Virulence Pattern of *Pyricularia oryzae* Pathotypes Towards Blast Monogenic Lines

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Highlights

- Virulence patterns of six pathotypes of *P. oryzae* (P0.0, P0.2, P1.0, P3.0, P7.0 and P9.0) in Peninsula Malaysia were evaluated using a set of 22 IRRI-bred blast resistance lines (IRBL) to determine the response of the resistance genes against specific lines.

- Based on disease severity patterns, the six pathotypes were avirulence towards seven IRBLs [IRBLi-F5, IRBLk-Ka, IRBLkh-K3, IRBLz-Fu, IRBLsh-S, IRBLPi7 (t) and IRBL9-W] of which these IRBLs harbouring *Pii*, *Pik*, *Pik-h*, *Piz*, *Pish*, *Pi7(t)* and *Pi9* resistance genes, respectively.

- To combat rice blast against current high frequencies of pathotypes P7.0, P0.0, P9.0 and P1.0, suitable resistance genes or donor to be incorporated for developing future rice blast resistant variety are *Pii*, *Pik*, *Pik-h*, *Piz*, *Pish*, *Pi7(t)* and *Pi9*. 
Virulence Pattern of *Pyricularia oryzae* Pathotypes Towards Blast Monogenic Lines

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Abstract: Rice blast caused by *Pyricularia oryzae* (*P. oryzae*) is one of the most serious diseases infecting rice worldwide. In the present study, virulence pattern of six *P. oryzae* pathotypes (P0.0, P0.2, P1.0, P3.0, P7.0 and P9.0) identified from the blast pathogen collected in Peninsular Malaysia, were evaluated using a set of 22 IRRI-bred blast resistance lines (IRBL) as well as to determine the resistance genes involved. The information on the virulence of the blast pathotypes and the resistance genes involved is important for breeding of new rice variety for durable resistance against blast disease. The IRBL was established from 22 monogenic lines, harbouring 22 resistance genes [Pia, Pib, Pii, Pit, Pi3, Pi5(t), Pish, Pi1, Pik, Pik-s, Pik-m, Pik-h, Pik-p, Pi7(t), Pi9, Piz, Piz-5, Piz-t, Pi19, Pi20(t), Pita-2, and Pita=Pi4(t)]. Based on the disease severity patterns, the tested pathotypes were avirulence towards seven IRBLs [IRBLi-F5, IRBLk-Ka, IRBLk-K3, IRBLz-Fu, IRBLsh-S, IRBLp17 (t) and IRBL9-W] of which these IRBLs harbouring *Pii*, *Pik*, *Pik-h*, *Piz*, *Pish*, *Pi7(t)* and *Pi9* resistance genes, respectively. Therefore, the results suggested that the seven IRBLs carrying seven resistance genes [Pii, Pik, Pik-h, Piz, Pish, Pi7(t) and Pi9] would be suitable candidates of resistance genes to be incorporated in new breeding lines to combat the current blast pathotypes in the field.

Keywords: *Pyricularia oryzae*, Rice Blast, Pathotypes, Virulence Patterns

Abstrak: Karah padi yang disebabkan oleh *Pyricularia oryzae* merupakan salah satu penyakit paling signifikan yang memberi kesan terhadap tanaman padi di seluruh dunia. Dalam kajian ini, corak kevirulenan enam patotip, P0.0, P0.2, P1.0, P3.0, P7.0 dan P9.0 yang dikenal pasti dari pencilan *P. oryzae* yang dikumpul dari Semenanjung Malaysia, dinilai menggunakan satu set 22 galur rintangan biakan karah-IRRI (IRBL) serta untuk

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Blast disease caused by *Pyricularia oryzae* (*P. oryzae*) (synonym *Magnaporthe oryzae*) is one of the most serious diseases of rice worldwide including Malaysia. The most effective and practical method to control blast disease is the use of resistant rice varieties. However, the resistance often breaks down or is lost in a few years after the rice variety was released. This is caused by the appearance of new virulence pathotypes or races of the blast pathogen that overcome the resistance (Zhou et al. 2007) as well as due to high variability of rice blast pathogen (Wang et al. 2013; Poonsin & Parinthawong 2020). In order to develop more effective resistance against blast pathogen, it is important to have the knowledge of the host resistance and the pathogen as well as to determine the resistance genes.

The interaction between rice plant and blast pathogen is based on gene-for-gene theory by Flor (1971) of which for every resistance gene (*R* gene) in the host, there is a corresponding avirulence gene (*AVR*) in the pathogen. Thus, in blast disease pathosystem, a major resistance gene confers specific resistance to a blast pathogen pathotype that contains a specific corresponding avirulence gene. In other words, every resistance gene in the host corresponds to an avirulence gene in the pathogen that acts as an effector to trigger the defence response (Wang et al. 2008; Huang et al. 2014).

