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Short Communication: *Sarocladium oryzae* associated with sheath rot disease of rice in Indonesia

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Abstract. *Pramunadipta S, Widiastuti A, Wibowo A, Suga H, Priyatmojo A. 2020. Short Communication:* Sarocladium oryzae *associated with sheath rot disease of rice in Indonesia. Biodiversitas 21: 1243-1249.* One of the obstacles in increasing rice production is the presence of sheath rot pathogen infection, which causes changes in color on the rice sheath to brown or reddish-brown, sometimes does not produce rice grain. The major fungal pathogens that cause sheath rot disease are *Sarocladium oryzae* and *Fusarium* spp. The loss of rice yields reaches 85%. The disease found in six provinces, some of which are the largest rice-producing centers in Indonesia. A total of twenty-four *Sarocladium* sp. were isolated from leaf sheath symptom on potato dextrose agar and water agar medium. Sheath rot pathogen identification based on molecular method was performed using internal transcribed spacer (ITS) rDNA gene sequencing. Necrosis occurs after artificial inoculation in Ciherang rice variety was observed and showed that all isolates were pathogenic. Morphological characterization of the isolates identified them as *Sarocladium* sp. Molecular identification showed that six representatives isolates belonging to *S. oryzae*. These findings are important information about the fungal pathogen that causes sheath rot disease in Indonesia, and in studies for formulating control measures of the pathogen in the future to prevent the disease epidemic on rice. This is the first report about the existence of sheath rot disease, morphological characterization and molecular identification of *S. oryzae* in various rice fields in Indonesia.

Keywords: Gene sequencing, ITS rDNA, rice, *Sarocladium*, sheath rot disease

INTRODUCTION

Rice (*Oryza sativa* L.) is the most important product of agriculture in Indonesia (Mau et al. 2017). In 2019, provinces in Indonesia, which have the largest rice production were Central Java, East Java, West Java and South Sulawesi (BPS 2020). The human need for rice always increases from year to year in line with the increase in population. Sheath rot disease possible to become an obstacle in efforts to increase rice productivity (Garcia et al. 2003; Shamsi and Chowdhury 2016). The main pathogens associated with this disease in some countries are fungal pathogens such as *Sarocladium oryzae* and *Fusarium* spp. that can spread by wind and seed and the bacterial pathogen such as *Pseudomonas fuscovaginae* (Bigirimana et al. 2015). These pathogens produce very similar symptoms.

Sarocladium oryzae was known to be the first major important pathogen of fungi that caused sheath rot disease of rice after been first isolated in 1922 in Taiwan (Mathur 1981; Mew and Gonzales 2002; Ayyadurai et al. 2005; Bigirimana et al. 2015). *S. oryzae* also is known to produce antimicrobial secondary metabolites such as helvolic acid and cerulenin (Bridge et al. 1989; Tschen et al. 1997; Ghosh et al. 2002; Hittalmani et al. 2016). *S. oryzae* develops well in rain-fed rice fields, and found in lowland and medium land environments (Pearce et al. 2001; Sarangi et al. 2019). Sheath rot disease symptom usually occurs on the leaf sheath which encloses panicles on rice plants. The infected leaf sheath will rot, turn grayish-brown or reddish-brown spot depending on rice cultivars and sometimes produce no grain of rice (Nair 1976; Ou 1985; Mvuyekure et al. 2017). The brown spot has a length of 0.5-1 cm and width of 0.2 to 0.5 cm, while the healthy sheath remains green (Amin et al. 1974). The disease spots are linear, have irregular margins and at the next stage, the disease spot will unite and cover the entire sheath (Srinivasachary et al. 2002). Pathogens that infect leaf sheath make the young panicles cannot get out of the leaf sheath and solidify or partly appear but produce empty, partly filled and turn into brown (Mvuyekure et al. 2017). The losses incurred were in the form of quantitative losses (loss of yields including discoloration of grain becomes unsuitable for sale) and qualitative losses (Gopalakrishnan et al. 2010; Zhang et al. 2019). Rice sheath rot causes yield losses that vary from 20% to 85% (Desjardins et al. 2000; Sakthivel 2001; Park et al. 2005; Balgude et al. 2019).

Moreover, it is important to understand the impact of the yield loss because of the infection of sheath rot disease, to formulating a method on how to control the disease on

future research. The pathogens that associated with sheath rot disease have not yet reported in Indonesia. Hence, this study will be conducted to generate information about the existence of *S. oryzae* in several rice fields in Indonesia, the pathogenicity and cultural characters of the pathogens through field survey, pathogenicity test, morphological characterization and ITS rDNA gene sequencing.

MATERIALS AND METHODS

Study area

Sheath rot disease samples were collected by purposive sampling in various varieties from several rice fields in six provinces in Indonesia including Banten, West Java, Central Java, East Java, Bali and South Sulawesi (Figure 1). Banten and Bali provinces were chosen as comparison regions from other provinces that had the largest rice production. The symptoms in the leaf sheath were cut from each rice tiller, then put in a paper bag, and stored in a cooler box before isolation process.

