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Research Article

History of Sheath Rot Disease in Indonesia and Disease Severity in Two Rice Production Centres of West Java

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ABSTRACT

The observation conducted from 2000 to 2018 found that rice sheath rot has become an emerging disease in several regions. The disease was able to reduce quality and quantity of rice yield. This research objective was to study the history and status of rice sheath rot caused by *Sarocladium oryzae.* Data was obtained from literature study, farmer interview, and field survey. Data were used to calculate disease incidence followed by visual observation of the symptoms caused by this disease. Identification of sheath rot pathogen was done using microscopic and molecular techniques using specific primers. Rice sheath rot was first reported in Indonesia as a minor disease in 1987 and has only currently become an important disease. This fungus generally causes rice sheath rot in Karawang and Cianjur Regency. Disease incidence of rice sheath rot was 12.56% without considering the varieties at all generative growth stages. Average disease incidence on all rice varieties observed was 12.64%, except for IR-42 (0%). The level of rice plants damage due to rice sheath rot in Cianjur was higher than Karawang with average disease incidence of 11.58% and 9.27%, respectively. Rice sheath rot symptoms have often been found in the last 10 years with average level of damage of 3%–18% and yield loss of 10%–15%. This disease has proven to be important and has become a challenge in rice cultivation. The fungus that infect rice plants in Karawang and Cianjur had different morphotypes, namely KP, KP2, KP3, PW3, and PW03. The amplification results showed that all fungal isolates were *S. oryzae*.

Keywords: disease incidence; rice; *Sarocladium oryzae*; sheath rot; status

INTRODUCTION

Rice sheath rot caused by *Sarocladium oryzae* (*Acrocilindrium oryzae*) (Garm and Hawksworth 1975) is a disease which causes 0.5–1.5 cm brown with inner grey lesion rot symptoms. These lesions later will enlarge and cover the whole sheath causing sheath not to develop, which later cause empty grain and rot. The infection of *S. oryzae* on rice causes failure of plant to develop panicles and up to 85% production loss (Webster & Gunnell, 1992).

The occurrence of *S. oryzae* in Indonesia, firstly reported by Ditjen Pangan (1992) in 1987, was not a concern due to its low severity at that moment. However, this disease is found more frequently in rice production districts in Indonesia, such as Karawang and Cianjur. This encourages the need for research on the status of this disease, its infection rate, and distribution in both districts. Both locations were chosen due tho the different altitude and rice growing condition from each location.

MATERIALS AND METHODS

Data Collection

A literature search, interview on knowledgable subjects and farmers were done to obtain primer and secondary data. Literatures used in this study were technical reports from the Indonesia–Japan technical cooperation on plant protection of staple food (ATA-162), which first reported the occurrence of *A. oryzae* (*S. oryzae*) in Indonesia, together with several scientific publications about the development of *S. oryzae.* Interviews were done on 54 famers regarding the occurrence time of rice sheath rot in Karawang and Cianjur. A report from Baskoro Sugeng Wibowo, a researcher at the Pest Forecasting Institute (BBPOPT) Jatisari, which focused on the observation of rice sheath rot in 2000 was also used as a source. Besides that, an interview with Baskoro Sugeng Wibowo was also done to support primary data regarding rice sheath rot in Indonesia.

Field observations were done to measure disease incidence in Karawang and Cianjur. The Regency of Karawang consisted of Jatisari (Jatiragas, Jatibaru, and Cikalongsari village) at altitude of 31.2 masl, District of Banyusari (Gempol, Jayamukti, and Pemekaran village) altitude of 16.2 masl, and District of Cilamaya (Cikalong, Rawagempol Kulon, and Sukatani village) altitude of 8.9 masl (BPS, 2016). The Regency of Cianjur consisted of the Disctrict of Warungkondang (Cikaraya, Jambudipa, and Bunikasih village) altitude of 900 masl, District of Cianjur City (Mekarsari, Nagrak, and Babakan Karet village) altitude 675 masl, and District of Gekbrong (Sukaratu, Bangbayan, and Songgom village) altitude of 900 masl (BPS, 2017). Rice fields observed in this study were fields where rice plants were in generative stages.

