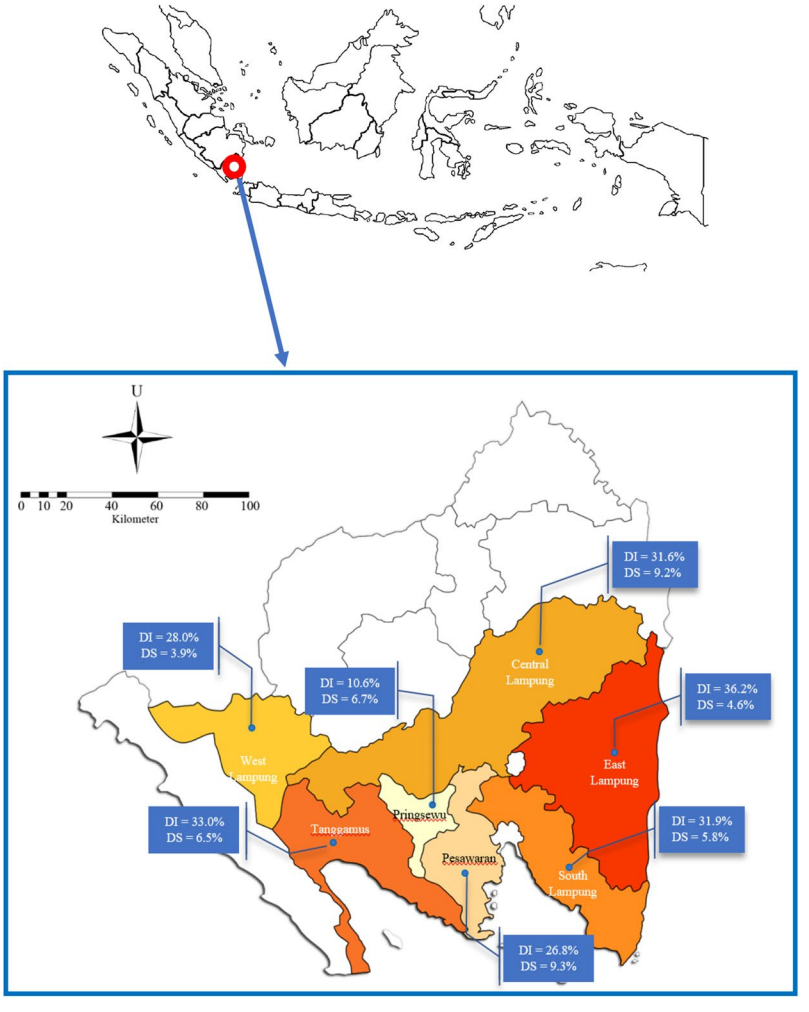

Figure 1. Distribution of rice sheath rot in Lampung Province. DI = Disease incidence, DS = Disease severity.

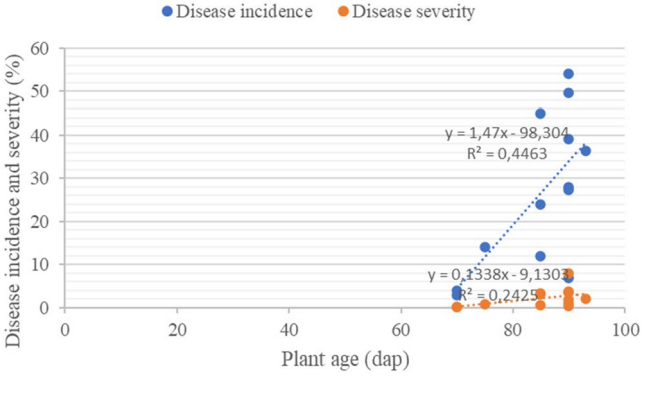

Figure 2. Disease incidence and severity of rice sheath rot on various plant ages.