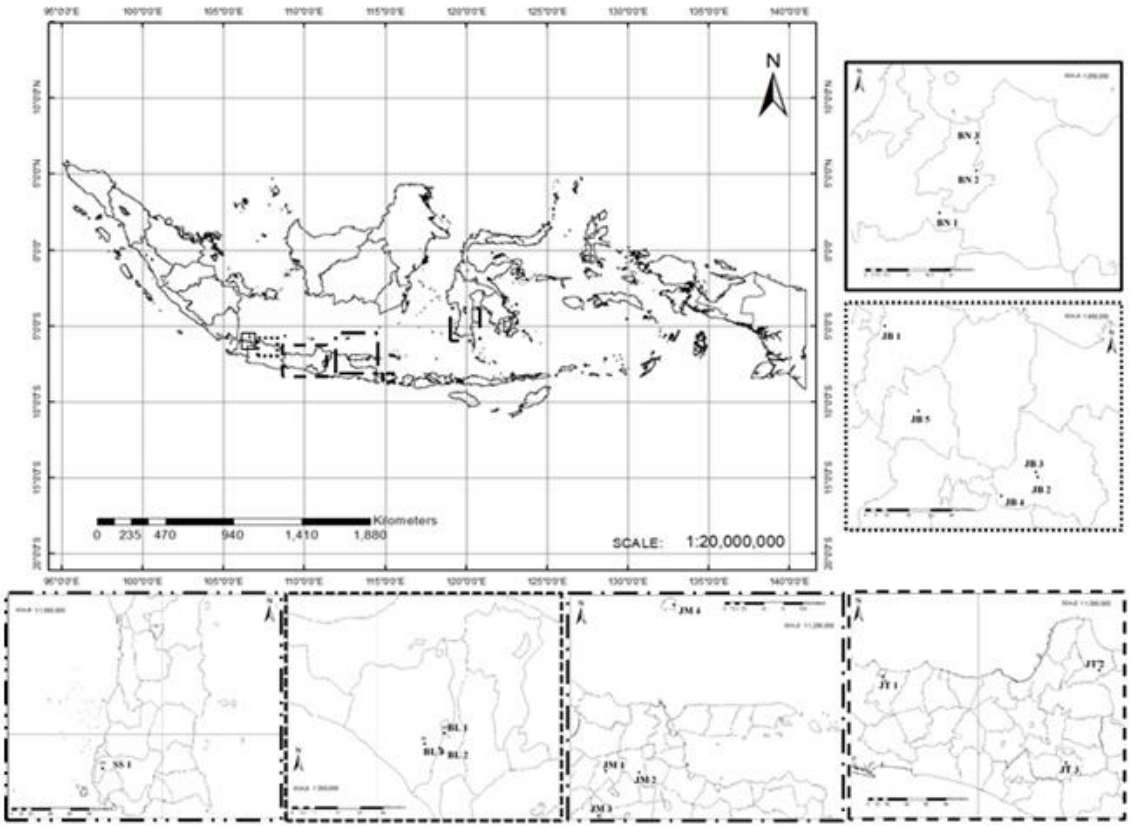
Procedures

Isolation of Sarocladium sp.

Isolation process was carried out by Chowdhury et al. (2015) with modification, the area between infected and healthy tissue was cut into small pieces (approx. 5mm^2), then sterilized with 1% sodium hypochlorite for 2 min, rinsed once with sterile water for 2 min, and dried up on sterile filter paper. The leaf sections were placed on PDA (Potato Dextrose Agar for microbiology, Millipore Sigma 110130) plates and incubated at 25^{o}C for 5-7d to observed fungal growth (Gnanamanickam and Mew 1991). Single spore of *Sarocladium* sp. were maintained on water agar (WA) (WA; agar, 20g; water to final volume of 1.000 ml) plates, then incubated in the dark at 25^{o}C for 12h to permit conidial germination. The germinate fungal spore then identified by examination through a light microscope and transferred into new PDA plates.

Pathogenicity test

Ciherang rice variety aged eight weeks after transplanting was used for artificial inoculation with single rice grain that colonized by *Sarocladium* sp. in 7d. The colonized of single rice grain, then inoculated on the leaf sheath of three rice tillers in each three clumps without wounding. The control of plant inoculated by single rice grain without colonizing by fungi. Then, rice sheath covered with cotton, soaked in sterile water and left overnight. The next day, the cotton was removed. The inoculated rice was placed under controlled greenhouse conditions. The symptoms were observed every day to note the time initial symptoms appear, and disease severity index (DSI; Narayanasamy and Viswanathan 1990) until the harvest period. *Sarocladium* sp. was re-isolated using PDA plates, and confirmed with inoculated isolates.



