SHEATH BROWN ROT DISEASE OF RICE CAUSED BY *PSEUDOMONAS FUSCOVAGINAE* IN THE PENINSULAR MALAYSIA

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Abstract: Relative to the established and well known rice diseases, sheath brown rot caused by *Pseudomonas fuscovaginae* can be considered new but getting widespread and serious all over the world. Our research was aimed to monitor and quantify the incidence and importance of the disease in Malaysia. A series of field monitoring and sampling were conducted to quantify the incidence and severity of the disease. Laboratory analysis of the collected diseased plant was done to identify the causal organism. Disease resistance screening of selected rice cultivars were also conducted to overcome the problem. The disease was found to become more important, prevalent and widely spread throughout rice growing areas in Peninsular Malaysia. Infected plants in the field became yellowish, lower leaf sheaths turned light or dark brown, while grains produced by an infected plant were discoloured, malformed and empty. The highest disease incidence was recorded in the state of Pahang (62%) and Selangor (62%), while the most severe infection was recorded in Pahang (55%) and Terengganu (61%). The evaluations of varietal resistance evaluation showed that the pathogen naturally infected all tested rice varieties at different levels of infection. Several rice varieties i.e. MR240, MR243, MR244, MR245, MR246, MR248 and MR249, classified as moderately resistant to the disease, could be recommended for planting in the next planting season.

Key words: rice, *Pseudomonas fuscovaginae*, bacterial disease, sheath brown rot, resistant varieties

INTRODUCTION

Pseudomonas spp. have been identified as a group of devastating plant pathogens on a wide range of economically important crops throughout the world. Among the earliest plant diseases reported, caused by *Pseudomonas* spp. was bacterial rot of onion (Burkholder 1949). On rice, several species of *Pseudomonas* were identified as a causal agents of a number of diseases and widely distributed throughout the world. Among major publications on rice diseases caused by *Pseudomonas* spp. were those by Mueller (1974), Agarwal *et al.* (1989), Webster and Gunnell (1992) and Shakya (1997).

In 1976, a new disease of rice namely sheath brown rot was detected and rated as the most important bacterial disease of rice in Hokkaido, Japan (Tanii *et al.* 1976). A number of reports on the new disease from other parts of the world i.e. Latin America (Zeigler and Alvarez 1987a, 1987b), Burundi (Duveiller *et al.* 1988), Brazil, Cameroon, Madagascar, Reunion Island and Rwanda (Rott *et al.* 1991), the Philippines (Cottyn *et al.* 1996) and China (Xie 2003) also were published. The incidence was also reported in Central Africa (Autrique and Maraite 1983). *Pseudomonas marginalis* (*Pseudomonas fluorescens* biovar II) was reported to be the causal agent of the disease. However, after more detailed studies, the bacterium was re-identified as a new species and was given a new name of *P. fuscovaginae* (Tanii *et al*. 1976).

The disease symptoms could be detected as early as at seedling stages and normally the infected seedlings died. However, if the infection occurs at a later growth stages, the field of infected rice plants become yellowish. Lower parts of the leaf sheath usually turn light or dark brown. At the later stages of infection, the whole leaf sheath becomes necrotic. Grains produced by infected panicles are discoloured, malformed and empty (Tanii *et al*. 1976; Webster and Gunnell 1992; Cottyn *et al.* 1994). Yield losses as high as 72.2% due to the disease were reported in Indonesia (Cahyaniati and Mortensen 1997). Severe losses caused by the disease also were reported by Notteghem (1998) in rice production areas at Central Africa and Madagascar. Under a very severe infection, total yield

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loss (almost 100%) could be observed as reported from the observation by Rott (1987) in Madagascar.

In Malaysia, first report of the sheath brown rot on rice was published in 1991 (Marzukhi *et al.* 1991). The incidence was observed at Seberang Perak rice estate in Perak causing the yield loss around 46.0% to farmers. Results from the latest observation conducted during the first growing seasons of 2003 throughout all major rice granary areas in the country showed that the disease had widely spread throughout Peninsular Malaysia (Saad *et al*. 2003). A total of 4,571 ha. of rice growing areas in Selangor, Perak and Johor were infected by the pathogenic bacterium. The highest yield loss (ca. 20.0%) was detected in Tanjung Piandang, Perak.

