



Pathotype Identification of Rice Blast Pathogen, *Pyricularia oryzae* Using Differential Varieties in Peninsular Malaysia

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Highlights

- Seven pathotypes (P0.0, P0.2, P1.0, P2.0, P3.0, P7.0 and P9.0) of *Pyricularia oryzae* were differentiated from blast disease using eight Malaysian differential rice varieties.
- The common pathotype is still pathotype P7.0, due to MR219 rice variety is planted in many rice granary areas
- New emergence of pathotype P0.2 was identified which was isolated from a new released rice variety, MR284.

SHORT COMMUNICATION

Pathotype Identification of Rice Blast Pathogen, *Pyricularia oryzae* Using Differential Varieties in Peninsular Malaysia

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Abstrak: Tujuh patotip *Pyricularia oryzae* telah dibezakan daripada sampel-sampel penyakit karah yang diperolehi dari 2014–2016 menggunakan lapan varieti padi pembeza Malaysia. Patotip P7.0 adalah patotip dominan yang dikenal pasti (33.9%) diikuti dengan P0.0, P1.0 dan P9.0 di mana ketika ini patotip-patotip ini didapati meningkat di lapangan dengan frekuensi 20.0% bagi P.00, dan 15.4% bagi kedua-dua P1.0 dan P9. Patotip P7.0 kebanyakannya dipencil daripada MR220CL2, MR263 dan MR219 yang merupakan varieti popular ditanam oleh petani di Semenanjung Malaysia. Menariknya, kemunculan patotip baru, P0.2 telah dikenalpasti dan dipencilkan daripada varieti baru yang diperkenalkan, MR284 dan titisan isogenik IRBL 20 yang membawa gen rintang karah *Pi5 (t)*. Tempoh penanaman melebihi 20 musim dan penanaman MR219 dan MR220 dalam skala besar dengan kawasan liputan lebih daripada 90% di Semenanjung Malaysia berkemungkinan menyebabkan patotip P7.0 menjadi dominan. Dengan peningkatan kawasan liputan penanaman varieti MR220CL2 dan MR263 menghampiri 50% ketika ini disyaki menyebabkan perubahan pada kedominan patotip patogen karah kepada P0.0, P1.0 dan P9.0.

Kata kunci: *Pyricularia oryzae*, karah padi, patotip, varieti pembeza

Abstract: Seven pathotypes of *Pyricularia oryzae* were differentiated from blast disease samples collected from 2014–2016, using eight Malaysian differential rice varieties. Pathotype P7.0 is the dominant pathotype identified (33.9%) followed by P0.0, P1.0 and P9.0 which are currently become more abundant in the field with frequency of 20.0% for P0.0, and 15.4% for both P1.0 and P9. Pathotype P7.0 was mostly isolated from MR220CL2, MR263 and MR219 rice varieties which are popular variety planted by farmers in Peninsular Malaysia. Interestingly, new emergence of pathotype P0.2 has been identified, which was isolated from a new released variety, MR284 as well as blast isogenic line IRBL 20 carrying

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Pi5(t) blast resistance gene. Prolong planting of more than 20 planting seasons and large scale planting of MR219 and MR220 with current varietal coverage areas of more than 90% in Peninsular Malaysia are suspected as possible reason for P7.0 become dominant. Varietal coverage of MR220CL2 and MR263 has reached about 50% might be the cause of changes in blast pathogen pathotype dominancy to P0.0, P1.0 and P9.0.

Keywords: *Pyricularia oryzae*, rice blast, pathotype, differential varieties

Pyricularia oryzae is a pathogen of rice blast disease worldwide and causing yield loss in epidemically favourable rice planting areas. In Malaysia, conventional screening of blast resistance in breeding lines is considered as one of the important strategies for development of new rice varieties. Although a number of varieties with resistance to blast have been released in Malaysia, the degree of the blast resistance has breakdown from year to year. The reason of this scenario is lack of information on pathotype composition and pathogen populations at the areas which the varieties are planted. Thus, the objective of this study was to identify current pathotypes of rice blast isolates from samples collected in 2014–2016.