Map Resource: RBI Map 2010 **Legend**: •: sampling site

Figure 1. Sampling sites for the location of sheath rot disease from several rice fields

Culture morphology characteristics

The morphological characteristics of the isolates were studied based on culture growth on PDA were incubated at 25ºC in the dark and examined each 7d up to 4 weeks (Giraldo et al. 2015; Liu et al. 2017). Fungal colony colors determined using the color charts by Kornerup and Wanscher (1978). Microscopic features were examined by slide cultures on oatmeal agar (OA) (OA; filtered oat flakes after 1h of simmering, 30g; agar, 20g; water to final volume of 1.000 ml) (Giraldo et al. 2015). Fifty conidia were observed for shape and size measures. The examination was done using Olympus CX21 Binocular Microscope, images captured by OptiLab Microscope Camera and Optilab viewer 2.2 software. Conidia measured by Image Raster 3.0 software. *Sarocladium* sp. was identified from cultures grown on PDA plates, according to the descriptions of Giraldo et al. (2015).

DNA extraction, amplification, and sequencing

Representative isolates were selected for molecular identification. Isolates were grown on potato dextrose broth (PDB) (PDB; potato 200g; dextrose 20g; water to final volume of 1.000 ml) for 3-4d at 25ºC by using potassium ethyl xanthogenate solution, as previously described (Suga et al. 2008). The final DNA pellet was dissolved in 400 µL of water. The ITS rDNA region was amplified with the primer pairs ITS1/ITS4 (White et al. 1990). Reactions were performed in BioRad T100™ Thermal Cycler using the following conditions: initial denaturation at 94ºC for 2 min, 30 cycles of denaturation at 94ºC for 1 min, annealing at 55ºC for 1 min, extension at 72°C for 2 min, final extension at 72°C for 7 min, followed by stored at 4°C. PCR products were directly sequenced as previously described (Suga et al. 2008). The sequence was obtained by an ABI 3100 genetic analyzer (Life Technologies).

Alignment and phylogenetic analysis

The results of the ITS rDNA gene sequence were then compared with sequences in the GenBank were performed in Nucleotide Basic Local Alignment Search Tool (BLASTn) (https://blast.ncbi.nlm.nih.gov/Blast.cgi). Sequences were assembled and manually edited in MEGAX. Maximum-likelihood (ML) analysis was implemented in MEGAX software (Kumar et al. 2018) with 1000 bootstrap replications and GTR+G+I model. Reference sequences retrieved from the BLASTn search based on ITS rDNA gene sequences (Bills et al. 2004; Giraldo et al. 2015) were used for phylogenetic analysis (Table 2; Figure 4). The outgroup of phylogenetic tree using *Acremonium curvulum* that not include in the Sarocladium clade but closely related to *Sarocladium* sp. (Giraldo et al. 2012). The phylogenetic tree was visualized using FigTree (http://tree.bio.ed.ac.uk/software/figtree/) software. Sample sequences are deposited in the GenBank database under accession numbers MT012231- MT012236.

Table 1. List of *Sarocladium* sp. isolates

Field code	District	Province	Varieties origin	Sample code	Coordinates
BN1	Serang	Banten	Inpari 22	SO12	6°13'18.1"S 106°07'27.3"E
BN2			Ciherang	SO16	6°07'49.9"S 106°12'32.7"E
BN3			Inpari 22	SO2	6°04'15.0"S 106°12'42.0"E
JB1	Karawang	West Java	Mekongga	SO1; SO4; SO11	6°15'12.5"S 107°17'42.6"E
JB2	Sumedang		Ciherang	SO3; SO10; SO21	6°50'24.1"S 107°55'44.9"E
JB3			IR 64	SO15; SO20	6°49'17.7"S 107°55'16.3"E
JB4			Ciherang	SO14	6°54'51.4"S 107°46'41.9"E
JB5	Purwakarta		Ciherang	SO22	6°35'00.3"S 107°26'07.8"E
JT1	Tegal	Central Java	Ciherang	SO9	6°54'54.6"S 109°07'32.9"E
JT2	Pati		IR 64	SO5	6°51'27.3"S 111°07'04.5"E
JT3	Sukoharjo		Mekongga	SO23	7°39'26.3"S 110°48'39.0"E
JM1	Nganjuk	East Java	Ciherang	SO24	7°36'07.9"S 111°55'21.1"E
JM2	Jombang		IR 64	SO7	7°36'59.8"S 112°18'26.0"E
JM3	Tulungagung		Memberamo	SO13	8°05'09.1"S 111°50'20.5"E
JM4	Gresik		Memberamo	SO17	5°48'19.8"S 112°42'21.3"E
BI1	Badung	Bali	Ciherang	SO18	8°28'45.8"S 115°11'14.5"E
BI2			Ciherang	SO19	8°31'08.0"S 115°10'29.7"E
BI3	Tabanan		Memberamo	SO6	8°30'25.8"S 115°07'58.5"E
SS1	Gowa	South Sulawesi	Ciherang	SO8	5°17'47.4"S 119°27'06.5"E

