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SHORT COMMUNICATION

Qualitative Resistance of Sarawak Rice Landraces Against *Pyricularia oryzae*

KIM YEN LAI[1], NOR AIN HUSSIN[1], NUR KARIMAH MOHAMAD[1], HUI YIN TEN[1], LEE SAN LAI[2], FREDDY KUOK SAN YEO*[1]

[1]Faculty of Resource Science and Technology, Universiti Malaysia Sarawak, 94300 Kota Samarahan, Sarawak, Malaysia; [2] Agriculture Research Centre, Semongok, 12th Mile, Kuching-Serian Road, P.O.Box 977, 93720 Kuching, Sarawak, Malaysia
*Corresponding author: yksfreddy@unimas.my
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ABSTRACT

Malaysia rice production is threatened by rice blast disease, caused by *Pyricularia oryzae*. Yield can be greatly reduced by this disease as it can attack all the aerial parts of rice including leaves, node, neck, and collar. The use of resistant cultivar, which can be produced from resistance breeding, can control the disease effectively. Sarawak, in Malaysian Borneo, has diverse rice landraces, which can be genetic resources for resistance breeding. Study on the resistance of Sarawak rice landraces against *P. oryzae*, is still limited. In this study, diseased leaf samples were collected from rice fields in Serian division, Sarawak. One isolate was successfully obtained and designated as B2PG. The morphological characteristics were documented. Six Sarawak rice landraces were challenged with isolate B2PG. Four of the rice landraces were resistant and might carry resistance gene(s), which can be utilised in future breeding program.

Keywords: Rice blast, Resistance, Sarawak rice

Rice (*Oryza sativa*) is the third most important crop in Malaysia after oil palm and rubber with areas of cultivation accumulated up to 698,702 ha and acts as the main staple food in Malaysia (Harun, 2015). However, rice production in Malaysia is threatened by one of the most destructive diseases, rice blast. Yield reduction due to rice blast can reach up to 70% (Gieanessi, 2014). This disease is caused by an ascomycete fungus named *Pyricularia oryzae* (teleomorph: *Maganoporthe oryzae*), which can strike any aerial parts of rice plant including leaves, node, neck, and collar, at all growth stages. The symptom of blast infection is typically observed on leaves and collar with elliptical shape of lesion with brownish or necrotic border and whitish grey area in centre. *P. oryzae* can evolve or adapt to dynamic environment, which resulted in high pathogenic variations (McDonald & Linde, 2002). The common way to control this disease is by using fungicides. The application of fungicides, however, only treats the disease temporarily by inhibiting the growth of fungi. Besides, the use of chemical in large-scale farm is neither practical nor environmentally friendly (Chaudhary *et al.*, 2005). Planting resistant variety is a recommended strategy to control the disease.

Some local rice varieties and landraces in Malaysia might carry blast resistance or defence genes in their genome. Those unknown resistances or defence genes can be utilized in rice resistance breeding. One of the resistant local varieties is Pongsu Seribu 2 originated from Peninsular Malaysia, which has been reported as the most resistant variety against 22 blast pathotypes and was used extensively in resistant breeding in Peninsular Malaysia (Hasan *et al.*, 2015). Sarawak, in Malaysian Borneo, has diverse rice landraces (Yeo *et al.*, 2018), which also can be exploited for rice resistance breeding. The resistance characterisation of Sarawak rice landraces against *P. oryzae* is however, lacking. This study reports the preliminary finding of the effort to characterise the resistance.

Diseased leaf samples were collected from lowland rice fields in Serian division, Sarawak. The leaf samples were cut into small fragments including lesion borders. The leaf segments were surface sterilised with 1% sodium hypochlorite for one minute and rinsed with sterilised distilled water for three times. The leaf segments from the same lesion were placed on the inner lid of water agar plate and incubated in a humid translucent box under room temperature. Single spore colonies were transferred onto oatmeal agar (OMA) and was incubated in dark condition for the first five days and placed in light condition for the subsequent days at room temperature. Spore and internal transcribed spacer (ITS) sequence (unpublished data) were used for fungal identification. One *P. oryzae* isolate was obtained from Kampung Paon Gahat (N0°56'41.5", E110°39'16.3"). The isolate was designated as B2PG.