So far, several management procedures to reduce the incidence of other bacterial diseases on rice were evaluated by many researchers in the world over. Among the most effective and popular approaches under taken for the management of the diseases was the use of tolerant or resistant varieties. This approach was categorized as the best and the results were very encouraging (Klement *et al*. 1990; Saddler 2002). As an example, successful control of bacterial blight disease on rice in the Philippines was achieved by using resistant variety (Mew 1987). Reports that discussed the importance of resistant varieties for control of sheath brown rot disease of rice were published by Batako *et al.* (1997), Sharma *et al*. (1997) and Malavolta *et al*. (1997). Specific reports on the use of resistant varieties for sheath brown rot disease management were published by Macapuguay and Mnzaya (1988), also by Tilquin and Detry (1993). Other publications in Japan by Kiyosawa (1972), and Horino (1980) also reported the use of resistant varieties for management of bacterial rice diseases. While in Malaysia, reports on the importance of tolerant or resistant varieties in rice disease management and sustainability of rice production were published by Ali (1995) and Othman and Saad (1998).

Therefore, based on the importance of the disease, a study was conducted to recognize typical disease symptoms and syndromes in the field and to quantify the distribution, incidence and severity of the disease in major rice growing areas in Peninsular Malaysia. Efforts also were undertaken to evaluate and identify suitable rice varieties for management of the disease in the field.

MATERIALS AND METHODS

Disease symptoms, distribution, incidence and severity

A field monitoring and sampling were conducted to characterise typical field symptoms and syndromes, and document the distribution of sheath brown rot disease of rice in Peninsular Malaysia. At the same time, assessment of the incidence and severity of the disease was also conducted. All rice granaries area in seven states i.e. Kedah, Pulau Pinang, Perak, Selangor, Pahang, Terengganu and Kelantan were visited during the field monitoring and sampling periods (Fig. 1). The first monitoring and collecting samples was carried out in April 2004 in Seberang Perak and Sungai Manik (Perak), Rompin (Pahang), Besut (Terengganu), Pasir Puteh and Tumpat (Kelantan), and Yan (Kedah). The second inspection was done in May 2004 in Seberang Perak (Perak), Sekincan and Sawah Sempadan (Selangor). The third inspection was in June 2004 at rice growing areas in Balik Pulau, Bukit Merah and Permatang Pauh (Pulau Pinang). The last monitoring and collecting samples was in July 2004 in Tikam Batu (Kedah).

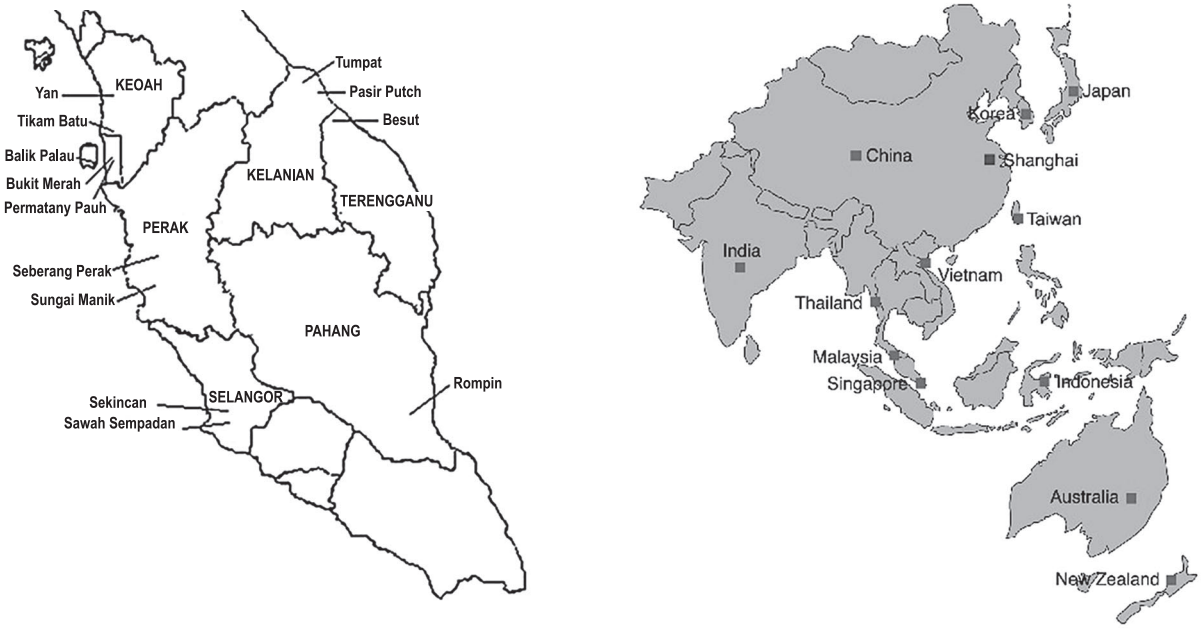

Fig. 1. Specific locations where field monitoring and samples collection of sheath brown rot disease of rice were conducted in Peninsular Malaysia