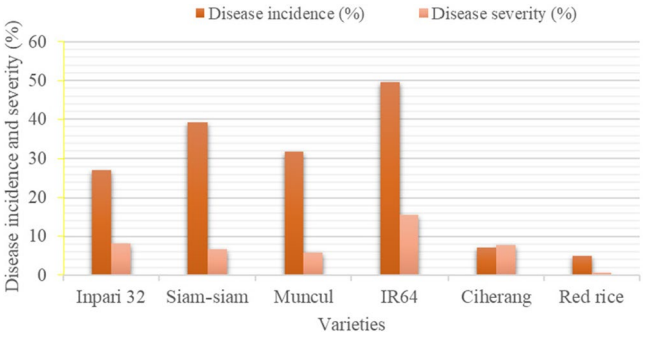

Figure 3. Disease incidence and severity of rice sheath rot in various varieties.

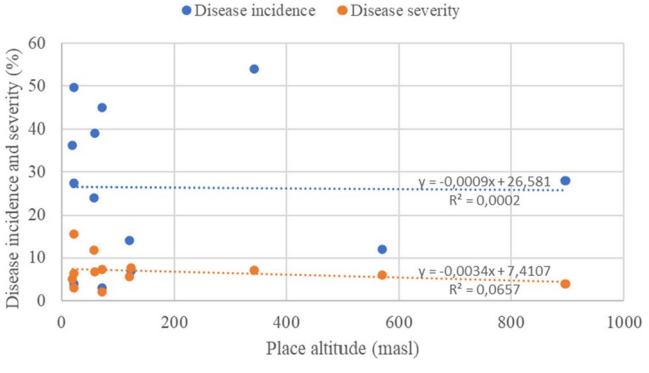

Figure 4. Disease incidence and severity of rice sheath rot in various place altitudes.

Pathogen of rice sheath rot

In the pathogenicity test, rot symptoms began to appear at 2 days after inoculation (DAI) and spread at 14 DAI. On the basis of pathogen isolation using Koch's postulates, molecular identification using BLASTn search, sequence analysis on ITS rDNA gene region, and phylogenetic analysis using ML, five isolates were identified as *Sarocladium oryzae* (Figure 5), one isolate as *Curvularia geniculata* (Figure 6), two isolates as *Alternaria padwickii* (Figure 7), and three isolates as *Setophoma poaceicola* (Figure 8). This study is the first to report that *S. poaceicola* is a pathogen of rice sheath rot. The TEF 1-α gene region indicated that the *Fusarium* isolates belonged to the FIESC, with one isolate *F. bubalinum* and three isolates *F. hainanense* (Figure 9). Information about each isolate is provided in Table 3.

The pathogens induced different sheath rot symptoms (Figure 10). *S. oryzae* caused early lesions in the form of oval or slightly irregular patches with brown edges and a gray or grayish-brown center. Symptoms similar to those caused by *S. oryzae* were also found in the plants