Table 2. Phylogenetic reference sequences isolates used in this study

Species	Strain (original identification)	Origin	GenBank acc. no.	Reference
Sarocladium bacillisporum	CBS 212.79	Insect, Romania	HG965002	Giraldo et al. 2015
	CBS 388.67	Soil, Netherlands	HG965003	Giraldo et al. 2015
Sarocladium bactrocephalum	CBS 749.69^{T}	Ustilago sp. Canada	HG965006	Giraldo et al. 2015
Sarocladium bifurcatum	CBS 383.73	Dead stem of bamboo, India	HG965008	Giraldo et al. 2015
Sarocladium gamsii	CBS 425.73	Dead petiole of *Pandanus lerum*, Sri Lanka	HG965014	Giraldo et al. 2015
Sarocladium glaucum	CBS 382.73	Dead stem of bamboo, India	HG965018	Giraldo et al. 2015
	CBS 100350	Dead stem of bamboo, Japan	HG965020	Giraldo et al. 2015
Sarocladium hominis	UTHSC 02-2564	Leg, USA	HG965011	Giraldo et al., 2015
Sarocladium implicatum	CBS 397.70A	*Saccharum officinarum*, Jamaica	HG965021	Giraldo et al. 2015
	CBS 959.72^{NT}	Dessert soil, Egypt	HG965023	Giraldo et al. 2015
Sarocladium ochraceum	CBS 428.67^{T}	*Zea mays*, Kenya	HG965025	Giraldo et al. 2015
Sarocladium oryzae	CBS 180.74^{ET}	*Oryza sativa*, India	HG965026	Giraldo et al. 2015
	CBS 399.73	*Oryza sativa*, India	HG965027	Giraldo et al. 2015
	CBS 414.81	*Oryza sativa*, Nigeria	HG965028	Giraldo et al. 2015
	CBS 361.75	*Oryza sativa*, Kenya	AY566993	Bills et al. 2014
Sarocladium pseudostrictum	UTHSC 02-1892^{T}	Sputum, USA	HG965029	Giraldo et al. 2015
Sarocladium strictum	CBS 346.70^{T}	*Triticum aestivum*, Germany	FN691453	Giraldo et al. 2015
Sarocladium subulatum	MUCL 9939^{T}	Soil, Egypt	HG965031	Giraldo et al. 2015
Sarocladium summerbellii	CBS 200.84	Water in air moistener, Netherlands	HG965033	Giraldo et al. 2015
	CBS 797.69	Decaying leaf of *Canna indica*, Netherlands	HG965035	Giraldo et al. 2015
	CBS 951.72	Agricultural soil, Netherlands	HG965037	Giraldo et al. 2015
Sarociadium terricola	MUCL 12011	Decaying leaf of *Milleta launentii*, D.R. Congo	HG965039	Giraldo et al. 2015
Sarocladium zeae	CBS 800.69^{T}	*Zea mays* stalk, USA	FN691451	Giraldo et al. 2015
Sarocladium sp. (= *Sarocladium oryzae*)	SO 2	*Oryza sativa*, Serang, Banten, Indonesia	MT012231	This study
	SO 3	*Oryza sativa*, Sumedang, West Java, Indonesia	MT012232	This study
	SO 5	*Oryza sativa*, Pati, Central Java, Indonesia	MT012234	This study
	SO 8	*Oriza sativa*, Gowa, South Sulawesi, Indonesia	MT012236	This study
	SO 11	*Oryza sativa*, Karawang, West Java, Indonesia	MT012233	This study
	SO 13	*Oryza sativa*, Tulungagung, East Java, Indonesia	MT012235	This study
Acremonium curvulum	CBS 430.66^{T}	Wheatfield soil, Germany	HE608638	Giraldo et al. 2012

Note: ET: Epitype strain; NT: Neotype strain ; T: type strain

Figure 2. Pathogenicity test of *Sarocladium* sp. 7DAI. Symptomatic leaf sheath, isolate SO2 (A); SO3(B); SO8 (C); control (D)