To study the resistance and avirulence genes interaction, differential system comprising rice differential varieties and blast pathogen pathotypes are often used. This system provide systematic method to characterise and to postulate the resistance genes as well as to determine the relationships between pathotypes and the resistance genes (Koide et al. 2011). The information can be used to strategise effective and lasting method to manage blast disease of rice.

Differential varieties distinguish pathotypes by their differential reactions to the blast pathogen. Current pathotype identification by using local differential varieties is useful to study the pathological diversity of *P. oryzae* population for pathotype identification and to characterise the blast isolates, but it is not sufficient to distinguish and characterise in detail blast pathotypes as the system
lack the information on the genes involved in these local differential varieties. Thus, a set of international differential varieties harbouring resistance genes is frequently used to determine blast pathotypes virulence patterns.

A set of international blast differential varieties were developed by collaborative effort between International Rice Research Institute (IRRI) and Japan International Research Center for Agricultural Sciences (JIRCAS). The international blast differential varieties consisted of 23 monogenic lines of IRRI-bred blast-resistant line (IRBL) that representing 23 resistance genes, namely Pish, Pia, Pib, Pit, Pii, Pi1, Pi3, Pi5(t), Pik, Pik-s, Pik-m, Pik-h, Pik-p, Pi7(t), Pi9, Piz, Piz-5, Piz-t, Pita-2, Pita, Pi12(t), Pi19(t) and Pi20(t) with the genetic background of a Chinese Lijiang Xintuan Heigu (LTH) rice variety from blast-susceptible Japonica variety (Tsunematsu et al. 2000; Kobayashi et al. 2007). This differential system is used for identification of pathotype virulence, virulence pattern between the blast pathotypes and predicting the resistance gene(s) in rice varieties (Telebanco-Yanoria et al. 2010).

In Peninsular Malaysia, six pathotypes P0.0, P0.2, P1.0, P3.0, P7.0 and P9.0 of P. oryzae were identified from blast disease samples collected from 2014–2016 (Siti Norsuha & Latiffah 2019). Thus, the objectives of this study were to evaluate the virulence patterns of the blast pathotypes using international differential varieties and to predict the resistance genes corresponded to the blast pathotypes.

MATERIALS AND METHODS

Blast Monogenic Lines

In this study, a set of international differential varieties consisting of 22 IRBLs carrying 22 resistance genes [Pia, Pib, Pii, Pit, Pi3, Pi5(t), Pish, Pi1, Pik, Pik-s, Pik-m, Pik-h, Pik-p, Pi7(t), Pi9, Piz, Piz-5, Piz-t, Pita, Pita-2, Pi12(t), Pi19(t) and Pita=Pi4(t) and a susceptible control, LTH (Table 1) were used to evaluate the virulence of six pathotypes identified in Peninsular Malaysia. The resistance genes present in each line was predicted based on their reaction patterns to the IRBL. Only 22 IRBL lines were used in the present study as the seeds of IRBL12-M did not germinate and thus was not included in the study.

Sowing of the seeds and inoculum preparation were synchronised. Seeds were pre-germinated and sown in a plastic tray (26 cm × 37 cm) with sieved top soil mixed with cow dung. The seedlings were fertilised with urea (150N kg/ha) 2–3 days before inoculation.
Table 1: International differential varieties consisting of 22 IRRI-bred blast resistance lines (IRBL) and target resistant genes.

| Designation of IRBLs | Target resistant gene |
|----------------------|-----------------------|
| IRBLa-A              | *Pia*                 |
| IRBLi-F5             | *Pii*                 |
| IRBLks-S             | *Pik-s*               |
| IRBLk-Ka             | *Pik*                 |
| IRBLkp-K60           | *Pik-p*               |
| IRBLkh-K3            | *Pik-h*               |
| IRBLz-Fu             | *Piz*                 |
| IRBLz5-CA            | *Piz-5*               |
| IRBLzt-T             | *Piz-t*               |
| IRBLta-K1            | *Pita=Pi4(t)*         |
| IRBLb-B              | *Pib*                 |
| IRBLt-K59            | *Pit*                 |
| IRBLsh-S             | *Pish*                |
| IRBL1-CL             | *Pi1*                 |
| IRBL3-C4             | *Pi3*                 |
| IRBLP5-M             | *Pi5(t)*              |
| IRBLPi7(t)           | *Pi7(t)*              |
| IRBL9-W              | *Pi9*                 |
| IRBL19-A             | *Pi19*                |
| IRBLPik-m            | *Pik-m*               |
| IRBL20-IR24          | *P20(t)*              |
| IRBLta2-Re           | *Pita-2*              |