Interview of 54 farmers was done using a structured questionnaire about plant stages, variety of rice plant, farmer's loss estimation due to rice sheath rot and farmer's knowledge on rice sheat rot by showing pictures and samples of rice plant infected with *S.oryzae*. Disease incidence observations and famer interviews were done from September to November (dry season) 2018. Observation locations in each region were randomly selected through Google Map. Ten rice plants were randomly selected at each field using a diagonal transect method. Disease incidence (DI) was calculated using the formula:

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

n = number of infected plants
N= number of observed plants

Isolation of <u>Sarocladium</u> <u>oryzae</u>

Pathogen was isolated from grains and sheaths of infected rice plant from 20 plants randomly selected from all observed locations. Collected plant tissues were rinse using running water to clean debris and surfaces were sterilized using NaOCl 2% (38 ml NaOCl mixed with 62 ml of sterile water) for 2 minutes, rinsed with sterile water twice and air-dried on tissue papers. Sterilized rice sheaths were cut into 1 cm pieces using sterile scissors. Grains were than germinated on potato dextrose agar (PDA) for 3–4 days (Ghosh *et al*., 2002). The growing fungus was purified as pure isolates for following tests.

Visual Observation of Rice Sheath Rot Symptoms

The aim of this observation is to ensure that sheath rot symptoms found on plants were caused by *S. oryzae*. Rice sheaths that showed rot symptoms, were picked and cut twice for each sample into approximately 0.3 cm from 20 randomly chosen samples using scissors. One cut of rice sheath was place on an object glass with sterile water drops and closed with a cover glass. Prepared specimens were observed under a compound microscope. Rice sheath infected with fungus will produce mycelium and conidia, while if infected with bacteria, plant tissue will excrete ooze from their perimeters. Remaining pieces of rice sheaths were then grown on PDA to ensure microbes that infected rice sheath (Shurtleff & Averre, 1996).

Fungi Identification

Microscopic identification was done by first using transparent tape to obtaine spore and mycelium structures. Lacthophenol cotton blue was placed on an object glass. Transparent tape was gently stuck to 14-day-old isolates grown on PDA until mycelium was stuck on the tape. Mycelium were then palced on the object glass. Observation on mycelium, conidia, conidiophore, and hyphae branches were done under a compound microscope and compared with a morphological key by Watanabe (2002).

The extraction of total fungal DNA was done using Qiamp DNA mini kit (Qiagen). The amplification of DNA was done using a Sensoquest Thermal Cycler PCR by following Q5 protocols from New England Biolabs (NEB). Amplification was done using a *S. oryzae* specific forward primer (5'-GATCTCTTGG CTCTGGCATC-3') (Aldakil *et al*., 2019) and reverse primer (5'-GCTCCTGAGGGTTGAAATGA-3') (Bigirimana *et al*., 2015) with the target amplification size of 157 pb. Reaction of DNA amplification was done using a total of 25 µL that consisted of 12.5 µL Q5 hot start high-fidelity 2X master mix, 1.25 µL forward primer, 1.25 µL reverse primer, 2 µL sample DNA, and 8 µL nuclease free water. Amplification of DNA used a Sensoquest Thermal Cycler PCR, initial heating was done until 98°C and required 30 s, the subsequent phase was a 35-amplification cycle where each cycle consisted of denaturation at 98°C for 10 s, followed by primer annealing at 60°C for 20 s, and extension at 72°C for 25 s. Final extension was done at 72°C for 2 minutes and the end of the cycle was maintained at 4–10°C.

Result products from the amplification process was analyzed on 2% gel agarose (0.4 g agarose and 20 mL buffer TAE 1X). Electrophoresis was done at 94 volts for 40 minutes. Agarose was later incubated in ethidium bromide (1%) for 40 minutes and rinsed with water. Electrophoresis results were visualized using ultraviolet transilluminator. The DNA bands formed where then documented using a digital camera.

Data Analysis

Farmer interview data, field survey, and visual observation of rice sheath rot symptoms were decribed using descriptive statistics and processed using *Microsoft Excel* 2013.

RESULTS AND DISCUSSION

The History of Rice Sheath Rot

The pathogen *S. oryzae* was first reported in Taiwan by Sawada in 1922 (Sigh & Dodan 1995). Pearch *et al*. (2001) stated that *S. oryzae* has been reported to spread in several rice-producing countries, such as Bangladesh, Cameroon, India, Korea, Japan, Peru, Philipines, Thailand, Venezuela, Vietnam, USA, Indonesia, Brunei Darussalam, China, Pakistan, Nepal, Malaysia, Saudi Arabia, Sri Lanka, Tajikistan, Uzbekistan, Burundi, Gambia, Cote d'Ivoire, Kenya, Madagaskar, Niger, Nigeria, Senegal, Tanzania, Mexico, China, Argentina, Brazil, and Australia. Rice sheath rot in Indonesia was first identified during the dry season of 1987 (May until August) through the technical cooperation between Indonesia–Japan in plant protection (ATA-162) leaded by Shizui Mogi. The cooperation between Indonesia and Japan at that moment resulted in methods to forecast blast disease on rice and to determine the status and distribution of rice diseases in Indonesia (Ditjen Pangan, 1992).