For morphological characterisation of B2PG, 10 potato dextrose agar (PDA) and 10 filtered OMA plates were prepared. Perpendicular lines were drawn on the reverse side of each Petri dish with 9 cm diameter. A 5 mm fungal plug of *P. oryzae* isolate B2PG was placed at the centre of each Petri dish and was incubated in dark condition for the first five days and placed in light condition for the subsequence days at room temperature. Colony diameter was measured at day 10 after incubation. It was measured from the centre of each Petri dish to the mycelium edge. Both macroscopic and microscopic characteristics were recorded.

The macromorphology of *P. oryzae* isolate B2PG showed no clear difference when cultured on different media. The colony of B2PG on OMA had light to dark grey aerial mycelium with entire margins reaching up to 6.6 cm diameter in average after 10 days of incubation, with smooth colonies but few plates showed rough surface formed by sparse white aerial mycelia (Figure 1a); reverse plate had dark grey colour with pigmentation (Figure 1b). On PDA, colony was whitish to grey colour with entire margin, reaching up to 6.9 cm diameter in average after 10 days, mostly smooth colonies but few plates had spiral form of white aerial mycelia (Figure 1c); reverse was olivaceous with pigmentation (Figure 1d). The conidia were pyriform shaped, 2- or 3- septa with rounded base, narrowed towards the tip, which was pointed or blunt, similar to the conidia description of rice blast by Hossain *et al.* (2004) and Vanaraj *et al.* (2013).

For resistance test, six lowland rice landraces i.e. Padi Bubuk collected from Sri Aman; Padi Semanggang, Padi Entangor collected from Serian; Padi Perintah collected from Samarahan, Padi Kuching and Padi Rendah collected from Kuching; were used. The name of each landrace is according to the name given by farmers. For each landrace, 20 germinated seeds were planted in a pot (20 replicates). Seedlings at 3-leaf stage were used for the test, which was performed twice. Spore suspension (2.5×10^5 spores mL^{-1}) of *P. oryzae* isolate B2PG was prepared and homogeneously sprayed onto seedlings until runoff (5 ml per pot). Inoculated pots were first incubated in an inoculation box under dark and high humidity condition overnight. Later, the seedlings were taken out and examined on day 7 after inoculation. A key modified from Yu *et al.* (1987) was used to score the resistance of seedlings.

The two repetitions of resistance test were consistent and are summarized in Table 1. There were four landraces found to be resistant to isolate B2PG, *viz*. Padi Semanggang, Padi Perintah, Padi Rendah and Padi Bubuk. Immune plants without any symptoms (type 0) and plants with brown specks (lesions type 1 & 2) were observed on these four landraces. Two landraces were scored as having mild resistance (with few susceptible seedlings) towards isolate B2PG, *viz*. Padi Entangor and Padi Hitam. The lesions of these two landraces ranged from type 3 to 4.

The resistant landraces may have qualitative resistance conferred by resistant gene(s) (R-gene), which results in either immune or showing hypersensitive response that inhibits the growth of pathogen (Scheuermann *et al.*, 2012). These resistant landraces may not carry the same R gene(s) even though they were found resistant towards the same isolate (Yu *et al.*, 1987).

Padi Hitam and Padi Entangor that were categorized as having mild resistance may have weak qualitative resistance (weak form of R gene) or maybe quantitative resistance (Poland *et al.*, 2009). Further resistance characterisation of these two landraces is needed.