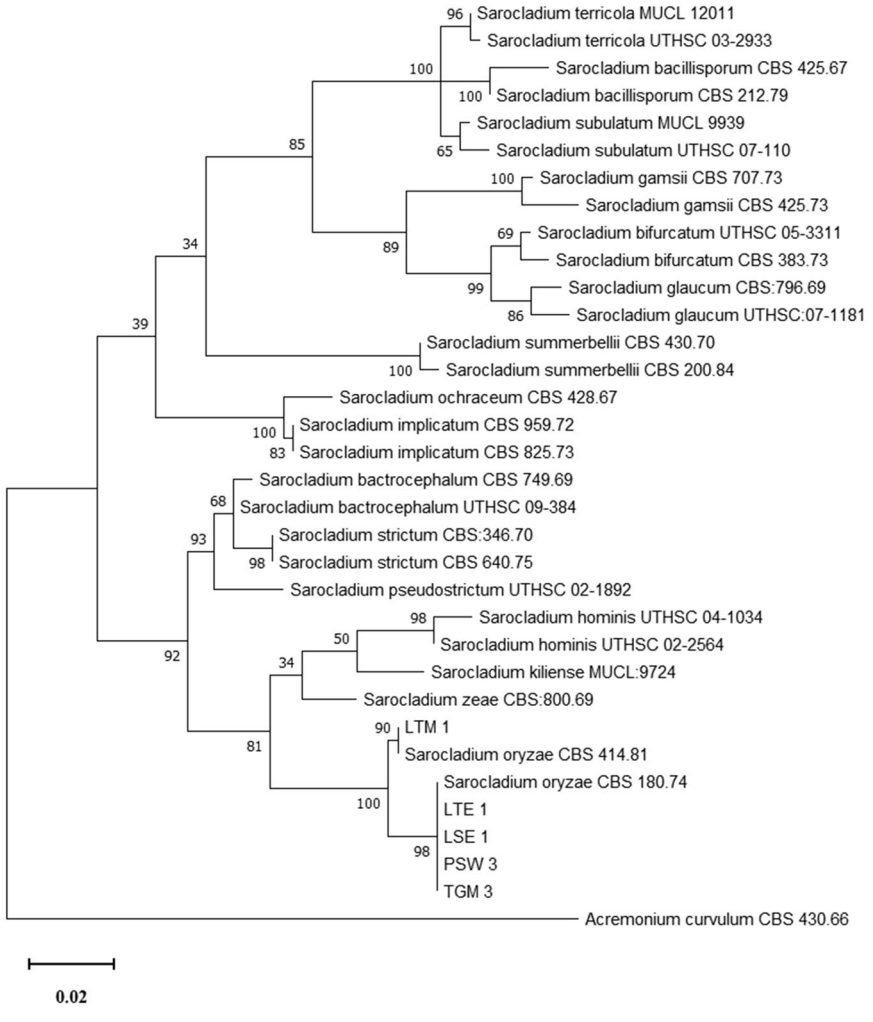

Figure 5. Phylogenetic analysis of *Sarocladium oryzae* based on ITS rDNA gene sequence. The tree was constructed using the Maximum-Likelihood method and implemented in MEGA 11 software with 1000 bootstrap replications. Bootstrap values ≥90%. *Acremonium curvulum* CBS 430.66 was used as an outgroup taxon.

inoculated with *S. poaceicola*, only that the development of lesions was not as fast as that in the plants inoculated with *S. oryzae*. Inoculation of *Fusarium* sp. on the sheath of rice plants caused reddish brown elongated lesions. Meanwhile, *E. padwickii* caused oval or circular spots with a grayish white center and brown dark edges.

Host range of rice sheath rot pathogens

S. oryzae has a wider host range than other pathogens. It was found in the IR 64, Ciherang, Muncul, Inpari 32, and Siam-Siam varieties.