Rice sheath rot (*S. oryzae*) during the dry season between May and August 1987 was considered mild. Observation showed that rice sheath rot (*S. oryzae*) has been widely spreaded to rice fields in Sumatera, Java, Bali, Lombok, South Kalimantan, and South Sulawesi. During the rainy season, distribution and infection levels of *S. oryzae* was found in several areas in East Java and from the 13 plots observed in that study, disease incidence reached 30.8%; North Sumatera and West Sumatera from 26 plots, disease incidence was approximately 3.8%, and in West Java to be around 2.3%. Rice variety planted in observed areas were IR-64, Cisadane, Cisokan, Cisanggarung, and Krueng Aceh (Ditjen Pangan, 1992). Field observation by Baskoro Sugeng Wibowo between 1990 and 2000, found that rice sheath rot was found to reach disease incidence of 5% in several areas. This was the reason why Baskoro Sugeng Wibowo focused the following observation on the disease incidence of rice sheath rot in several areas around Indonesia from 2000 to 2010. He found that disease incidence of rice sheath rot in Tangerang (Banten) was 50%, Subang was 25%, Purwakarta reached 100%, Bekasi was 40%, Bangli (Bali) was 10%, and Siak (Riau) was 50%. After closely examined, disease symptoms were identified to be caused by *S. oryzae*.

After 2010, research on *S. oryzae* started to thrive in Indonesia. Some of the research evaluated disease incidence in the District of Tanggul, Region of Jember with average disease incidence of rice sheath rot caused by *S. oryzae* to be 28.67–35.19% (Wijaya, 2017). Pramunadipta (2017) evaluated the diversity of pathogens that caused rice sheath rot and the environmental factors that affected them and their severity. Rosaliyana (2017) studied the severity of rice sheath rot caused by *S. oryzae* and *Fusarium* sp. at different growth stages. Pratiwi (2018) identified and characterized rice sheath rot causing pathogens in the Region of Serang and Yulia *et al*. (2019) examined methanol extracted from binahong leaves (*Anredera cordifoli*) ability to suppress the growth of *S. oryzae*. Results showed that methanol extracted from binahong leaves were not able to significantly suppress *S. oryzae* colonies.

The interview with 54 farmers was done to identify the development of rice sheath rot and showed that 42% of the respondents stated that this disease was frequently found from 2000–2010, and 54% of the respondents stated that this disease was frequently found between 2012 and 2018 (Figure 1). This shows the condition and status of rice sheath rot in becoming a major disease in Indonesia. All repondents did not know the cause of rice sheath rot before being informed. They assumed that rice sheath rot was caused by rice bugs (*Leptocorisa oratorius*), high rainfall, or nutrient deficiency.

Symptoms and Incidence of Disease in the Field

Rice plants in the field showed brown lesions on sheaths with grey centers and white powdery spores.

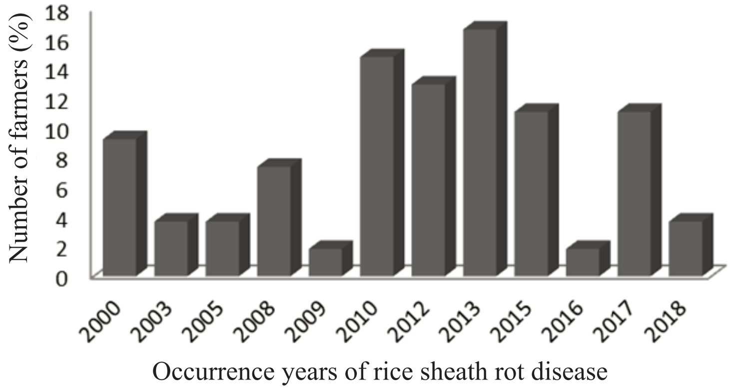

Figure 1. First occurrence of rice sheath rot according to farmers in Karawang and Cianjur