Other varieties evaluated

| Lijiang Xintuan Heigu (LTH) | Susceptible |
| MR211 (MARDI released varieties) | Susceptible check |
| MR84 (MARDI released varieties) | Resistant check |

Inoculum preparation

*P. oryzae* pathotypes used in this study were P13-1.3 (P0.0), K3-1.1 (P0.2), K11-8.1 (P1.0), B10-2.3 (P3.0), K8-1.2 (P7.0) and P14-4.2 (P9.0). Culture of the six *P. oryzae* pathotypes were sub-cultured and grown on oatmeal agar (OMA) at room temperature for 14 days. The OMA was manually prepared and to prepare 1L of the medium, rolled oats (50 g), sucrose (5 g) and agar (16 g) were used and distilled water was added to made up 1L of the medium. The medium was sterilised at 121°C for 15–20 min.
The inoculum preparation was done according to Hayashi et al. (2009). Mycelia grown on OMA were scraped with spatula and the plates were left open in a tray covered with wrapping plastic. The plates were then placed under exposure of fluorescent light at 25°C ± 2°C for 4–7 days in order to induce sporulation. To prepare a conidial suspension, the plates were flooded with distilled water and the conidia were gently scraped using a brush. The conidial suspensions were filtered through nylon mesh and the concentrations of the suspensions were adjusted to 1×10^5 spores/ml by using a haemocytometer.

**Inoculation of P. oryzae pathotypes**

Rice seedlings at 3–4 leaf stage were inoculated with different pathotypes of blast isolates by spraying the conidial suspension simultaneously using a motorised sprayer. The inoculated seedlings were placed in a dark chamber with a moisture-saturated atmosphere at 25°C–30°C for 24 hr. The seedlings were then transferred to a mist room with high humidity for 6 days (Hayashi et al. 2009). The seedlings were arranged in complete randomised design with three replicates in a plant house at MARDI Seberang Perai, Pulau Pinang, Malaysia. The inoculation and evaluation were repeated twice.

**Disease assessment**

Disease severity was assessed 7 days after inoculation using disease scale as described by Goto and Yamanaka (1968) and Mackill and Bonman (1992) (Table 2). Disease assessment scored as 0 to 2 is categorised as resistant (R) and scored 3 to 5 is categorised as susceptible (S) as described by Hayashi et al. (2009). Percentage of resistant or susceptible reaction was calculated as follows:

\[
\text{Percentage of resistant or susceptible reaction} = \frac{\text{No. of IRBL showed resistant or susceptible reactions}}{\text{Total number of IRBL evaluated}} \times 100
\]

**Statistical analysis**

SAS statistical software package version 9.4 (SAS Institute, Cary, NC) was used for statistical analysis. Disease score for virulence analysis was found to be non-normally distributed, therefore the data was analysed using nonparametric method: Kruskal-Wallis test \( p \leq 0.05 \).

Cluster analysis was conducted to classify the pathotypes based on the disease severity patterns. For cluster analysis, disease scores were converted to binary system where the resistant reaction was scored as 0 while the susceptible reaction was scored as 1. The binary data of the disease score was entered for analysis using NTSYS-pc 2.2 software (Rohlf 2005). Dendogram of cluster
Analysis was performed based on matrix of similarities between all pair of isolates (Jaccard coefficient) by Unweighted Pair Group Method with Arithmetic Mean (UPGMA). Jaccard coefficient was chosen as the coefficient measured similarity between all pair of isolates and negative matches are not counted (Romesburg 1984).

**Table 2:** Disease scale for blast disease assessment on IRBLs.

| Scale | Blast symptom |
|-------|---------------|
| 0 | No evidence of infection. |
| 1 | Brown specks smaller than 0.5 mm in diameter. No sporulation. Uniform or scattered brown specks. |
| 2 | Brown specks about 0.5 mm–1.0 mm in diameter. Small lesions with distinct tan centre which surrounded by a darker brown margin approximately 1 mm in diameter. No sporulation. |
| 3 | Roundish to elliptical lesion about 1 mm–3 mm in diameter with grey centre surrounded by brown margins. Small eyespot lesions less than one and a half times the interval between thin veins or less than 1.5 mm in diameter surrounded by dark brown, lesions capable of sporulation. |
| 4 | Typical spindle shaped blast lesion capable of sporulation, 3 mm or longer with necrotic gray centre and water soaked brown margins with little or no coalescence of lesion. Intermediate size eyespot lesions less than twice the size of interval between thin veins or less than 2 mm in diameter. |
| 5 | Lesions as in scale 4 but about half of one or two leaf blade killed by coalescence of lesion. Large eyespot lesions sized more than twice the size of interval between thin veins or more than 2 mm in diameter. |

**RESULTS AND DISCUSSION**

Disease severity pattern of IRBL to the six blast pathotypes identified in Peninsular Malaysia is shown in Table 3. Determination on the virulence pattern of the six pathotypes (P0.0, P0.2, P1.0, P3.0, P7.0 and P9.0) would help to reveal the response of the resistance genes against specific lineages by using a set of international differential variety.