Diseased samples were collected from various infected rice varieties, rice lines used for research purposes and weedy rice from Malaysian Agricultural Research and Development Institute (MARDI) trial plots and farmers' field in rice granary areas in Peninsular Malaysia, in the states of Kedah, Penang, Perlis, Kelantan, Terengganu and Selangor. The infected rice varieties sampled included MR219, MR220, MR220CL1, MR220CL2, MR263 and MR284 which were resistant towards rice blast during its release but have become susceptible after a few cropping seasons (Zainudin *et al.* 2012; Zainal Abidin 2015). Diseased leaves, panicle nodes, collars and panicles were separately bagged and brought to the laboratory and subjected to monoconidial isolation as described by Hayashi *et al.* (2009). Pure cultures of rice blast isolates were then sub-cultured into slant Potato Dextrose Agar (PDA) as stock cultures for inoculum preparation. From the sampling, 65 isolates were obtained, and these isolates were used for pathotype identification. List of *P. oryzae* isolates used for pathotype identification using Malaysian Differential Varieties (DVs) is shown in Table 1.

Malaysian DVs used to identify the pathotypes were Mahsuri, Setanjung (MR1), Bahagia, Engkatek, Seribu Gantang, Tadukan, Pankhari 203 and Pongsu Seribu 2 (Table 2). The Malaysian DVs consisted of two sets which are the susceptible range, Mahsuri, Setanjung (MR1), Bahagia and Engkatek; and the resistant range, Seribu Gantang, Tadukan, Pankhari 203 and Pongsu Seribu 2. Inoculation of blast isolates on the DVs was based on the methods by Hayashi *et al.* (2009). Disease reaction was assessed on the 7 days after inoculation using a 0–9 scale as described by IRRI Standard Evaluation System (SES) for rice (Anon 2013).

Table 1: List of *P. oryzae* isolates used for pathotype identification using Malaysian Differential Varieties

No	Isolate	Variety	Plant parts	Location	State
1	B8-3.1	MR278	Node	MARDI Serdang	Selangor
2	B8-5.2	MR278	Node	MARDI Serdang	Selangor
3	B9-1.3	MR220CL2	Node	Kg Sawah Sempadan, Tg Karang	Selangor
4	B10-1.3	MR263	Node	Sekinchan	Selangor
5	B10-2.3	MR263	Node	Sekinchan	Selangor
6	B10-3.1	Unknown	Node	Sekinchan	Selangor
7	D1-5.3	MR219	Panicle	Ladang Merdeka Senior, Pasir Mas	Kelantan
8	D1-10.2	MR219	Panicle	Ladang Merdeka Senior, Pasir Mas	Kelantan
9	D1-11.1	MR219	Panicle	Ladang Merdeka Senior, Pasir Mas	Kelantan
10	D2-1.1	MR304	Node	Ladang Merdeka Mulong	Kelantan
11	D2-2.3	MR304	Node	Ladang Merdeka Mulong	Kelantan
12	D2-3.5	MR308	Node	Ladang Merdeka Mulong	Kelantan
13	T1-9.1	MR284	Node	Kg Gong Guchil, Jerteh	Terengganu
14	K1-3.2	MR220CL2	Node	Kg Matang Kerengga, Kerpan	Kedah
15	K2-5.1	MR220CL2	Collar	Kg Pida 4, Kodiang	Kedah
16	K3-1	MR220CL2	Panicle	Kg Pida 4 Lama, Kodiang	Kedah
17	K5-2.1	MR220CL2	Node	Kg Bukit Hantu, Kodiang	Kedah
18	K5-5.3	Weedy rice	Node	Kg Bukit Hantu, Kodiang	Kedah
19	K5-5.4	Weedy rice	Node	Kg Bukit Hantu, Kodiang	Kedah
20	K5-7.1	MR220CL2	Node	Kg Bukit Hantu, Kodiang	Kedah
21	K7-1.1	MR220CL2	Collar	Kg Tok Mengkula, Ayer Hitam	Kedah
22	K7-2.3	MR220CL2	Node	Kg Tok Mengkula, Ayer Hitam	Kedah
23	K8-1.2	MR263	Node	Alor Serdang, Pendang	Kedah
24	K8-2.1	MR263	Node	Alor Serdang, Pendang	Kedah
25	K8-3.1	MR263	Rachis	Alor Serdang, Pendang	Kedah
26	K11-1.1	MR284	Panicle	Bt 18, Sg Baru, Guar Chempedak	Kedah
27	K11-3.2	MR284	Node	Bt 18, Sg Baru, Guar Chempedak	Kedah
28	K11-4.1	MR284	Panicle	Bt 18, Sg Baru, Guar Chempedak	Kedah
29	K11-8.1	MR263	Node	Bt 18, Sg Baru, Guar Chempedak	Kedah
30	K15-1.1	MRQ89	Node	Simpur, Tikam Batu	Kedah
31	K15-2.1	MRQ89	Node	Simpur, Tikam Batu	Kedah
32	K15-7.1	MRQ74	Node	Simpur, Tikam Batu	Kedah