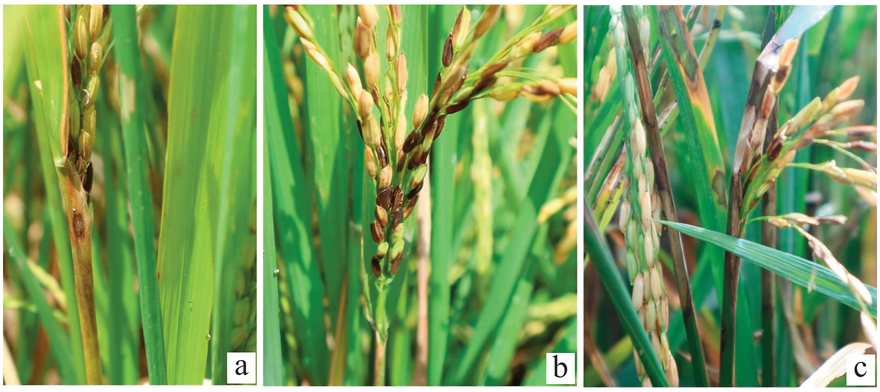

Figure 2. Rice sheath rot symptom caused by *Sarocladium oryzae*: (a) symptom on rice sheath, (b) symptom on rice grain, and (c) severely infected rice grain

Table 1. Rice sheath rot symptoms from various locations

Observation method	Identified fungi(%)		
	S. oryzae	*S. oryzae* and *Fusarium* sp.	*Fusarium* sp.
Microscopic	65	20	15
Growth on PDA	60	30	10
Average	62.5	25	12.5

These lessions were elongated with irregular or oval shaped. Infected rice plants had grains that were black and empty. Severe infection caused grain to not fully develop (Figure 2). Sheath and grain decay are caused by the main secondary metabolites (helvolic acid and cerulenin) produced by *S. oryzae* to disturb membrane rigorouness and cause electrolyte leaks which makes plants susceptible to rice sheath rot (Sakthivel *et al.*, 2002). Helvolic acid is a tetracyclic triterpenoid that disturbes plant's chlorophyll biosynthesis (Ayyadurai *et al.*, 2005), while cerulenin is a hexaketida amide that prevents polyketide by malonyl-ACP-acyl inhibition, inhibit fatty acid synthesis, and disrupts flavenoid biosynthesis (Omura, 1967). Rice grain decay that are infected by *S. oryzae* caused decrease of sugar levels, carbohydrates, and protein, while increased the composition of phenol.

Rice sheath rot symptoms observed in Karawang and Cianjur were caused by *S. oryzae*. These results were confirmed by the observation under the microscope and isolates grown on PDA (Table 1). The observation of plant tissue infected by fungi showed necrotic oval, elongated, and irregular shaped lessions. Under the microscope, conidia and spores were hyaline coloured, not segmented, and oval shaped.

Table 2. Disease incidence of rice sheath rot at different ages during the generative stage

No.	Plant age (DAP)	DI(%)
1	55	5.37
2	60	13.78
3	63	16.04
4	65	11.33
5	70	2.58
6	72	10.73
7	75	14.77
8	78	8.68
9	80	4.95
10	83	63.78
11	85	6.73
12	87	15.94
13	90	6.52
14	95	10.30
15	98	7.96
16	100	5.46
17	105	8.67
	Average	12.56

Information: DAP = days after planting; DI = disease incidence

Table 3. Disease incidence (DI) rice sheath rot on different varieties

No.	Rice variety	DI (%)
1	Ciherang	12.09
2	Sintanur	7.48
3	Mujaer Mundur	0.30
4	Inpari 32	11.13
5	IR-64	28.83
6	Tarabas	63.78
7	Situbagendit	4.52
8	Pandan Wangi	8.96
9	Inpari 30	7.33
10	Mekongga	5.80
11	BTN	11.53
12	Lamdour	2.59
13	IR-42	0.00
	Average	12.64

Isolates grown on PDA were orange. Field observation in Karawang and Cianjur, showed that disease infection occurred during the generative stage from 55–105 days after planting (DAP) (Table 2). This shows that generative stages of rice are susceptible to *S. oryzae* infection. Rice variety observed in Karawang and Cianjur were Ciherang, Sintanur, *Mujaer Mundur* (local variety), Inpari 32, IR-64, Tarabas, Situbagendit, Pandan Wangi, Inpari 30, Mekongga, BTN, IR-42, and Lamdour. Rice sheath rot occurred on all varieties, except IR-42, with an average disease incidence of 12.64% (Table 3). IR-42 may be more resistant against *S. oryzae* compared to other varieties. Rice variety IR-42 grows upright, can grow from 90 upto 105 cm, have upright flag leaves, produce slender and oval and yellow grain, are resistant against collapsing, brown plant hopper biotype 1 and 2, bacterial blight, and with potential yield upto 7 ton/ha (Suprihatno *et al*., 2009).