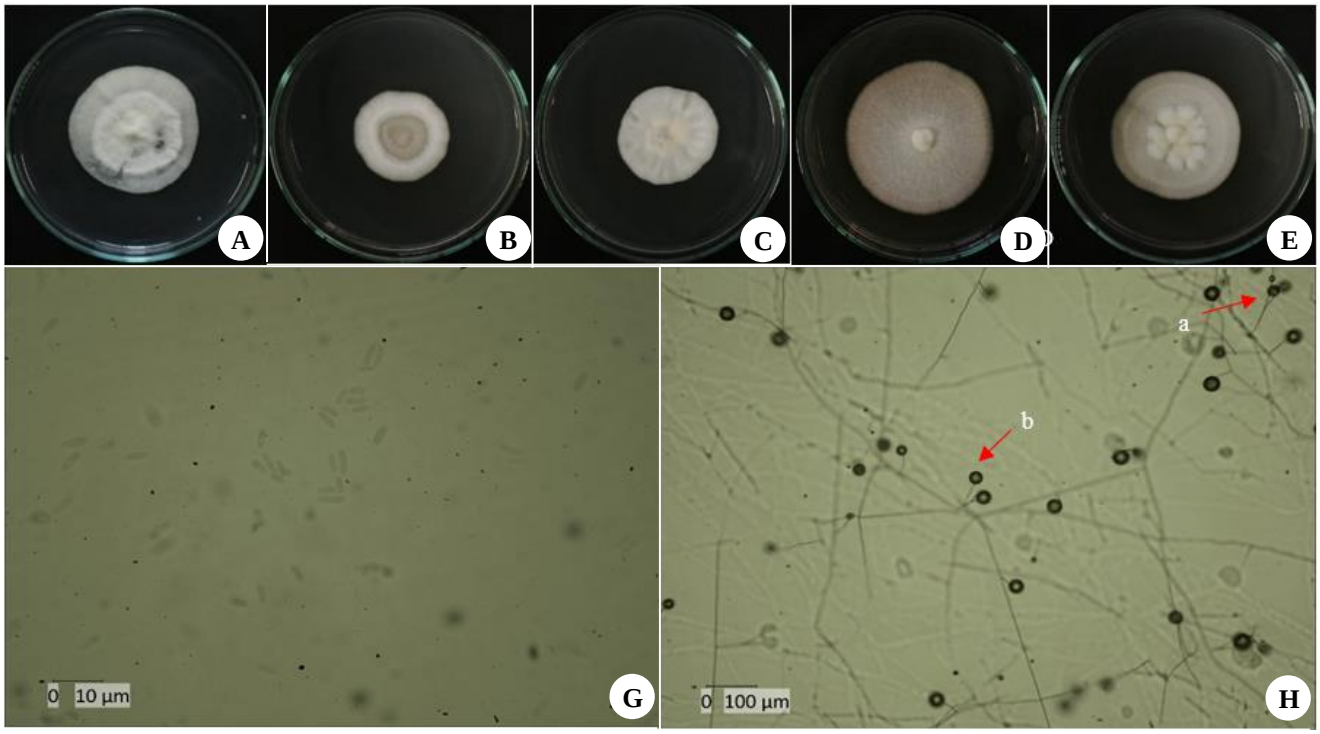


Figure 3. Morphology of *Sarocladium* sp.: cultural colony morphology isolate SO 2 (A); SO 3 (B); SO 5 (C); SO 8 (D); SO 13 (E); 14d completely dark on PDA; Conidia of *Sarocladium* sp. (G); Conidia aerial mycelium presentation branched conidiophore (H.a); slimy head conidia formed (H.b)

RESULTS AND DISCUSSION

Pathogenicity test and culture morphology characteristics

Sheath rot disease was found in the rice field at the sampling site. A total of twenty-four *Sarocladium* sp. isolates were collected from nineteen sampling locations in six provinces in Indonesia (Table 1). Artificial inoculation of Ciherang rice variety without wounding, showed that sheath necrosis occurred in all *Sarocladium* sp. isolates which varied in the disease severity index range from 300-500 (data not shown). Necrosis were first noted within 48-72 hours after inoculation varied in each isolates (data not shown). DSI index was affected by the level virulence of pathogen infection and host response. Pathogens that have a high level of virulence in susceptible hosts will produce high DSI values and a faster time for symptoms to appear. The symptoms obtained are in accordance to Nair (1976); Ou (1985) and Mvuyekure et al. (2017), where the rot starts with irregular small spots and brown margins and occurs on the leaf sheaths enclosing the young panicles. The spot then enlarges and changes color to reddish-brown and the stems will rot. Symptoms caused causes panicles changes color to blackish-brown and not completely exerted. The control plants remained asymptomatic (Figure 2).