Based on the reactions of 22 IRBL harbouring 22 resistance genes \([Pia, Pib, Pii, Pit, Pi3, Pi5(t), Pish, Pi1, Pik, Pik-s, Pik-m, Pik-h, Pik-p, Pi7(t), Pi9, Piz, Piz-5, Piz-t, Pi19, Pi20(t), Pita-2, and Pita=Pi4(t)]\) and LTH, the results showed that seven IRBLs, IRBLi-F5, IRBLk-Ka, IRBLKh-K3, IRBLz-Fu, IRBLsh-S, IRBLPi7(t) and IRBL9-W, harbouring \(Pii, Pik, Pik-h, Piz, Pish, Pi7(t)\) and \(Pi9\), respectively were resistant to all pathotypes evaluated.

Meanwhile, IRBL carrying resistance genes, \(Pib\) (IRBLb-B) and \(Pit\) (IRBLt-K59) were susceptible to all pathotypes tested. This suggested that the resistance genes are not suitable to be incorporated in developing new resistant variety.
Table 3: Disease severity pattern of IRBL to six *P. oryzae* pathotypes identified in Peninsular Malaysia.

| Designation of IRBL | Resistance Gene | P0.0 | P0.2 | P1.0 | P3.0 | P7.0 | P9.0 |
|---------------------|-----------------|------|------|------|------|------|------|
| IRBLa-A             | Pia             | R    | S    | S    | R    | S    | S    |
| IRBLi-F5            | Pii             | R    | R    | R    | R    | R    | R    |
| IRBLks-S            | Pik-s           | R    | R    | R    | R    | S    | S    |
| IRBLk-Ka            | Pik             | R    | R    | R    | R    | R    | R    |
| IRBLkp-K60          | Pik-p           | S    | R    | R    | R    | R    | R    |
| IRBLkh-K3           | Pik-h           | R    | R    | R    | R    | R    | R    |
| IRBLz-Fu            | Piz             | R    | R    | R    | R    | R    | R    |
| IRBLz5-CA           | Piz-5           | R    | R    | R    | R    | S    | S    |
| IRBLzt-T            | Piz-t           | S    | R    | S    | S    | S    | S    |
| IRBLta-K1           | Pita=P4(t)      | R    | R    | S    | R    | R    | R    |
| IRBLb-B             | Pib             | S    | S    | S    | S    | S    | S    |
| IRBLt-K59           | Pit             | S    | S    | S    | S    | S    | S    |
| IRBLsh-S            | Pish            | R    | R    | R    | R    | S    | S    |
| IRBL1-CL            | Pi1             | R    | S    | R    | R    | S    | R    |
| IRBL3-C4            | Pi3             | R    | S    | S    | S    | S    | S    |
| IRBLP5-M            | Pi5(t)          | S    | R    | R    | R    | R    | R    |
| IRBLPi7(t)          | Pi7(t)          | R    | R    | R    | R    | R    | R    |
| IRBL9-W             | Pi9             | R    | R    | R    | R    | R    | R    |
| IRBL19-A            | Pi19            | S    | R    | S    | R    | R    | R    |
| IRBLPik-m           | Pik-m           | S    | R    | R    | R    | R    | R    |
| IRBL20-IR24         | Pi20(t)         | S    | S    | R    | R    | S    | R    |
| IRBLta2-Re          | Pita-2          | R    | S    | S    | R    | R    | R    |
| Lijiang Xintuan Heigu (LTH) | | | | | | | |
| MR 211              | –               | S    | R    | S    | R    | S    | S    |
| MR 84               | –               | R    | R    | R    | R    | R    | R    |

*Note: R = Resistant, S = Susceptible, ‘-‘ = information on the resistance gene is not available.*

Virulence of the pathotypes was determined by analysing the disease score scale using Kruskal-Wallis test. The analysis showed that the difference in median of disease score were significant among the six pathotypes (*p* ≤ 0.001) as shown in Table 4. Among the pathotypes evaluated, pathotype P7.0 was the most virulent based on the highest mean ranking value of 248.01. The second virulent pathotype based on the mean ranking value was P1.0 followed by pathotypes P0.0, P9.0 P0.2 and P3.0.
Table 4: Disease score based on disease severity of IRBL against six pathotypes of *P. oryzae*.

| Pathotype | N   | Median | Mean rank |
|-----------|-----|--------|-----------|
| P7.0      | 74  | 2.00   | 248.01    |
| P1.0      | 75  | 1.70   | 245.87    |
| P0.0      | 75  | 2.00   | 233.93    |
| P9.0      | 75  | 1.60   | 226.39    |
| P0.2      | 74  | 1.20   | 220.41    |
| P3.0      | 75  | 0.40   | 191.22    |

Note: Chi-square, H = 17.33 with df = 5 at *p* ≤ 0.05; Disease score = 0 to 5.