Rice sheath rot incidence were higher in Cianjur compared to Karawang, where disease incidence in Cianjur was 1158% and 9.27% in Karawang (Table 4). Lower air temperature and higher RH levels in Cianjur were considered to be the better condition for *S. oryzae* development. Singh & Dodan (1995) stated that incidence and severity of rice sheath rot caused by *S. oryzae* is affected by temperature and humidity with optimum air temperature was 17–20^{o}C and minimum humidity 40–50%.

Rice sheath rot was a minor disease in Indonesia at 1987, implying that this disease exists in Indonesia, but its development was slow. Interviews with farmers showed that rice sheath rot could easily be found between 2000 and 2018 (Figure 1). Damage in rice fields is estimated to reach 3–18%. Loss due to rice sheath rot (*S. oryzae*) may increase if immediate management practices are not done (Figure 3). This implies that this disease is growing into a major rice disease. Field observation done by Baskoro Sugeng Wibowo from 2000-2010 showed that the increase of rice sheath rot infection in several areas in Indonesia, with the average disease incidence to reach ± 45.83%.

Identification of S. oryzae

Observation under the microscope showed *S. oryzae* cell structures, including hypha, conidia, conidiophore, and the cells that structure conidigineous cells. Hyphae of *S. oryzae* are not segmented, conidia of the fungi are hyaline colored, cylindrical or oval, were not segmented, conidiogen appeared to show a complete fungi structure, conodiophore appear from mycelium with 1 or 2 branches (Figure 4). Colonies of *S. oryzae* were white on top and generally orange on its bottom. These findings were similar with the study from Bigirimana *et al*. (2015) where *S. oryzae* colonies were slow growth, approximately 2.5 mm each day on PDA at 28ºC, and in general were orange.

Table 4. Incidence of rice sheath rot on different rice variety

Region	District	Altitude (masl)	Disease incidence (%)	Average each region (%)
Karawang	Cilamaya	8.9	0.76	9.27
	Jatisari	31.2	18.51	
	Banyusari	16.2	8.53	
Cianjur	City of Cianjur	675.0	1.66	11.58
	Gekbrong	900.0	10.79	
	Warungkondang	900.0	22.29	