(Continue on next page)

Table 1 (Continued)

No	Isolate	Variety	Plant parts	Location	State
33	P1-4.1	MR263	Node	Blok 4, Mardi Seberang Perai	Pulau Pinang
34	P1-26.1	IRBL20	Node	Blast Screening Nursery	Pulau Pinang
35	P1-27.3	IRBL20	Node	Blast Screening Nursery	Pulau Pinang
36	P1-28.1	MR263	Leaf	Blast Screening Nursery	Pulau Pinang
37	P1-29.2	MR263	Leaf	Blast Screening Nursery	Pulau Pinang
38	P1-30.3	MR263	Leaf	Blast Screening Nursery	Pulau Pinang
39	P1-31.4	MR263	Leaf	Blast Screening Nursery	Pulau Pinang
40	P1-33.1	MR220CL1	Node	Blok 2, Mardi Seberang Perai	Pulau Pinang
41	P6-3.1	MR220	Node	Kg Setol, Pinang Tunggal, Kepala Batas	Pulau Pinang
42	P6-4.1	Weedy Rice	Panicle	Kg Setol, Pinang Tunggal, Kepala Batas	Pulau Pinang
43	P6-5.1	MR219	Node	Kg Setol, Pinang Tunggal, Kepala Batas	Pulau Pinang
44	P7-1.1	MR219	Node	Kg Padang Merdeka, Pinang Tunggal	Pulau Pinang
45	P7-1.1	MR219	Collar	Kg Padang Merdeka, Pinang Tunggal	Pulau Pinang
46	P7-2.1	MR219	Panicle	Kg Padang Merdeka, Pinang Tunggal	Pulau Pinang
47	P8-1.1	MR269	Node	Bumbung Lima, Kepala Batas	Pulau Pinang
48	P8-2.1	MR269	Node	Bumbung Lima, Kepala Batas	Pulau Pinang
49	P8-3.1	MR269	Panicle	Bumbung Lima, Kepala Batas	Pulau Pinang
50	P9-1.3	MR220CL2	Panicle	Permatang Tok Loba, Penaga	Pulau Pinang
51	P11-4.2	MR269	Node	Paya Keladi Hujung, Kepala Batas	Pulau Pinang
52	P11-5.3	MR269	Node	Paya Keladi Hujung, Kepala Batas	Pulau Pinang
53	P11-6.1	MR269	Node	Paya Keladi Hujung, Kepala Batas	Pulau Pinang
54	P12-1.2	MR284	Node	Kg Paya Keladi, Kepala Batas	Pulau Pinang
55	P13-1.3	MR220CL2	Node	Permatang Tinggi B, Kepala Batas	Pulau Pinang
56	P13-2.2	MR220CL2	Node	Permatang Tinggi B, Kepala Batas	Pulau Pinang
57	P13-3.2	MR220CL2	Node	Permatang Tinggi B, Kepala Batas	Pulau Pinang
58	P14-3.1	MR220CL2	Node	Kg Tok Bedu, Tasek Gelugor	Pulau Pinang
59	P14-4.2	MR220CL2	Node	Kg Tok Bedu, Tasek Gelugor	Pulau Pinang
60	P18-1.1	MR263	Node	Bumbung Lima, Kepala Batas	Pulau Pinang
61	P18-2.1	MR263	Node	Bumbung Lima, Kepala Batas	Pulau Pinang
62	P18-4.1	MR263	Node	Bumbung Lima, Kepala Batas	Pulau Pinang
63	R1-4.2	MR220CL2	Node	Kg Behor Lalang, Kangar	Perlis
64	R1-5.2	MR220CL2	Node	Kg Behor Lalang, Kangar	Perlis
65	R1-6.1	MR220CL2	Node	Kg Behor Lalang, Kangar	Perlis