Morphological characteristics for all *Sarocladium* sp. was observed. *Sarocladium* sp. isolates grows slowly (about 1.9 mm/r/day) on PDA at 25°C in the dark. Colony characteristics of *Sarocladium* sp. were cottony, produces mycelia color varies from white to light yellow, sometimes turn into pale orange with age (Figure 3) and pale orange color of reverse views. Vegetative hyphae of *Sarocladium* sp. are septate, hyaline, smooth and thin-walled. Conidiphores of *Sarocladium* sp. are hyaline, smooth-walled and can be simple or branched. Conidia are cylindrical, hyaline and aseptate, 1.6-4.8µm x 0.6-1.7µm in size and arranged in slimy heads (Figure 3). Chlamydospores not observed. These characters are similar to morphological features of *S. oryzae*, which is slow growth fungi (Bigirimana et al. 2015), has white, orange-white colonies on PDA at 25^{o}C with the formation of conidia in slimy heads and not produce chlamydospores (Giraldo et al. 2015). In addition to *S. oryzae*, which has an important role in causing sheath rot disease, another member of the genus Sarocladium has a role in causing disease in rice is *Sarocladium synense* that causing rice purple sheath disease in China (Giraldo et al. 2015). However, *S. oryzae* is a common pathogen in rice and in several species of bamboo (*Bambusa balcooa, Bambusa tulda, Bambusa vulgaris*) (Boa and Brady 1987).

Morphological observation of fungi is less credible because there are several fungi that cannot be distinguished morphologically. Morphological data are not enough as a basis for determining a species because it can lead to improper identification. Implementation of molecular fungal identification needs to be done to identify fungal species through phylogenetic analysis to get correct results. However, morphological observation of fungi can be used as supporting data for the characteristics of a fungus (Sarwar et al. 2019). Fungal isolates that based on morphological identified as *Sarocladium* sp., DNA sequencing were also carried out by ITS rDNA gene sequence to correctly identify species.

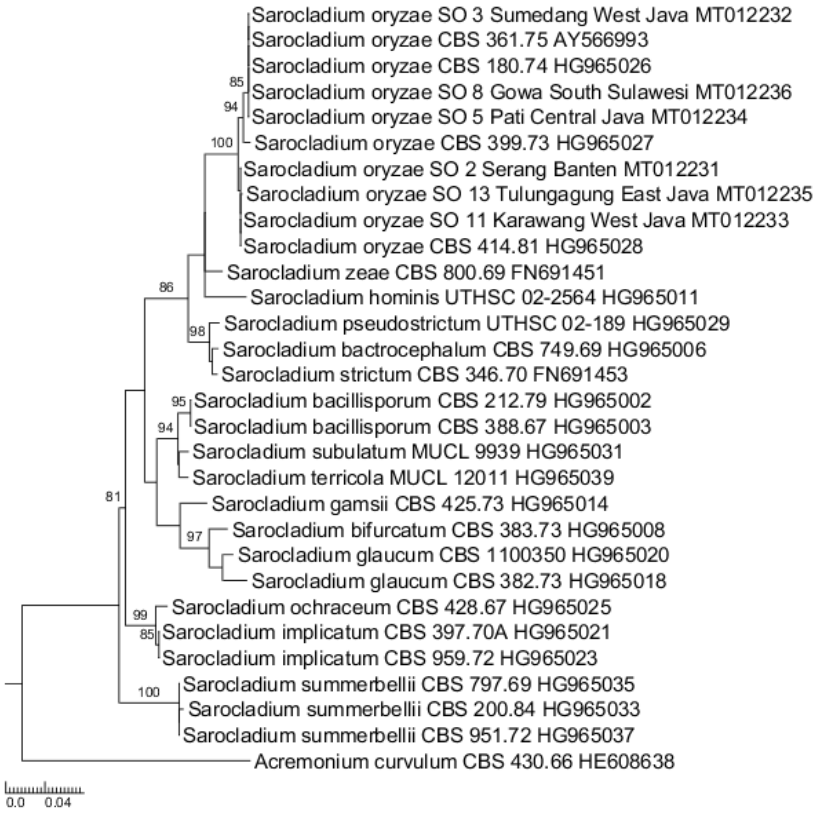


Figure 4. Maximum-likelihood phylogenetic tree based on comparative ITS rDNA gene sequence analysis of *Sarocladium* sp. showing the phylogenetic affiliation of *Sarocladium oryzae* strains. *Acremonium curvulum* CBS 430.66 HE608638 was used as outgroup

Phylogenetic analysis

The ITS rDNA region is a marker for identification of fungi with high probability (Schoch et al. 2012). In this research, six representative *Sarocladium* sp. isolates selected for molecular identification by ITS rDNA gene sequencing. The ITS rDNA gene sequences of six *Sarocladium* sp. were successfully identified by using BLASTn based on GenBank databases. The phylogenetic analysis of six *Sarocladium* sp. construct with ITS rDNA gene sequence by reference sequence (Table 2). Based on Maximum-Likelihood (ML) phylogenetic analysis, six *Sarocladium* sp. isolates were identified as *S. oryzae* and were close to references isolates (Figure 4).

It is the first report about the existence of sheath rot disease in various rice fields in Indonesia. Our result showed that one of the major fungal pathogen of sheath rot disease in Indonesia caused by *S. oryzae*. It is the first report for the existence of *S. oryzae* in various rice fields in Indonesia. These findings are important information about *S. oryzae* in Indonesia. The presence of *S. oryzae* in countries around Indonesia such as Malaysia, Brunei Darussalam, the Philippines and Thailand has been reported, but there have been no more detailed reports (EPPO 2014).

