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

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Manuscript received: 9 December 2019. Revision accepted: 27 February 2020.

**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; Sakhthivel 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. 5mm²), 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°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°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 25°C in the dark and examined each 7d up to 4 weeks (Giraldo et al. 2015; Liu et al. 2017). Fungal colony colors 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). Fungal colony colors were identified from cultures grown on PDA plates, measured by Image Raster 3.0 software.

**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) (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).

**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        | Ciharan    | Ciharan  | 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| SO1; SO4; SO11  | 6°15′12.5″S 107°17′42.6″E |
| JB2        | Ciharan    | 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        | Ciharan    | SO14     | 6°54′51.4″S 107°46′41.9″E |
| JB5        | Ciharan    | SO22     | 6°35′00.3″S 107°26′07.8″E |
| JT1        | Tegal      | Central Java | Ciharan  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| Ciharan  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     | Ciharan  | SO18            | 8°28′45.8″S 115°11′14.5″E |
| BI2        | Ciharan    | 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 | Ciharan  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 & CBS 388.67          | Insect, Romania                     | HG965002         | Giraldo et al. 2015        |
| Sarocladium bactrocephalum      | CBS 749.69T                      | Soil, Netherlands                   | HG965006         | Giraldo et al. 2015        |
| Sarocladium bifurcatum          | CBS 383.73                       | Dead stem of bamboo, India          | HG965008         | Giraldo et al. 2015        |
| Sarocladium gamssii             | CBS 425.73                       | Dead petiole of Pandanus lorum, Sri Lanka | HG965014 | Giraldo et al. 2015        |
| Sarocladium gamsii              | CBS 382.73                       | Dead stem of bamboo, India          | HG965018         | Giraldo et al. 2015        |
| Sarocladium hominis             | CBS 100350                       | Dead stem of bamboo, Japan          | HG965020         | Giraldo et al. 2015        |
| Sarocladium implicatum          | CBS 397.70A & CBS 959.72NT       | Saccharum officinarum, Jamaica      | HG965021         | Giraldo et al. 2015        |
| Sarocladium oryzae              | CBS 428.67T & CBS 180.74ET       | Zea mays, Kenya                     | HG965025         | Giraldo et al. 2015        |
| Sarocladium oryzae              | CBS 399.73                       | Oryza sativa, India                | HG965026         | Giraldo et al. 2015        |
| Sarocladium oryzae              | CBS 414.81                       | Oryza sativa, Nigeria              | HG965027         | Giraldo et al. 2015        |
| Sarocladium oryzae              | CBS 361.75                       | Oryza sativa, Kenya                | HG965028         | Giraldo et al. 2015        |
| Sarocladium pseudostrictum      | UTHSC 02-1892T                   | Sputum, USA                        | HG965029         | Giraldo et al. 2015        |
| Sarocladium strictum            | CBS 346.70T                      | Triticum aestivum, Germany         | FN691453         | Giraldo et al. 2015        |
| Sarocladium subulatum           | MUCL 9939NT                      | Soil, Egypt                        | HG965031         | Giraldo et al. 2015        |
| Sarocladium summerbellii        | CBS 200.84                       | Water in air moistener, Netherlands | HG965033         | Giraldo et al. 2015        |
| Sarocladium terricola           | CBS 797.69                       | Decaying leaf of Canna indica, Netherlands | HG965035 & HG965037 | Giraldo et al. 2015        |
| Sarocladium zeae                | MUCL 12011                       | Decaying leaf of Milletta launtenii, D.R. Congo | HG965039 | 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                            | Oryza 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 430.66T                      | Wheatfield soil, Germany            | HE680838         | 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)
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 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 counties 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 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 spread of disease to the other rice production field to decreasing yield loss that can maybe happen in future.

ACKNOWLEDGEMENTS

This study has been funded by Ministry of Research, Technology and Higher Education of the Republic of Indonesia by PMDSU number 2964/UN1.DITLIT/DIT-LIT/LT/2019.

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