Both monitoring and collecting samples were carried out in the field when the crops were almost ready for harvesting, around 90–100 days after sowing. All collecting procedures were based on procedures established by Cottyn *et al*. (1996) and Jaunet *et al*. (1995), with some modification to suite the local environment. Ten hills of rice plants were randomly selected and observed closely at every field inspection. Infected plant parts i.e. leaf sheath and grains showing sheath brown rot symptoms were collected in separate paper bag and given a reference number. Aseptic procedures were followed in every sample collection activities. Background information of each sample i.e. location, plant growth stage and disease symptoms were recorded. All samples were then taken to the laboratory at School of Biological Sciences, University Sains Malaysia, Pulau Pinang for isolation of the causal organisms and other further studies. Laboratory procedures and techniques such as isolation of the causal organisms were based on a standard laboratory guide (Mortensen 1994). The causal disease agents were isolated from collected plant samples using nutrient agar and King's medium B (King *et al.* 1954). Morphological characteristics of bacterial colonies on these media were examined for microscopic and cultural appearance, followed by several biochemical tests for species identification such as Gram stain, potassium hydroxide solubility, Kovac's oxidase test and several others (Mortensen 1994).

Disease incidence was calculated base on the plant samples analysis i.e. percentage of isolation of the causal organism i.e. *P. fuscovaginae* from the samples for each state. On the other hand, disease severity was calculated base on the percentage of discoloured rice grains folllowing the standard scale of grain discolouration of the International Rice Research Institute (IRRI) Standards Evaluation System for Rice (Anon. 2002a) as shown in table 1.

Table 1. Disease severity scale (0–9) based on grains discoloration (Anon. 2002a)

Scale	Discoloured grains
0	Nil
1	less than 1%
3	1–5%
5	6–25%
7	26–50%
9	51–100%

Varietal resistant evaluation

Evaluation of varietal resistance to natural infection by *P. fuscovaginae* was carried out at Permatang Pauh, Pulau Pinang by testing ten new varieties i.e. MR240 – MR249. Two recommended varieties i.e. MR211 and MR219, widely planted by the rice growers, were also tested for comparison. Plot size for each treatment was 5 x 5 m with three replicates. Cultural practices as recommended by the Malaysian Agricultural Research and Development Institute (MARDI) (Anon. 2002b) were strictly followed in this experiment. Disease progress was monitored and recorded throughout the experiment. Disease incidence and grain discolouration percentage was observed, recorded and calculated before harvesting the crop.

RESULTS AND DISCUSSION

Disease symptoms, distribution, incidence and severity

General field observation on all crops during monitoring and collecting periods revealed that infected rice plants become yellowish and show different types of symptoms. Lower parts of the infected leaf sheath appeared light or dark brown. Several leaf sheaths became necrotic and dried. A number of flag leaves also turned light or dark brown. Observation of infected panicles showed that rice grains were abnormal and discoloured i.e. colour changed to brownish or dark brown, grains were malformed, and empty (Fig. 2). This findings confirmed that the plants were infected by *P. fuscovaginae* because all disease symptoms observed were similiar to the symptom descriptions reported by earlier researches such as Tanii *et al*. (1976), and Zeigler and Alvarez (1987a). Indeed, the same disease symptoms were also observed when the *P. fuscovaginae* infected wheat crop (Duveiller and Maraite 1990).

Compared to the earlier reports by Marzukhi *et al.* (1991) and Saad *et al.* (2003), the results of the current investigation showed that the disease was not restricted but widely spread throughout all growing areas in the Peninsular Malaysia. Infected plants could be found at every sampling area in the states of Kedah, Pulau Pinang (Pulau Pinang and Seberang Perai), Perak, Selangor, Pahang, Terengganu and Kelantan. The spread of the disease was expected to be very quick due to the use of infected rice seeds for planting in almost all visited areas. This could simply happen because sheath brown rot disease was confirmed as a seedborne disease (Duveiller *et al.* 1988; Xie *et al.* 2002). Results of the experiment carried-out by Duveiller and Martinez (1990) showed that disease symptoms were observed on plants raised from infected seeds. Earlier research also proved that the disease could be established from inoculum in infected seeds carried-over from previous crops (Tsushima *et al*. 1985).

On the other hand, results from our field monitoring and plant samples analysis also showed that the level of the disease incidence varied between 5–62% depending on location of the inspected areas and states. The lowest disease incidence was observed in Kedah (5%), while the highest was recorded in Pahang and Selangor, both at 62%. Disease incidence for other states is shown in table 2.