To summarise, the macro- and micromorphology of *P. oryzae* isolate B2PG was described on different media. No clear difference

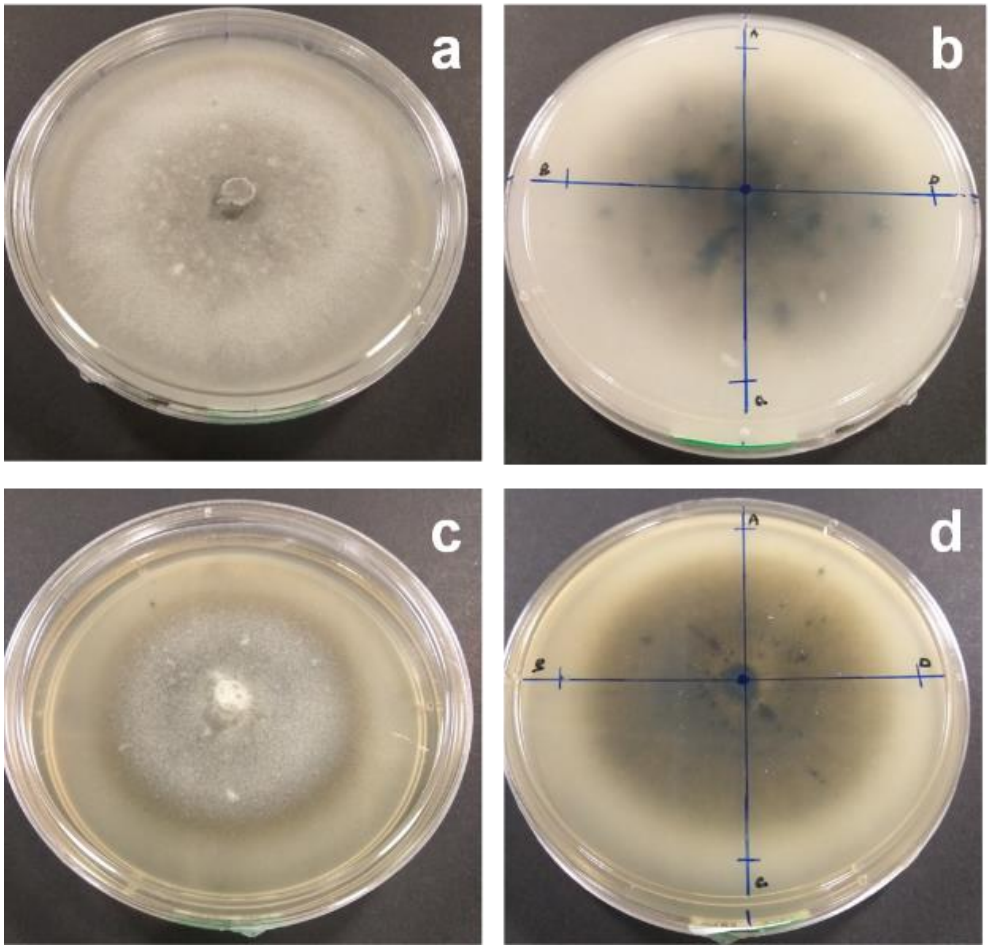

Figure 1: Colony morphology of *P. oryzae* isolate B2PG on OMA: (a) OMA front plate; (b) OMA reverse plate; on PDA: (c) PDA front plate; (d) PDA reverse plate.

Table 1: Resistance of six Sarawak rice landraces against *P. oryzae* isolate B2PG based on scoring key modified from Yu *et al.* (1987).

Variety	B2PG Isolate							
	Number of plants with lesion type[!]						Range	Final score
	0	1	2	3	4	5		
Padi Bubuk	11	7	2				0-2	Resistant
Padi Semanggang	12	8					0-1	Resistant
Padi Entangor				16	4		3-4	Mild resistant to susceptible
Padi Perintah	10	10					0-1	Resistant
Padi Hitam				12	8		3-4	Mild resistant to susceptible
Padi Rendah	12	8					0-1	Resistant

0-2: Resistant, 3: Mild Resistant, and 4-5: Susceptible

[!] Twenty seedlings (3-leaves stage) were scored for each rice landrace.

was observed. Out of six Sarawak lowland rice landraces challenged with isolate B2PG, Padi Semanggang, Padi Perintah, Padi Bubuk and Padi Rendah were found to be resistant, whereas Padi Hitam and Padi Entangor were having mild resistance.

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