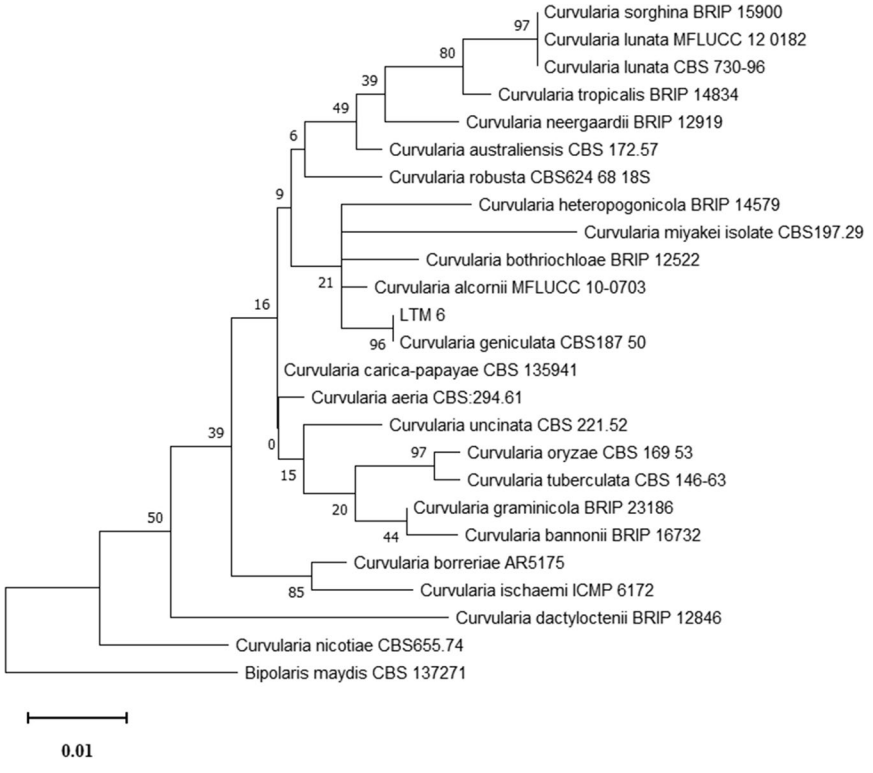

Figure 6. Phylogenetic analysis of *Curvularia geniculata* based on ITS rDNA gene sequence. The tree was constructed using the Maximum-Likelihood method and implemented in MEGA 11 software with 1000 bootstrap replications. Bootstrap values ≥96%. *Bipolaris maydis* CBS 137271 was used as an outgroup taxon.

Meanwhile, *S. poaeceicola* was found in the Ciherang, Inpari 32, and Muncul varieties; *A. padwickii* in the Ciherang, Inpari 62, and red rice varieties; *F. hainanense* in the Inpari 32 and red rice varieties; *F. bubalinum* in the Muncul variety; and *C. geniculata* in the Inpari 32 variety.

Discussion

Plant diseases can occur when virulent pathogen, susceptible host plants, and a conducive environment interact (Agrios 2005). In the concept of epidemiology proposed by Fones et al. (2020), the parameters that affect plant susceptibility, pathogen virulence, and the enabling environment may change from time to time. Important factors that influence changes in pathogen virulence over time are virulence genes, speed of the life cycle, number of spores, and population size. In terms of host plants, factors that affect plant susceptibility are host availability, stages of plant

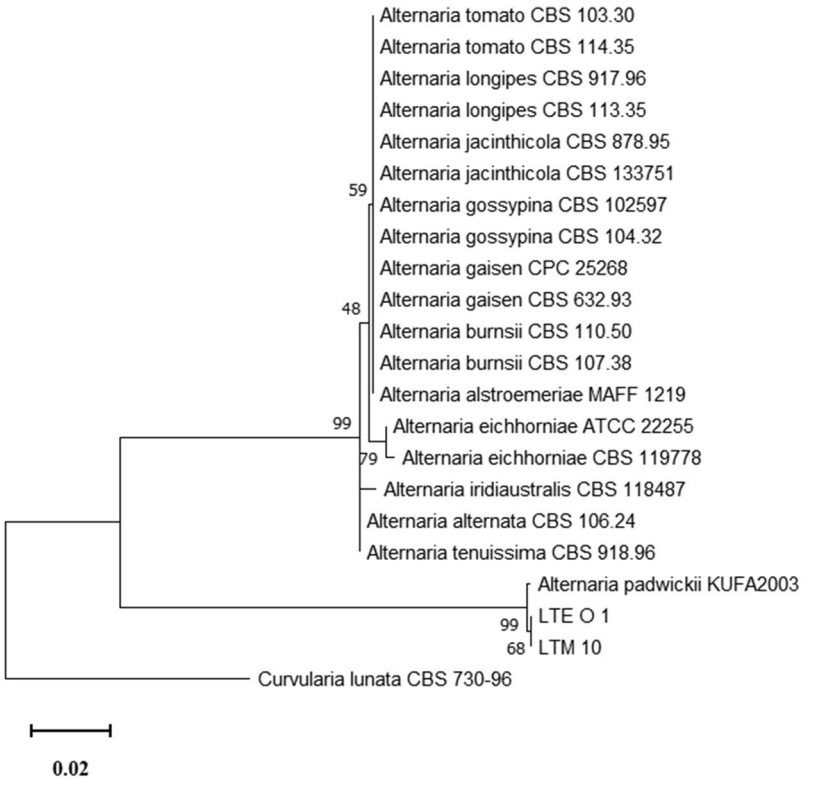

Figure 7. Phylogenetic analysis of *Alternaria padwickii* based on ITS rDNA gene sequence. The tree was constructed using the Maximum-Likelihood method and implemented in MEGA 11 software with 1000 bootstrap replications. Bootstrap values ≥99%. *Curvularia lunata* CBS 730.96 was used as an outgroup taxon.