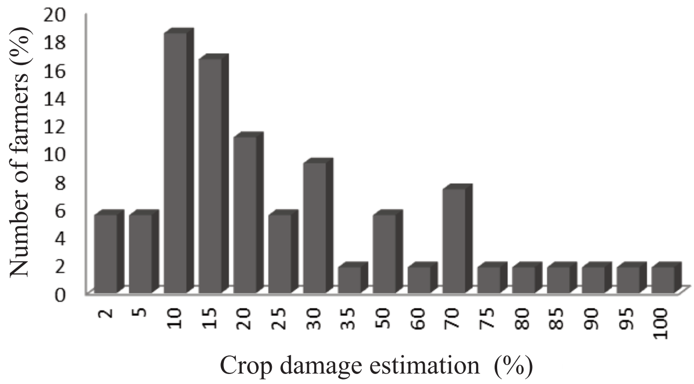

Figure 3. Estimated yield loss caused by rice sheath rot

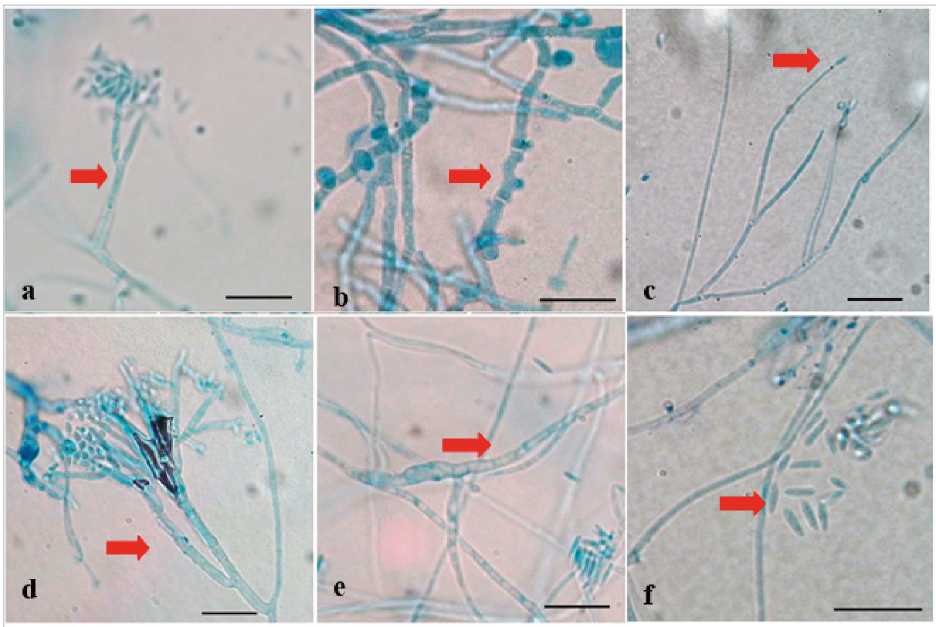

Figure 4. Morphological structure of *Sarocladium oryzae*: (a) conidiophore, (b) gnarled hyphae, (c) conidiogenous cell, (d) conidiophore, (e) hypha, and (f) conidia; scale bar size 10 µm

Isolates from rice sheath and grain showing rot symptoms resulted in 5 fungal colonies with different appearances (morphotypes), including KP, KP2, KP3, PW03, and PW3 (Figure 5). Naming of each morphotype was based on collection location, morphotype KP, KP2, and KP3 were from Karawang, while PW03 and PW3 were from Cianjur. From all obtained isolates, 2 isolates (KP3 and PW3) had

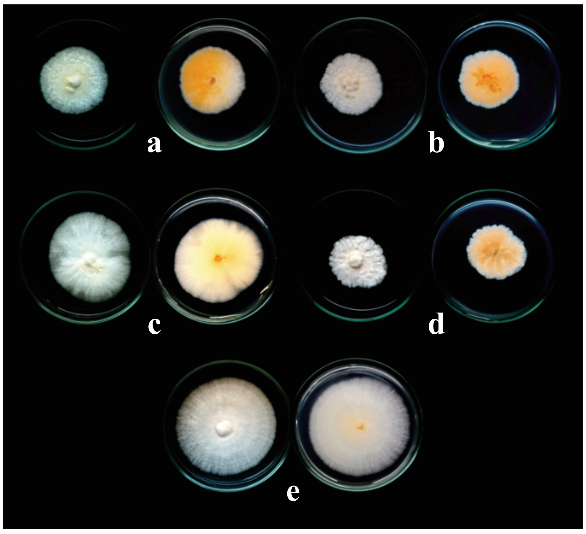

Figure 5. Morphotypes of *Sarocladium oryzae* isolates: (a) KP, (b) KP2, (c) KP3, (d) PW03 and (e) PW3 grown on PDA at temperature fo 28ºC; isolates are 14 days old

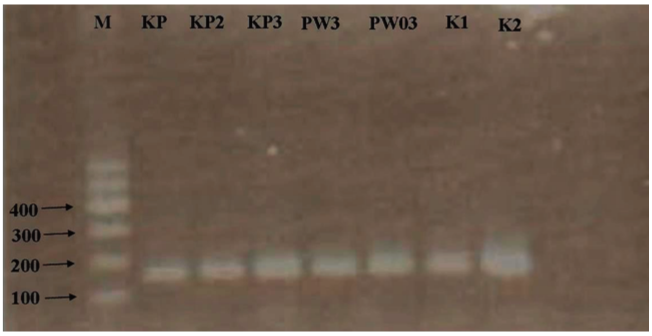

Figure 6. DNA amplification of *Sarocladium oryzae* isolates sequenced using *S. oryzae* specific primer: DNA marker (M), positive control (K1), positive control (K2)

yellowish colors and 3 isolates (KP, KP2, and PW03) were orange. However, morphotypes had oval conidia, were not segmented, had hyaline colors, and showed cell structures of *S. oryzae*. Mycelium growth of each morphotype were different at 14 days. Isolate KP, KP2 and PW03 mycellium grew slower than KP3 and PW3. Amplification results with amplification target of 157 pb, showed that all isolates analyzed were *S. oryzae* based on bands that appeared visually (Figure 6).

CONCLUSION

Rice sheath rot has widely spread in the rice producing districts, Cianjur and Karawang. This disease is being found more frequently over time causing it to become an important disease on rice.

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