Based on the disease severity pattern, the number and percentage of IRBL showed resistant or susceptible reactions to the six blast pathotypes is shown in Table 5. The results indicated similar ranking patterns of virulence from Kruskal-Wallis test where pathotype P7.0 showed the highest virulence among the pathotypes with 40.9% of the IRBL were susceptible, followed by pathotypes P0.0 and P1.0 with the percentage of susceptible reaction of 36.4%. Both pathotypes P0.2 and P9.0 were considered as intermediate virulence to the IRBL tested with percentage of susceptible reaction of 31.8%. Subsequently, pathotype P3.0 was the least virulent pathotype where 81.8% of the IRBL were resistant.

Table 5: Number and percentage of IRBL showed resistant or susceptible reactions to six *P. oryzae* pathotypes.

| Pathotype | Resistant | | Susceptible |
|-----------|-----------|------|-------------|
|           | Number    | %    | Number      | %    |
| P0.0      | 14        | 63.6 | 8           | 36.4 |
| P0.2      | 15        | 68.2 | 7           | 31.8 |
| P1.0      | 14        | 63.6 | 8           | 36.4 |
| P3.0      | 18        | 81.8 | 4           | 18.2 |
| P7.0      | 13        | 59.1 | 9           | 40.9 |
| P9.0      | 15        | 68.2 | 7           | 31.8 |

The most virulent pathotype, P7.0 which also the dominant pathotype in rice field in Peninsular Malaysia (Siti Norsuha & Latiffah 2019) was avirulent to 13 IRBL, namely IRBLi-F5, IRBLk-Ka, IRBLkh-K3, IRBLz-Fu, IRBLta-K1, IRBLsh-S, IRBLP5-M, IRBLPi7(t), IRBL9-W, IRBL19-A, IRBLPik-m and IRBLta2-Re which harbour the resistance genes *PiI*, *Pik*, *Pik-p*, *Pik-h*, *Pita*, *Pish*, *Pi7(t)*, *Pi9*, *Pi19*, *Pik-m* and *Pita-2*, respectively. In particular, IRBL carrying *PiI*, *Pik*, *Pik-h*, *Piz*, *Pish*, *Pi7(t)* and *Pi9* were observed to be resistant to all pathotypes.
evaluated. The least virulent pathotype, P3.0 was virulent to IRBLzt-T, IRBLb-B, IRBLt-K59 and IRBL3-C4, carrying resistance genes Piz-t, Pib, Pit and Pi3, respectively.

Pathotype P0.0 was avirulent to susceptible control variety, LTH. The results suggested that LTH may harboured the genes that confer specific resistance to pathotype P0.0. Similar results were reported by Fukuta et al. (2014) of which 3.3% of blast isolates tested in Cambodia showed avirulence to LTH, suggesting that the susceptible variety may contain resistance genes in its genetic background. However, according to Ling et al. (1995) the resistance genes in LTH were of minor importance, and Tsunematsu et al. (2000) reported major resistance genes have not been identified in LTH.

Based on the disease severity pattern, each pathotype was either virulent or avirulent on specific monogenic lines and the monogenic lines were also susceptible or resistant against specific pathotypes. Ellingboe and Chao (1994) described that the ability of a plant to express resistance depends on the genotype of the pathogen. Thus, the results from this study was an agreement with the gene-for-gene hypothesis by Flor (1971) of which the presence of major resistance gene (R gene) in the plant is effective in recognising avirulence gene (AVR gene) in P. oryzae pathotypes (Jones & Dangl 2006; Lu et al. 2019). Subsequently, the race-specific pathogen recognition will trigger the signal transduction events that lead to pathogen invasion and their virulence functions.

Results from this study shown the avirulence of all pathotypes evaluated to IRBL harbouring R genes, Pii, Pik, Pik-h, Piz, Pish, Pi7(t) and Pi9. The results suggested that the six pathotypes may contain the AVR gene, avr-Pii, avr-Pik, avr-Pik-h, avr-Piz, avr-Pish, avr-Pi7(t) and avr-Pi9 that recognised R gene in their respective IRBL. Therefore, these R genes, namely Pii, Pik, Pik-h, Piz, Pish, Pi7(t) and Pi9 could be suitable candidates of resistance genes to be incorporated in the new breeding lines in terms of combating the current pathotypes in the field.