Table 2. Disease incidence [%] and severity of sheath brown rot on rice based on frequency of *P. fuscovaginae* isolation from collected samples and discoloured grains [%] in different states in Peninsular Malaysia

State	Number of samples	Disease incidence [%]	Discoloured grains [%]	Disease severity
Perak	26	15	29	7
Pahang	21	62	55	9
Terengganu	4	25	61	9
Kelantan	15	20	40	7
Selangor	66	62	35	7
Pulau Pinang	92	35	26	7
Kedah	42	5	32	7

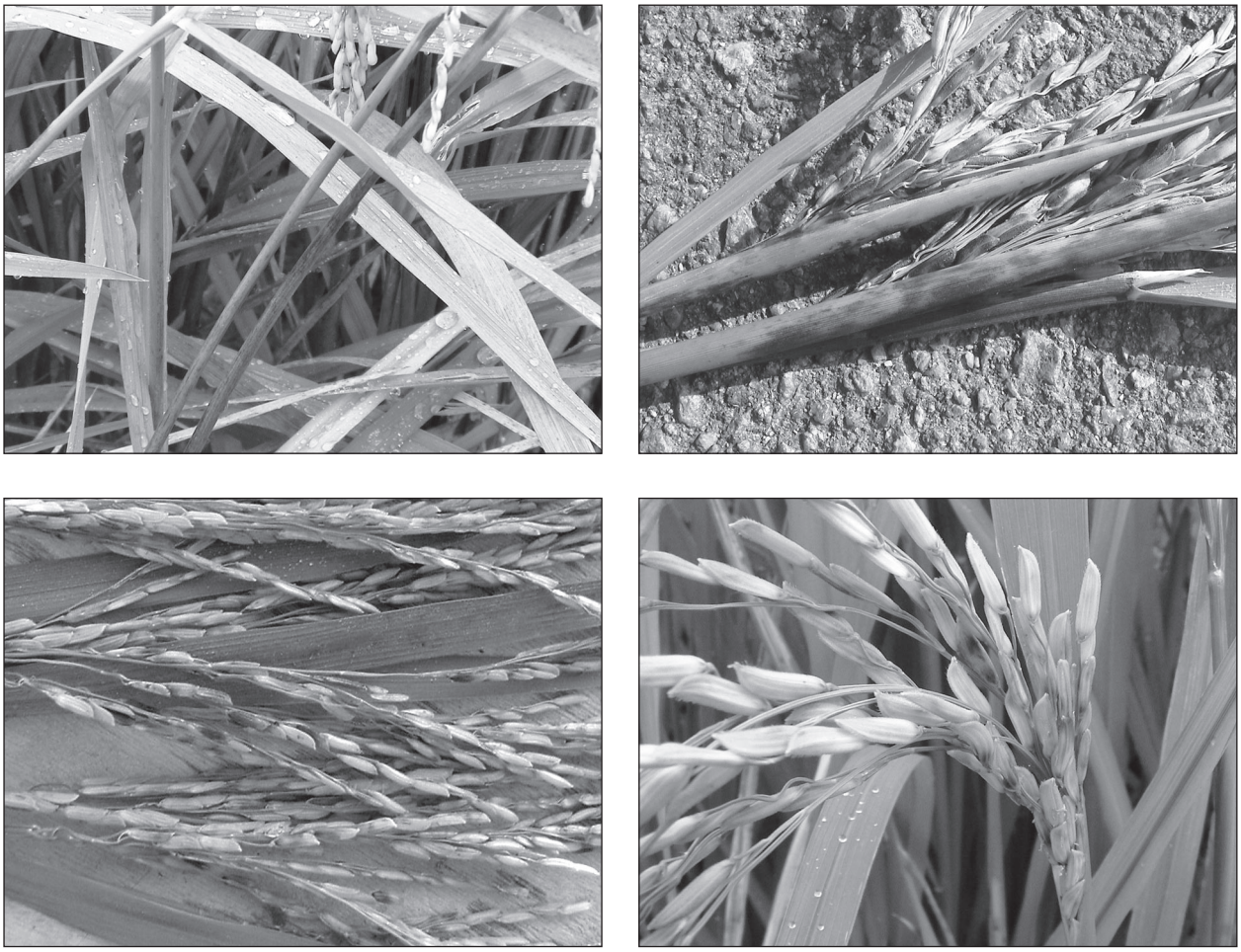

Fig. 2. Mature rice plant with brown leaf sheath (Top left and right), discoloured grains (Bottom left and right) naturally infected by the pathogen in sampling areas

The results also showed that severity of the disease varied for each inspected area and states. This value was based on mean of discoloured grains percentage as shown in table 2. The highest severity showed by 9 grade scale was observed in the state of Pahang and Terengganu. The percentages of discoloured grains for both states were 55% and 61% for Pahang and Terengganu, respectively. Rice plants in other states showed the disease severity of grade 7, whereas the percentages of discoloured grains were lower, between 26–40%. However, there is no information available how disease severity varied in different states.