Table 2: Malaysian Differential Varieties (DVs) used to identify pathotypes of *P. oryzae* isolates

Differential Varieties	Mahsuri	Setanjung	Bahagia	Engkatek	Seribu Gantang	Tadukan	Pankhari 203	Pongsu Seribu 2
Origin	Malaysia	Malaysia	Malaysia	Malaysia	Malaysia	Philippines	India	Malaysia
Numeric code value for resistant	0	0	0	0	0	0	0	0
Numeric code value for susceptible	1	2	4	8	1	2	4	8
Range	←—————				—————→			
	Most susceptible				Susceptible			
					Resistant			
					Most resistant			

The *P. oryzae* isolates were differentiated as pathotypes based on the disease reaction in the eight Malaysian DVs and identified according to a modified octal notation by Limpert *et al.* (1994). The designation of a pathotype is the summation of numeric code value from each DVs in the same set which the isolates produces compatible reaction.

Seven pathotypes were differentiated using the eight Malaysian DVs namely P0.0, P0.2, P1.0, P2.0, P3.0, P7.0 and P9.0 (Table 3). Pathotype P7.0 is a dominant pathotype and was mostly isolated from three popular varieties MR219, MR220CL2, and MR263 planted by farmers in Peninsular Malaysia since their released until now. Pathotype P7.0 was also isolated from MR284, MR220, MR220CL1, MR269 and other rice varieties which are used as research purposes but was not released to the farmers'. Pathotype P0.0 is currently become more abundant in the rice field with the frequency of 20.0% followed by P1.0 and P9.0 with the frequency of both pathotypes was 15.4% (Table 4).

Based on the current pathotypes identified, the common pathotype is still P7.0. Samples collected during blast outbreaks in 2004–2006 indicated that P0.0 and P7.0 were the most common pathotypes with frequency of 26.67% and 25.86%, respectively. On the other hand, during 2010–2012 the frequency of P7.0 was at 43.37%, an increment from 2004–2006. Other pathotypes with increased frequency during 2010–2012 were P15.0 (12.43%) and P9.0 (6.35%) (Personal communication). The occurrence of pathotype P7.0 might be due to MR219 is planted in many rice granary areas as this variety remains a popular variety among the farmers' in Peninsular Malaysia.

During the released of MR219 in 2001, the variety was resistant to blast disease and moderately resistant to brown plant hopper. The demand of this variety was high due to its high yield potential up to 10 t/ha (Alias *et al.* 2002; Alias 2002). The performance of MR219 decreased not only because of blast disease outbreak but also causes by weedy rice infestation in the rice granary areas. Therefore, MR220CL2 was released in 2010 which is resistant to blast as well as tolerant to imidazolinone herbicides and weedy rice infestation. The donor parent of MR220CL2 was MR220 which had the same genetic background of MR219 (Azmi *et al.* 2012).