Cluster analysis carried out using binary data of disease score based on Jaccard similarity coefficient is presented in a dendrogram (Fig. 1). The cluster analysis suggested the disease score can be grouped into two main clusters, I and II at similarity coefficient of 0.25. Cluster I was divided into two sub-clusters, A and B, while cluster II comprising five sub-clusters, C, D, E, F and G.

Sub-cluster A consisted of IRBLs, IRBLa-A (Pia) and IRBL3-C4 (Pi3) as well as LTH which were resistant to pathotype P0.0. Sub-cluster B consisted of IRBLt-K59 (Pit) and IRBLb-B (Pib) which were susceptible to all pathotypes tested and IRBLzt-T (Piz-t) was resistant to only P0.2.

Sub-cluster C comprised IRBLi-F5 (Pii), IRBLk-Ka (Pik), IRBLkh-K3 (Pik-h), IRBLz-Fu (Piz), IRBLsh-S (Pish), IRBLpi-7(t) [Pi7(t)] and IRBL9-W (Pi-9) which were resistant to all pathotypes tested. Another group in this cluster includes IRBLkp-K60 (Pik-p), IRBLP5-M [Pi5(t)] and IRBLPik-m (Pik-m) of which these lines were susceptible to only pathotype P0.0 and resistant to the rest of the pathotypes evaluated.
Sub-cluster D included IRBLta-K1 (*Pita*) and IRBL19-A (*Pi19*), both susceptible to pathotype P1.0. Only IRBLta2-Re (*Pita2*) was grouped in sub-cluster E of which the line was susceptible to pathotypes P0.2 and P1.0. Both sub-clusters D and E were resistant to pathotypes P3.0, P7.0 and P9.0.

Sub-cluster F contained two lines, IRBLks-S (*Pik-s*) and IRBLz5-CA (*Piz-5*) which were susceptible to pathotypes P7.0 and P9.0. Two lines, RBL1-CL (*Pi1*) and IRBL20-IR24 [*Pi20(t)*] susceptible to pathotypes P0.2 and P7.0 were grouped in sub-cluster G.

Pathogenicity and virulence patterns of blast isolates in many countries have been identified using differential varieties with different types and numbers of resistance gene. The present study was similar with a study conducted by Khan *et al.* (2014) on the inoculation of blast isolates from fragrant rice in Bangladesh, on a set of international differential varieties consisting of 32 monogenic lines. In the study, *Pish, Pi9, Pita-2* and *Pita* were estimated as the effective resistance genes against tested blast isolates, and 80%–90% resistance frequencies were observed.

In Japan, pathogenicity study was carried out on 310 blast isolates from eight regions then challenged to a set of differential varieties consisted of 23 monogenic lines (carrying 21 resistance genes) and two near-isogenic line with
the LTH genetic background. From the 310 blast isolates tested, 306 isolates (98.7%) were found to be virulent to IRBLsh-S \( (Pish) \) (Kawasaki-Tanaka & Fukuta 2014). In contrast, the results of the present study revealed that all the pathotypes tested were avirulent to IRBLsh-S \( (Pish) \).

The resistance gene \( Pii \) which could be one of suitable candidates of resistance genes to be used to develop new breeding lines, has been deployed for more than two decades in China (Duan et al. 1990). However, Zhu et al. (2000) and Li et al. (2007) reported the rice cultivars that harbored \( Pii \) gene were resistant to only 54.6% of blast isolates evaluated in Yunnan province and 15.1% isolates from Guangdong province, respectively. Subsequently, studies conducted by Huang et al. (2014) on blast isolates from various rice-producing regions in China revealed that 80% of the isolates were found to have complete deletions of \( AVR-Pii \). The results suggested breakdown of \( Pii \) resistance in the rice cultivars. The \( Pii \) gene composed of five exons encoding a putative CC-NBS-LRR protein with 1025 amino acids (Takagi et al. 2013). The \( AVR-Pii \) gene of blast pathogen corresponds with the host resistance gene \( Pii \), subsequently triggers the defence response (Yoshida et al. 2009).

Studies conducted by Silva et al. (2004) and Yasuda et al. (2006) revealed that a loss of the \( AVR-Pii \) gene transforms an avirulent to a virulent fungal strain. Moreover, the \( AVR-Pii \) gene is located on chromosome 7 which is a highly unstable chromosome segment (Yasuda et al. 2006; Yoshida et al. 2009) which suggested a risk of gene loss and horizontal transfer events (Silva et al. 2004; Rehmeyer et al. 2006).

Although in the present study, the blast isolates used were limited, determination on the virulence patterns of the six pathotypes of \( P. oryzae \) \( (P0.0, P0.2, P1.0, P3.0, P7.0 \text{ and } P9.0) \) help to determine the response of the resistance genes against specific lines. Thus, it is an initial step to elucidate the diversity and differentiation of blast pathotypes virulence using differential varieties. The knowledge on the virulence of the blast pathotypes and the resistance genes is important for breeding of new rice variety for durable resistance against blast disease.