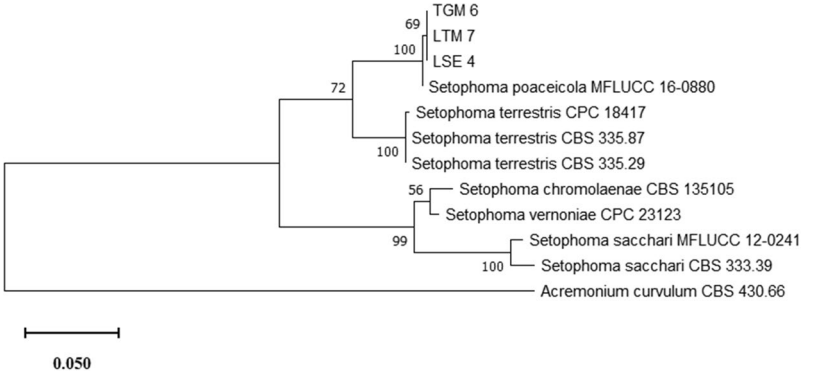

Figure 8. Phylogenetic analysis of *Setophoma poaceicola* based on ITS rDNA gene sequence. The tree was constructed using the Maximum-Likelihood (ML) method and implemented in MEGA 11 software with 1000 bootstrap replications. Bootstrap values 100%. *Acremonium curvulum* CBS 430.66 was used as an outgroup taxon.

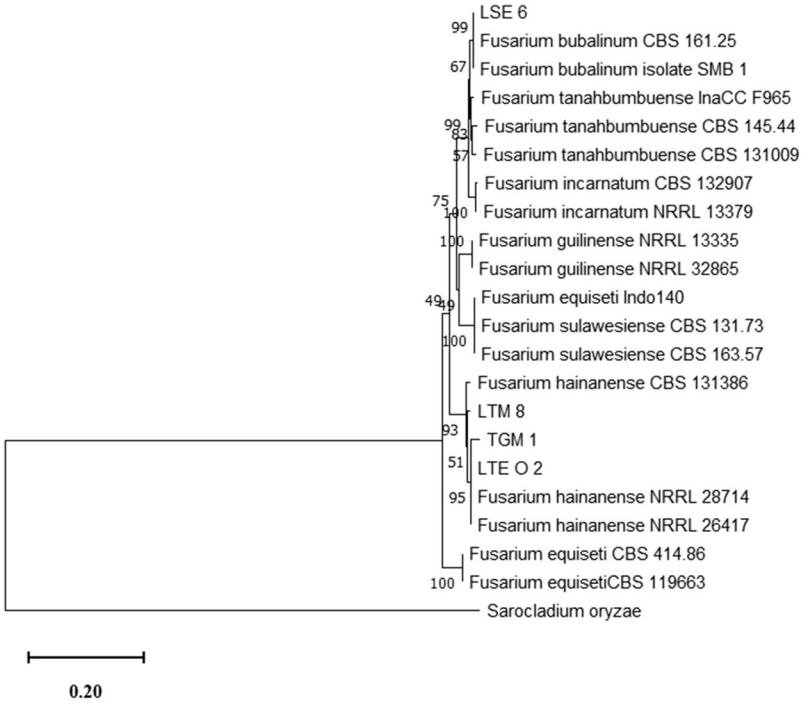

Figure 9. Phylogenetic analysis of *Fusarium hainanense* and *F. bubalinum* within the *Fusarium incarnatum-equiseti* species complex based on TEF 1-α gene sequence. The tree was constructed using the Maximum-Likelihood (ML) method and implemented in MEGA 11 software with 1000 bootstrap replications. Bootstrap values >80%. *Sarocladium oryzae* CBS 430.66 was used as an outgroup taxon.