The further studies about developing control measures to prevent the disease on the field through the implementation of biological control of the pathogen to prevent the disease epidemic on rice are necessary. Sheath rot has become an important disease in rice plants (Bigirimana et al. 2015). The occurrence and severity index of sheath rot disease are affected by external factors such as environmental conditions and farming practices, and internal factors such as varietal susceptibility (Pramunadipta et al. 2017). More attention must be given to this disease to prevent the epidemic and the spread of disease to the other rice production field to decreasing yield loss that can maybe happen in future.

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REFERENCES

Amin KS, Sharma BD, Das CR. 1974. Occurrence in India of sheath rot of rice caused by Acrocylindrium. Plant Dis Rep 58: 358-360.

Ayyadurai N, Kirubakaran SI, Srisha S, Sakthivel N. 2005. Biological and molecular variability of *Sarocladium oryzae*, the sheath rot pathogen of rice (*Oryza sativa* L.). Curr Microbiol 50: 319-323.

Balgude YS, Kshirsagar CR, Gaikwad AP. 2019. Evaluation on the efficacy of modern fungicides against blast and sheath rot of rice. Int J Curr Microbiol Appl Sci 8: 83-88.

Bigirimana VP, Hua GKH, Nyamangyoku OI, Höfte M. 2015. Rice Sheath Rot: An Emerging Ubiquitous Destructive Disease Complex. Front Plant Sci 6: 1-16.

Bills GF, Platas G, Gams W. 2004. Conspecificity of the cerulenin and helvolic acid producing "*Cephalosporium caerulens*", and the hypocrealean fungus *Sarocladium oryzae*. Mycol Res 108: 1291-1300.

Boa ER, Brady BL. 1987. *Sarocladium oryzae* associated with blight of Bambusa species in Bangladesh. Trans B Mycol Soc 89: 161-166.

BPS. 2020. Harvested Area, Productivity, and Production of Paddy by Province, 2018-2019. https://www.bps.go.id/dynamictable/2019/04/15/1608/luas-panen-produksi-dan-produktivitas-padi-menurut-provinsi-2018-2019.html.

Bridge PD, Hawksworth DL, Kavishe DF, Farnell PA. 1989. A revision of the species concept in *Sarocladium*, the causal agent of sheath-rot in rice and bamboo blight, based on biochemical and morphometric analyses. Plant Pathol 38: 239-245.

Chowdhury MTI, Mian MSM, Mia MAT, Rafi MY, Latif MA. 2015. Agro-ecological variations of sheath rot disease of rice caused by *Sarocladium oryzae* and DNA fingerprinting of the pathogen's population structure. Genet Mol Res 14: 18140-18152.

Desjardins AE, Manandhar KH, Plattner RD, Manandhar GG, Poling SM, Maragos CM. 2000. Fusarium species from Nepalese rice and production of mycotoxins and gibberellic acid by selected species. Appl Environ Microbiol 66: 1020-1025.

EPPO. 2014. EPPO Global database (available online). Paris, France: EPPO. https://gd.eppo.int/.

Garcia DM, Diaz CH, Artiles YC, Ramos RA, Rubi JA. 2003. Characterization of the proteinases secreted by *Sarocladium oryzae*. Biotecnología Aplicada 20: 170-172.

Ghosh MK, Amudha R, Jayachandran S, Sakthivel N. 2002. Detection and quantification of phytotoxic metabolites of *Sarocladium oryzae* in sheath rot-infected grains of rice. Lett Appl Microbiol 34: 398-401.

Giraldo A, Gené J, Cano J, De Hoog S, Guarro J. 2012- Two new species of Acremonium from Spanish soils. Mycologia 104: 1456-1465.

Giraldo A, Gené J, Sutton DA, Madrid H, De Hoog GS, Cano J, Decock C, Crous PW, Guarro J. 2015. Phylogeny of Sarocladium (Hypocreales). Persoonia 34: 10-24.

Gnanamanickam SS, Mew TW. 1991. Interactions between *Sarocladium oryzae* and stem attacking fungal pathogens of rice. Plant Soil 138: 213-219.

Gopalakrishnan C, Kamalakannan A, Valluvaparidasan V. 2010. Effect of seed-borne *Sarocladium oryzae*, the incitant of rice sheath rot on rice seed quality. J Plant Prot Res 50: 98-102.

Hittalmani S, Mahesh HB, Mahadevaiah C, Prasannakumar MK. 2016. De novo genome assembly and annotation of rice sheath rot fungus *Sarocladium oryzae* reveals genes involved in helvolic acid and cerulenin biosynthesis pathways. BMC Genomics 17: 1-13.

Kornerup A, Wanscher JH. 1978. Methuen handbook of colour. 3rd ed. Eyre Methuen, London.