The variability in virulence patterns of the blast pathotypes would greatly help researchers in selecting suitable donors in breeding for resistance of rice blast of which particular functioning set of genes are incorporated into a desirable rice variety for durable resistance against the blast pathogen.

**CONCLUSION**

In conclusion, to combat rice blast against current high frequencies of pathotypes P7.0, P0.0, P9.0 and P1.0, suitable resistance genes or donor to be incorporated for developing future rice blast resistant variety are \( Pii, Pik, Pik-h, Piz, Pish, Pi7(t) \) and \( Pi9 \). Meanwhile, \( Pib \) and \( Pit \) are not suitable resistance genes to be incorporated for developing new resistant variety considering their susceptible response to all pathotypes evaluated.
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REFERENCES

Duan Y, Zhu Y and Liu E. (1990). Genetic studies on the resistance of rice to the blast. In L Zhu (Ed.). Advances in research on resistance to disease in major crops. Nanjing, China: Jiangsu Science-Technology Publishing House, 116–123.

Ellingboe A H and Chao C T. (1994) Genetic interaction in Magnaporthe grisea that affect cultivar specific avirulence/virulence on rice. In R S Zeigler, S A Leong S A and P S Teng (eds.). Rice blast disease. Philippines: International Rice Research Institute, 51–63.

Flor H H. (1971). Current status of the gene-for-gene concept. Annual Review of Phytopathology 9: 275–296. https://doi.org/10.1146/annurev.py.09.090171.001423

Fukuta Y, Koga I, Ung T, Sathya K, Kawasaki-Tanaka A, Koide Y, Kobayashi N, Obara M, Yagana H and Hayashi N. (2014). Pathogenicity of rice blast (Pyricularia oryzae Cavara) isolates from Cambodia. Japan Agricultural Research Quarterly 48: 155–166. https://doi.org/10.6090/jarq.48.155

Goto K and Yamanaka S. (1968). Studies on the race of rice blast fungus. Bulletin of the College of Agriculture, Utsunomiya University 7(2): 21–71.

Hayashi N, Kobayashi N, Vera Cruz C M and Fukuta Y. (2009). Protocols for the sampling of diseased specimens and evaluation of blast disease in rice. JIRCAS Working Report No. 63, 17–33.

Huang J, Si W, Deng Q, Li P and Yang S. (2014). Rapid evolution of avirulence genes in rice blast fungus Magnaporthe oryzae. BMC Genetics 15: 45. https://doi.org/10.1186/1471-2156-15-45

Jones J D and Dangl J L. (2006). The plant immune system. Nature 444: 323–329. https://doi.org/10.1038/nature05286

Kawasaki-Tanaka A and Fukuta Y. (2014). Genetic variation in resistance to blast disease (Pyricularia oryzae Cavara) in Japanese rice (Oryza sativa L.) as determined using a differential system. Breeding Science 64: 183–192. https://doi.org/10.1270/jsbbs.64.183

Khan M A I, Sen P P, Bhuiyan R, Kabir E, Chowdhury A K, Fukuta Y, Ali A and Latif M A. (2014). Phenotypic screening and molecular analysis of blast resistance in fragrant rice for marker assisted selection. Comptes Rendus Biologies 337: 318–324. https://doi.org/10.1016/j.crvi.2014.02.007

Kobayashi N, Yanoria M J T, Tsunematsu H, Kato H, Imbe T and Fukuta Y. (2007). Development of new sets of international standard differentials varieties for blast resistance in rice (Oryza sativa L.). Japanese Agricultural Research Quarterly 41: 31–37. https://doi.org/10.6090/jarq.41.31
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Koide Y, Ebron L A, Kato H, Tsunematsu H, Yanoria M J T, Kobayashi N, Yokoo M, Maruyama S, Imbe T and Fukuta Y. (2011). A set of near-isogenic lines for blast resistance genes with an indica-type rainfed lowland elite rice (Oryza sativa L.) genetic background. Field Crop Research 123: 19–27. https://doi.org/10.1016/j.fcr.2011.04.005

Li Y, Wang L, Jing J X, Li Z Q, Lin F, Huang L F and Pan Q H. (2007). The Pikm gene, conferring stable resistance to isolates of Magnaporthe oryzae, was finely mapped in a crossover-cold region on rice chromosome 11. Molecular Breeding 20: 179–188. https://doi.org/10.1007/s11032-007-9118-6

Ling Z Z, Mew T, Wang J L and Lei C L. (1995). Development of near-isogenic lines as international differential of the blast pathogen. International Rice Research Notes 20: 13–14.