Varietal resistant evaluation

Results of the experiment carried-out at Permatang Pauh, Pulau Pinang showed that all tested varieties were infected naturally by *P. fuscovaginae*. It was confirmed by disease symptoms present on the rice plants whose leaf sheath turned brown and produced grains that were discoloured, malformed and some of them empty. However, the results showed that tested rice varieties had different level of resistance to the disease. Rice plants of variety MR247 were found to be most susceptible with disease incidence more than 50%. For the other varieties, the level of infection varied between 10 and 40% (Table 3).

Table 3. Percentage of discoloured grains and disease score of tested rice varieties naturally infected by *P. fuscovaginae*, Permatang Pauh, Pulau Pinang

Variety	Discoloured grains [%]	Disease score
MR240	20	5
MR241	30	7
MR242	30	7
MR243	10	5
MR244	20	5
MR245	20	5
MR246	10	5
MR247	50	7
MR248	10	5
MR249	20	5
MR211	10	5
MR219	40	7

Based on the above findings, it was clearly shown that using of selected varieties has some potential for the control of sheath brown rot disease on rice in Peninsular Malaysia. Although all tested varieties were infected naturally by the bacterial pathogen, the level of infection showed some differences. Basically, the plant diseases will be more severe when a susceptible variety will be planted. Study by Duveiller *et al.* (1990) gave some explanation on this finding i.e. sheath brown rot disease severity was significantly influenced by variety planted. Klement *et al.* (1990) stated that the use of resistant variety for the disease control was the most effective. Earlier findings by Macapuguay and Mnzaya (1988), Sharma *et al.* (1997) and Carisse *et al*. (2000) also showed that planting of a few selected varieties could control or at least reduce the infection in the field.

So, the use of resistant rice varieties MR240, MR243, MR244, MR245, MR246, MR248 and MR249 for the disease control has a potential significance and this should be further evaluated in different environment and cultural systems throughout Malaysia (including Sabah and Sarawak). This study showed promising results, no extra cost was involved, and the use of resistant varieties is not hazardous to the growers compared to chemical control. The obtained results also showed that the currently widely planted variety MR211 is suitable for planting.

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POLISH SUMMARY

BRĄZOWA ZGNILIZNA POCHEW LIŚCIOWYCH RYŻU WYWOŁYWANA PRZEZ *PSEUDOMONAS FUSCOVAGINAE* NA PÓŁWYSPIE MALAJSKIM

Brązowa zgnilizna pochew liściowych ryżu wywoływana przez *Pseudomonas fuscovaginae* może być uznana w porównaniu do innych, powszechnie występujących i dobrze znanych chorób ryżu za nową, ale rozprzestrzeniającą się i ważną na całym świecie. Podjęte badania miały na celu monitorowanie i zbadanie jej występowania, i znaczenia na Malajach. W celu określenia występowania i nasilenia choroby wykonano seryjny monitoring oraz przeprowadzono pobieranie prób. Wykonano analizy labolatoryjne zebranych roślin w celu określenia sprawcy choroby. Przeprowadzono testowanie wybranych odmian ryżu pod kątem ich odporności na patogena celem ograniczenia jego występowania. Wykazano, że choroba nabiera znaczenia i często występuje we wszystkich rejonach uprawy ryżu na półwyspie Malajskim. W polu zakażone rośliny żółkną, dolna pochwa liściowa staje się jasno- i ciemnobrązowa, a ziarno wytworzone przez chorą roślinę jest przebarwione, zniekształcone i puste. Najczęstsze występowanie choroby zanotowano w stanie Pahang (62%) i Selangor (62%), podczas gdy najsilniejsze porażenie stwierdzono w Pahang (55%0 i Terengganu (61%). Ocena odporności odmian wykazała, że patogen porażał naturalnie wszystkie odmiany ryżu, w zróżnicowanym stopniu. Kilka odmian zaklasyfikowano jako średnio odporne, t.j.: MR240, MR243, MR244, MR245, MR246, MR248 i MR 249, mogło być polecane do sadzenia w następnym sezonie wegetacyjnym.