Kumar S, Stecher G, Li M, Knyaz C, Tamura K. 2018. MEGA X: molecular evolutionary genetics analysis across computing platforms. Mol Biol Evol Soc 35: 1547-1549.

Liu XB, Guo ZK, Huang GX. 2017. *Sarocladium brachiariae* sp. nov., an endophytic fungus isolated from *Brachiaria briz*. Mycosphere 8: 827-834.

Mathur SC. 1981. Observations on diseases of dryland rice in Brazil. In: International Rice Research Newsletter. IRRI, Manila, Philippines.

Mau YS, Markus JER, Oematan SS, Ndiwa ASS, Handoko DD, Nasution A, Makbul K. 2017. Genetic diversity of red and black upland rice accessions from East Nusa Tenggara, Indonesia as revealed by agro-morphological characters. Biodiversitas 18: 197-211.

Mew TW, Gonzales P. 2002. A Handbook of Rice Seedborne Fungi. Science Publishers, Philippines.

Mvuyekure SM, Sibiya J, Derera J, Nzungize J, Nkima G. 2017. Genetic analysis of mechanisms associated with inheritance of resistance to sheath rot of rice. Plant Breed 136: 509-515.

Nair R. 1976. Incidence of sheath rot in rice a potential problem for Sambalpur, Orissa. In: International Rice Research Notes. IRRI, Manila, Philippines.

Narayanasamy P, Viswanathan R. 1990. A new scoring system for sheath rot of rice. Madras Agric J 77: 256-257.

Ou SH. 1985. Rice Diseases. 2nd ed. The Cambrian News, UK.

Park JW, Choi SY, Hwang HJ, Kim YB. 2005. Fungal mycoflora and mycotoxins in Korean polished rice destined for humans. Int J Food Microbiol 103: 305-314.

Pearce DA, Bridge PD, Hawksworth DL. 2001. Species concept in Sarocladium, the causal agent in sheath rot in rice and bamboo blight. In: Major Fungal Diseases of Rice. Springer, Dordrecht.

Pramunadipta S, Widiastuti A, Priyatmojo A. 2017. Environmental Factors Affecting the Severity of Sheath Rot Disease (*Sarocladium oryzae* and *Fusarium* spp.) on Paddy. Abstract. 2nd International Conference of Tropical Agriculture. Sustainable Tropical Agriculture Symposium. Yogyakarta, Indonesia, 26- 27 October 2017.

Sakthivel N. 2001. Sheath rot disease of rice: current status and control strategies. In: Major Fungal Diseases of Rice. Springer, Dordrecht.

Sarangi SK, Maiji B, Mahanta KK, Digar S, Burman D, Mandal S, Mandal UK, Sharma PC, Mainuddin M, Bell RW. 2019. Alternate Kharif rice crop establishment methods and medium duration varieties to enable cropping system intensification in coastal saline regions. J Indian Soc Coast Agric Res 37: 115-122.

Sarwar S, Firdous Q, Khalid AN. 2019. Importance of molecular and phylogenetic analyses for identification of basidiomycetes. In: Recent Advances in Phylogenetics. IntechOpen, UK.

Schoch CL, Seifert KA, Huhndorf S, Robert V, Spouge JL, Levesque CA, Chen W. Fungal Barcoding C. 2012. Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for Fungi. Proc Nat Acad Sci 109: 6241-6246.

Shamsi S, Chowdhury P. 2016. In vitro evaluation of fungicides and some plant extracts against rice sheath rot pathogen *Sarocladium oryzae*. Bangladesh J Sci Res 29: 47-54.

Srinivasachary SH, Kumar KG, Shashidhar HE, Vaishali MG. 2002. Identification of quantitative trait loci associated with sheath rot resistance (*Sarocladium oryzae*) and panicle exsertion in rice (*Oryza sativa* L.). Curr Sci 82: 133-135.

Suga H, Karugia GW, Ward T, Gale LR, Tomimura K, Nakajima T, Miyasaka A, Koizumi S, Kageyama K, Hyakumachi M. 2008. Molecular characterization of *Fusarium graminearum* species complex in Japan. Phytopathology 98: 159-166.

Tschen J, Chen L, Hsieh S, Wu T. 1997. Isolation and phytotoxic effects of helvolic acid from plant pathogenic fungus *Sarocladium oryzae*. Bot Bull Acad Sin 38: 251-256.

White TJ, Bruns T, Lee S, Taylor J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: PCR Protocols: A Guide to Methods and Applications. Academic Press, Inc., United State.

Zhang J, Pan Y, Li Y, Ren T, Cong R, Lu J, Li X. 2019. Low grain sink activity imposed by potassium deficiency aggravates loss in quality of rice (*Oryza sativa* L.) infected with natural sheath rot disease. J Cereal Sci 87: 31-38.