Table 3. Pathogen isolate of rice sheath rot in Lampung Province, Indonesia.

No	Isolate codes	Varieties origin	Regency	Species
1	LSE 6	Muncul	South Lampung	*Fusarium bubalinum*
2	LTM 8	Inpari 32	East Lampung	*Fusarium hainanense*
3	LTE O 2	Beras merah	Central Lampung	*Fusarium hainanense*
4	TGM 1	Inpari 32	Tanggamus	*Fusarium hainanense*
5	PSW 3	IR 64	Pesawaran	*Sarocladium oryzae*
6	TGM 3	Ciherang	Tanggamus	*Sarocladium oryzae*
7	LSE 1	Muncul	South Lampung	*Sarocladium oryzae*
8	LTM 1	Inpari 32	East Lampung	*Sarocladium oryzae*
9	TGM 6	Ciherang	Tanggamus	*Setophoma poaceicola*
10	LTE 1	Siam-siam	Central Lampung	*Sarocladium oryzae*
11	LTM 7	Inpari 32	Central Lampung	*Setophoma poaceicola*
12	LSE 4	Muncul	South Lampung	*Setophoma poaceicola*
13	LTM 6	Inpari 32	East Lampung	*Curvularia geniculata*
14	PRW 1	Ciherang	Pringsewu	*Alternaria padwickii*
15	LTE O 1	Beras merah	Central Lampung	*Alternaria padwickii*
16	LTM 10	Inpari 32	East Lampung	*Alternaria padwickii*

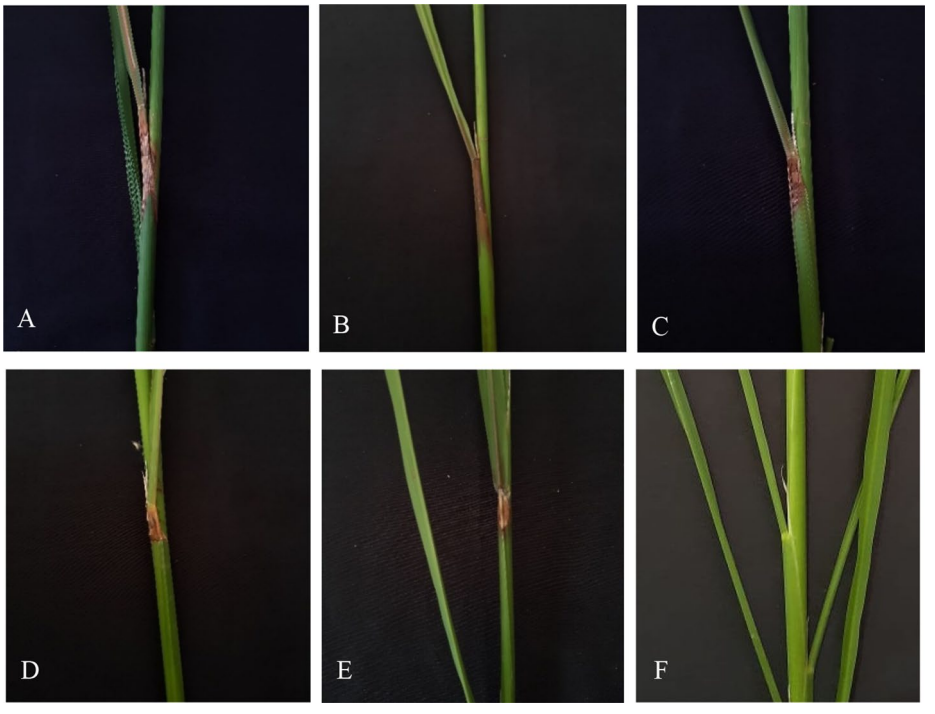

Figure 10. Symptom on pathogenicity test. (A) *Sarocladium oryzae*, (B) *Fusarium* sp., (C) *Setophoma poaceicola*, (D) *Curvularia geniculata*, (E) *Alternaria padwicki,* (F) Control.

development, plant health and nutrition, plant resistance genes, and abiotic stresses. Meanwhile, environmental factors that influence disease occurrence include air and soil quality, weather, temperature, light intensity, humidity, soil nutrient content, and water availability.