Table 3: Pathotypes of *P. oryzae* identified using Malaysian DVs

No	Isolate	MAH	MR1	BAH	ENG	SERIBU GANTANG	TAD	PAN 203	PONGSU SERIBU 2	MR 211	MR 84	Pathotype
1	B8-3.1	R	R	R	R	R	R	R	R	R	R	0.0
2	B8-5.2	R	R	R	R	R	R	R	R	R	R	0.0
3	B9-1.3	S	R	R	S	R	R	R	R	S	R	9.0
4	B10-1.3	R	R	R	R	R	R	R	R	R	R	0.0
5	B10-2.3	S	S	R	R	R	R	R	R	S	R	3.0
6	B10-3.1	S	R	R	R	R	R	R	R	R	R	1.0
7	D1-5.3	S	S	S	R	R	R	R	R	S	R	7.0
8	D1-10.2	S	S	S	R	R	R	R	R	S	R	7.0
9	D1-11.1	S	S	S	R	R	R	R	R	S	R	7.0
10	D2-1.1	S	R	R	R	R	R	R	R	R	R	1.0
11	D2-2.3	S	S	R	R	R	R	R	R	S	R	3.0
12	D2-3.5	S	S	S	R	R	R	R	R	S	R	7.0
13	T1-9.1	R	R	R	R	R	R	R	R	R	S	0.0
14	K1-3.2	R	R	R	R	R	S	R	R	R	S	0.2
15	K2-5.1	R	R	R	R	R	R	R	R	R	S	0.0
16	K3-1.1	R	R	R	R	R	S	R	R	R	S	0.2
17	K5-2.1	R	R	R	R	R	R	R	R	R	R	0.0
18	K5-5.3	S	S	S	R	R	R	R	R	R	R	7.0
19	K5-5.4	S	S	S	R	R	R	R	R	S	R	7.0
20	K5-7.1	R	R	R	R	R	R	R	R	R	R	0.0
21	K7-1.1	S	S	S	R	R	R	R	R	S	R	7.0
22	K7-2.3	S	S	S	R	R	R	R	R	S	R	7.0
23	K8-1.2	S	S	S	R	R	R	R	R	S	R	7.0
24	K8-2.1	S	S	S	R	R	R	R	R	S	R	7.0
25	K8-3.1	S	S	S	R	R	R	R	R	S	R	7.0
26	K11-1.1	R	R	R	R	R	R	R	R	R	R	0.0
27	K11-3.2	S	S	S	R	R	R	R	R	S	R	7.0
28	K11-4.1	S	S	S	R	R	R	R	R	S	R	7.0
29	K11-8.1	S	R	R	R	R	R	R	R	R	R	1.0
30	K15-1.1	S	R	R	R	R	R	R	R	R	R	1.0
31	K15-2.1	S	S	S	R	R	R	R	R	S	R	7.0
32	K15-7.1	S	R	R	S	R	R	R	R	S	R	9.0
33	P1-4.1	S	R	R	R	R	R	R	R	S	R	1.0
34	P1-26.1	R	R	R	R	R	S	R	R	R	R	0.2

(Continue on next page)

Table 3 (Continued)

No	Isolate	MAH	MR1	BAH	ENG	SERIBU GANTANG	TAD	PAN 203	PONGSU SERIBU 2	MR 211	MR 84	Pathotype
35	P1-27.3	R	R	R	R	R	S	R	R	R	R	0.2
36	P1-28.1	S	R	R	R	R	R	R	R	R	R	1.0
37	P1-29.2	S	R	R	S	R	R	R	R	S	R	9.0
38	P1-30.3	S	R	R	S	R	R	R	R	S	R	9.0
39	P1-31.4	S	R	R	R	R	R	R	R	S	R	1.0
40	P1-33.1	S	S	S	R	R	R	R	R	S	R	7.0
41	P6-3.1	S	S	S	R	R	R	R	R	S	R	7.0
42	P6-4.1	R	R	R	R	R	R	R	R	R	R	0.0
43	P6-5.1	S	S	S	R	R	R	R	R	S	R	7.0
44	P7-1.1	S	S	S	R	R	R	R	R	S	R	7.0
45	P7-1.1	S	S	S	R	R	R	R	R	S	R	7.0
46	P7-2.1	S	S	S	R	R	R	R	R	S	R	7.0
47	P8-1.1	R	S	R	R	R	R	R	R	R	R	2.0
48	P8-2.1	S	S	S	R	R	R	R	R	S	R	7.0
49	P8-3.1	S	S	R	R	R	R	R	R	S	R	3.0
50	P9-1.3	S	R	R	R	R	R	R	R	S	R	1.0
51	P11-4.2	S	R	R	S	R	R	R	R	S	R	9.0
52	P11-5.3	S	R	R	S	R	R	R	R	S	R	9.0
53	P11-6.1	S	R	R	S	R	R	R	R	S	R	9.0
54	P12-1.2	R	R	R	R	R	S	R	R	R	R	0.2
55	P13-1.3	R	R	R	R	R	R	R	R	R	S	0.0
56	P13-2.2	R	R	R	R	R	R	R	R	S	R	0.0
57	P13-3.2	R	R	R	R	R	R	R	R	R	S	0.0
58	P14-3.1	S	R	R	R	R	R	R	R	S	R	1.0
59	P14-4.2	S	R	R	S	R	R	R	R	S	R	9.0
60	P18-1.1	S	R	R	R	R	R	R	R	S	R	1.0
61	P18-2.1	S	R	R	S	R	R	R	R	S	R	9.0
62	P18-4.1	S	R	R	S	R	R	R	R	S	R	9.0
63	R1-4.2	S	S	S	R	R	R	R	R	S	R	7.0
64	R1-5.2	S	S	S	R	R	R	R	R	S	R	7.0
65	R1-6.1	R	R	R	R	R	R	R	R	R	S	0.0