Lu L, Wang Q, Jia Y, Bi Y Q, Li C Y, Fan H C and Li J. B. (2019). Selection and mutation of the avirulence gene AVR-Pii of the rice blast fungus Magnaporthe oryzae. Plant Pathology 68(1): 127–134. https://doi.org/10.1111/ppa.12935

Mackill D J and Bonman J M (1992). Inheritance of blast-resistance in near-isogenic lines of rice. Phytopathology 82: 746–749. https://doi.org/10.1094/Phyto-82-746

Poonsin R and Parinthawong N. (2020). Investigation of rice blast resistant genes in Thai elite rice varieties (Oryza sativa L.) for improvement of broad-spectrum blast disease resistance. International Journal of Agricultural Technology 16(1): 109–118.

Rehmeyer C, Li W, Kusaba M, Kim Y S, Brown D, Staben C, Dean R and Farman M. (2006). Organization of chromosome ends in the rice blast fungus, Magnaporthe oryzae. Nucleic Acids Research 34: 4685–4701. https://doi.org/10.1093/nar/gkl588

Rohlf F J. (2005). NTSYS-PC: Numerical taxonomy and multivariate analysis system, Version 2.2. Exeter Software, Setauket.

Romesburg H C. (1984). Cluster analysis for researchers. Belmont, CA: Lifetime Learning Publications.

Silva J C, Loreto E L and Clark J B. (2004). Factors that affect the horizontal transfer of transposable elements. Current Issues in Molecular Biology 6: 57–72.

Siti Norsuha M and Latiffah Z. (2019). Pathotype identification of rice blast pathogen, Pycnoria oryzae using differential varieties in Peninsular Malaysia. Tropical Life Sciences Research 30(2): 181–190. https://doi.org/10.21315/tlsr2019.30.2.13

Takagi H, Uemura A, Yaegashi H, Tamiru M, Abe A, Mitsuoka C, Saitoh H, Utsushi H, Natsume S, Kanzaki H, et al. (2013). MutMap-Gap: Whole-genome resequencing of mutant F2 progeny bulk combined with de novo assembly of gap regions identifies the rice blast resistance gene Pli. New Phytologist 200: 276–283. https://doi.org/10.1111/nph.12369

Telebanco-Yanoria M J, Koide Y, Fukuta Y, Imbe T, Kato H, Tsunematsu H and Kobayashi N. (2010). Development of near-isogenic lines of Japonica type rice variety Lijiangxintuanheigu as differentials for blast resistance. Breeding Science 60: 629–638. https://doi.org/10.1270/jsbbs.60.629

Tsunematsu H, Yanoria M J T, Ebron L A, Hayashi N, Ando I, Kato H, Imbe T and Khush G S. (2000). Development of monogenic lines of rice for blast resistance. Breeding Science 50: 229–234. https://doi.org/10.1270/jsbbs.50.229

Wang J C, Jia Y, Wen J W, Liu W P, Liu X M, Li L, Jiang Z Y, Zhang J H, Guo X L and Ren J P. (2013). Identification of rice blast resistance genes using international monogenic differentials. Crop Protection 45: 109–116. https://doi.org/10.1016/j.cropro.2012.11.020
Wang X, Jia Y, Shu Q Y and Wu D. (2008). Haplotype diversity at the Pi-ta locus in cultivated rice and its wild relatives. *Phytopathology* 98: 1305–1311. https://doi.org/10.1094/PHYTO-98-12-1305

Yasuda N, Noguchi M T and Fujita Y. (2006). Partial mapping of avirulence genes AVR-Pii and AVR-Pia in the rice blast fungus *Magnaporthe oryzae*. *Canadian Journal of Plant Pathology* 28: 494–498. https://doi.org/10.1080/07060660609507325

Yoshida K, Saitoh H, Fujisawa S, Kanzaki H, Matsumura H, Yoshida K, Yukio Y, Chuma I, Takano Y, Win J, Kamoun S and Terauchi R. (2009). Association genetics reveals three novel avirulence genes from the rice blast fungal pathogen *Magnaporthe oryzae*. *Plant Cell* 21(5): 1573–1591. https://doi.org/10.1105/tpc.109.066324

Zhou B, Dolan M, Sakai H and Wang G. (2007). The genomic dynamics and evolutionary mechanism of the Pi2/9 locus in rice. *Molecular Plant-Microbe Interaction* 20: 63–71. https://doi.org/10.1094/MPMI-20-0063

Zhu Y Y, Chen H R, Fan J H, Wang Y Y, Li Y, Chen J B, Fan J X, Yang S S, Hu L P, Leung H, Mew T W, Teng P S, Wang Z H and Mund C C. (2000). Genetic diversity and disease control in rice. *Nature* 406: 718–722. https://doi.org/10.1038/35021046