The description above indicates that the stages of plant development or plant age affect the high and low intensity of plant diseases. Observations of rice sheath rot in the field revealed that the critical phase of the plant is in the generative phase; in addition, the older the plant, the higher the intensity of the disease. Pathogen attacks at the panicle filling phase can cause rotting of the rice panicle wrapping leaves, preventing the panicle leaves from opening and causing the rice grains to become empty. Meanwhile, an attack that occurs during panicle formation causes the rice grains to change color and influences the nutritional and viability of the seeds (Gopalakrishnan et al. 2010).

Sheath rot pathogens can be transmitted through seeds and infect seedlings. Infected seedlings may die and become remains of infected plants or survive on these plants. Plants at the panicle filling stage are very susceptible to infection. Symptoms include decay in the sheath that covers the panicle and color change of the rice grains that are formed. In addition, the grains cannot be used as seeds. Furthermore, secondary infection occurs through conidia spread by wind or rain from infected

plants and infect healthy plants. After harvest, the infected plants are consistently left in the field and used as the next inoculum (Bigirimana et al. 2015).

Observations of disease intensity at various altitudes (57.9–896 m) of rice planting locations showed that altitude exerted no effect on the intensity of rice sheath rot. The insignificant difference in disease intensity at various altitudes may be attributed to moderate range of altitude variations. At this altitude variation, the temperature and humidity did not vary significantly. The temperature at 20–120 masl ranged from 23 to 30 °C with a relative humidity of 75–100% that at 300–600 masl ranged from 20 to 30 °C with a relative humidity of 85–100%, and that at 800–900 masl ranged from 15–28 °C with a relative humidity of 80–100%. This condition is still within the range supporting rice sheath rot, namely, 20–30 °C temperature and 65–85% relative humidity (Sakthivel 2001). High humidity and temperature support plant disease progression (Mew et al. 2004, Velásquez et al. 2018). Musonerimana et al. (2020) observed the microbiome composition of rice plants with symptoms of rice sheath rot at altitudes of 1534.15 and 849 m. Results showed that sheath rot pathogens *S. oryzae, Alternaria* sp., and *Fusarium* sp. were more abundant at an altitude of 849 m than at 1534.15 m.

Sarocladium oryzae is the main pathogen of rice sheath rot worldwide (Bigirimana et al. 2015). In this study, *S. oryzae* had a wider host range than other pathogens because these pathogens could be found associated with almost all varieties of rice plants observed, including IR64, Inpari 32, Ciherang, Muncul, and Siam-Siam. Aside from having a wider host range, results of the pathogenicity test in the greenhouse showed that the symptoms caused by *S. oryzae* were also more severe than those caused by other pathogens. *S. oryzae* has also been found in some common weeds of rice (Deka and Phookan 1992) and bamboo in Bangladesh and India (Pearce et al. 2001).

The molecular identification of the TEF 1α gene sequence showed that *F. bubalinum* and *F. hainanense* belonged to the FIESC. In this study, no *Fusarium* belonging to the FFSC was associated with rice sheath rot. *Fusarium* was identified as having a narrower host range than *S. oryzae. Fusarium* sp. was only associated with Inpari 32 and Muncul rice varieties. However, the symptoms caused by the pathogenicity test showed that the symptoms were as severe as those caused by *S. oryzae. Fusarium* sp. transmit through seeds, and infected seeds contain mycotoxins (Wulff et al. 2010; Sunani et al. 2020). In infected seeds under stressful conditions, *F. proliferatum* can survive and reisolate from seeds treated at 4 °C–5 °C for 6 months (Kushiro et al. 2012).

Alternaria padwickii is a common rice seed pathogen. It can cause seed rot, seed discoloration, and seedling blight; however, it has also

been detected as a sheath rot pathogen (Naeimi et al. 2003). In this study, *A. padwickii* has associated with rice sheath rot in Ciherang, Inpari, and red rice varieties. Symptoms caused by this pathogen are milder than those caused by *S. oryzae* and *Fusarium* sp. Use of healthy, clean, and testy seeds can prevent the transport and transmission of the pathogen in new areas. Fungi can survive as sclerotia in soil and plant debris. Application of seed treatment can reduce DIs when the pathogen is already present.