Note: MAH – Mahsuri, BAH – Bahagia, ENG – Engkatek, TAD – Tadukan, PAN 203 - Pankhari 203, R – resistance, S – susceptible

Table 4: Frequency of pathotypes differentiated using eight Malaysian Differential Varieties

Rice Variety	Pathotype							Total
	P0.0	P0.2	P1.0	P2.0	P3.0	P7.0	P9.0	
MR219	1	0	0	0	0	6	0	7
MR220	0	0	0	0	0	1	0	1
MR220CL1	0	0	0	0	0	1	0	1
MR220CL2	6	3	2	0	0	4	2	17
MR263	1	0	5	0	1	3	4	14
MR269	0	0	0	1	1	1	3	6
MR284	2	1	0	0	0	2	0	5
MRQ74	0	0	0	0	0	0	1	1
Other varieties	2	2	3	0	1	2	0	10
Weedy rice	1	0	0	0	0	2	0	3
No. of isolates	13	6	10	1	3	22	10	65
Frequency (%)	20.0	9.2	15.4	1.5	4.6	33.9	15.4	100.0

Interestingly, new emergence of pathotype P0.2 was identified which was isolated from a new released variety, MR284 as well as from blast isogenic line IRBL 20. Rice variety MR284 is a variety released by MARDI in 2015 but recently affected by bacteria leaf blight and blast outbreak. Meanwhile, IRBL 20 is one of international rice blast isogenic lines (IRBL) developed by IRRI which has *Pi5(t)* blast resistance gene (Telebanco-Yanoria *et al.* 2010).

MARDI released MR263 in 2010 and MR269 in 2012 as an alternative to increase production and food security requirement besides to overcome the problem of disease infestation. Rice variety MR263 is suitable to be planted in moderate fertile area with comparable yield performance as MR219 and was moderately resistant to leaf blast disease (Zainudin *et al.* 2012). Furthermore, MR269 is resistant to leaf blast and moderately resistant to panicle blast.

In addition to MR219, two other varieties MR220CL2 and MR263 are also widely planted by farmers and the most common pathotype P7.0 was also isolated. Pathotypes P0.0, P1.0 and P9.0 were also frequently isolated from varieties MR220CL2 and MR263. These pathotypes were already existed during samples collection in 2004–2006 as well as in 2010–2012 although the pathotypes were mostly isolated from MR219 (Personal communication). Being a major rice variety planted in a large area for a long period caused MR219 and MR220CL2 more vulnerable to rapid adaptation of high variability of the pathotypes population in the field. The abundance of dominant pathotypes population which has the ability to adapt and develop new virulent pathotypes might turned these varieties to became susceptible to blast disease after a few years it was released.

Hence, this is an indication of breakdown in resistance of MR219 and most likely the breakdown of MR220CL2 and MR263 considering its cultivation has dominating the rice planted areas. Planting a monovariety for a long period of time in a large area will caused pest and disease outbreak. Therefore, if the resistant varieties is used as a strategy to control disease, it is suggested that the varietal coverage in a certain area is not more than 50% (Ali *et al.* 1995).

Due to the high variability of the pathotypes, continuous research on development of durable resistant variety is needed. Durable resistance is influenced by epidemiological factors as well as interaction between host and pathogen. It is therefore necessary to monitor the population of blast pathogen by classifying the blast pathotypes using differential varieties. Thus, pathotype identification is still important for characterisation of *P. oryzae* population, thus the results from this study provided useful information on rice blast disease management using resistance variety as the pathotype identified can give information on the changes of dominant pathotypes as well as the emergence of new pathotypes over the years.

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