Curvularia sp. is a seedborne pathogen causing leaf spot on rice, but the present study found that *Curvularia geniculata* can also cause rice sheath rot. Species of *Curvularia* Boedijn (1933) and its anamorphs *Cochliobolus* Drechsler (1934) and *Bipolaris* Shoemaker (1959) are worldwide pathogens in grasses (Poaceae) (Manamgoda et al. 2011). A previous study reported that *C. geniculata* is also associated with leaf spot in maize in China (Zhang et al. 2019). *C. geniculata* (Tracy & Earle) Boedijn is the same biological species as *Cochliobolus geniculatus*. The main propagules of *Curvularia* for dispersing and surviving are the conidia Primary infection of this pathogen can occur through infected plant debris, infected seeds, alternative hosts, and dormant conidia in the soil (Reis and Wunschr 1984; Zenghai et al. 2002).

Setophoma poaceicola has not been reported as a pathogen in rice plants, but it has been reported to cause leaf spot in grass (Thambugala et al. 2017). However, in the present study, this pathogen was associated with rice sheath rot. *Setophoma* species are characterized by the presence of setose pycnidia, aseptate conidia, phialidic conidiogenous cells, and hyaline, ellipsoidal to subcylindrical, (de Gruyter et al. 2010, Quaedvlieg et al. 2013). Recognized *Setophoma* species are S. poaceicola in grass (Thambugala et al. 2017), S. chromolaenae in Chromolaena odorata (Quaedvlieg et al. (2013), S. cyperi in Cyperus sphaerocephalus (Crous et al. 2016), S. sacchari in Saccharum officinarum, S. terrestris in Allium cepa (de Gruyter et al. 2010), S. vernoniae in Vernonia polyanthes (Crous et al. 2014), and S. antiqua, S. endophytica, S. longinqua in Camellia sinensis (Liu et al. 2019).

Rice sheath rot is a disease with more than one type of pathogen. In the present study, pathogenicity test showed that various pathogens cause similar disease symptoms, such as necrosis. This similarity may be attributed to the fact that rice sheath rot pathogen produces phytotoxins that cause necrosis (Bigirimana et al. 2015). However, there are some different characters of necrosis. Phytotoxins play an important role in the progression of plant disease symptoms, including necrosis, chlorosis, leaf spots, wilting, and growth inhibition (Pontes et al. 2020; Chen et al. 2020). Phytotoxins produced by sheath rot are shown in Table 4.

Table 4. Pathogen phytotoxin of rice sheath rot.

Pathogen	Phytotoxin	Reference
Sarocladium oryzae	Cerulenin, helvolic acid	Bridge et al. 1989; Tschen et al. 1997; Ghosh et al. 2002, Hittalmani et al. 2016
Fusarium sp.	Fumonisin, moniliformin, and fusaric acid	Fotso et al. 2002; Wulff et al. 2010
Alternaria sp.	Host-specific toxins (HSTs): AAL-, ACR-, ACT-, AF-, AK-, AM-, AT-toxins	Otani et al. 1995; Yamagishi et al. 2006; Meena et al. 2017
Curcularia sp.	Dehydrocurvularin, curvularin	Jiang et al. 2008; Meepagala et al. 2016

Rice sheath rot is a very damaging disease, with yield loss of 20–85%. This disease is caused by various pathogens depending on the plant variety, environmental condition, farming system, other pests, region, and so on. Thus, the disease etiology is hard to specify and its spread difficult to control. The control strategies of this complex disease should follow the integrated pest management (IPM) approach, which apply various control techniques that are combined in one unit to prevent damage to plants and the emergence of economic losses and prevent environmental and ecosystem damage. Monitoring is the key to IPM. Thus, losses caused by plant diseases can be minimized by observing early symptoms.

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