Pathotypes and virulence of *Xanthomonas oryzae* causing bacterial blight disease of rice in Peninsular Malaysia

(Patotip dan virulens *Xanthomonas oryzae* yang menyebabkan penyakit hawar bakteria padi di Semenanjung Malaysia)

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Keywords: rice, bacterial blight, Xanthomonas oryzae pv. oryzae, pathotype, Malaysia

Abstract

A study was conducted on *Xanthomonas oryzae* isolates collected from major granary areas in Peninsular Malaysia. Study on the 346 isolates indicated that most of them (29.5%) could be grouped into pathotype $P_{1.0}$. Pathotype $P_{1.0}$ was only virulent against rice variety IR 8 which carried the resistant gene Xa-11 but remained avirulent against other differential varieties. Pathotype $P_{1.0}$ was detected in all states. The most virulent pathotype was $P_{7.7}$ which was capable of causing severe lesion on all differential varieties tested. However, most of the virulent pathotypes were only represented by a small number of isolates and limited to certain localities only. Rice variety MR 84 which was popular in the 90s was susceptible to the common isolates. However, other differential varieties such as Cempo Selak, Zenith, IR 20, DV 85 and Patong 32 were resistant, and most of the tested isolates remained avirulent against them.

Introduction

The bacterium Xanthomonas oryzae pv. oryzae (Xoo) or previously known as Xanthomonas campestris pv. oryzae is the causal pathogen of the bacterial blight disease (BLB) of rice in Asia (Swings et al. 1990). It is considered as one of the most serious and widespread diseases affecting rice industry in Asia since the 1960s (Ou 1985). It causes yellowing of the leaves, followed by death of infected leaf tissues, which usually starts from the tip of the leaves (*Plate 1*). Due to the reduction in the leaf area size, an infected susceptible plant may suffer a yield reduction of as high as 50% (Saad 1995). Incidence of the disease was observed in the rice fields of Peninsular Malaysia in the early 80s, but was in a limited widespread (Saad 1995). However, it became more widespread recently, which was believed to be partly due to the wide scale planting of a susceptible rice variety, MR 84, for many long consecutive years. The disease also caused an estimated loss of about RM50 million during the period of 1982 to 1994 (Saad 1994).

Chemical control of the disease is impractical due to unavailability of a suitable bactericide to suppress the disease development and its effect. Hence, breeding rice varieties resistant to the disease

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Plate 1. Rice leaves with lesions caused by **Xanthomanas oryzae**. Rice plants in the field with symptom of bacterial blight infection (left) and leaf cuts showing variable degree of lesion length, classified into different resistance classes

becomes the most logical approach, which is cheaper and environmentally acceptable. Previously in the 70s, breeding of rice varieties resistant to bacterial blight was only an optional objective of the overall rice breeding programme of MARDI (Ho 1976). Lately, it has become one of the criteria to be taken into consideration before a variety is released. The efficiency of breeding for disease resistant programme is dependent on two important variables, namely the availability of resistant genetic sources and the understanding of the variation within the pathogen population concerned. Understanding of the mechanism and host-pathogen relationship is essential in the formulation of a long-term disease management strategy.

Plant pathogens were deemed to be able to adapt rapidly following the introduction of any resistant genes, and this is due to or as a consequence of their populations variability. The population level where selection and adaptation occurred is called pathotype (Limpert et al. 1994), and various pathotypes of *Xanthomonas* *campestris* pv. *oryzae* (Xoo) had been identified in different Asian countries (Mew and Vera Cruz 1979).

The introduced resistant gene is only meaningful if it remains resistant and effective against local population of *X. oryzae* at a location where the gene is introduced. So, information on the variability and virulence of the local *X. oryzae* populations need to be obtained. A total of 346 *X. oryzae* isolates were collected from several rice growing regions in Peninsular Malaysia. The variability and virulence of these isolates against differential varieties, their classification into various pathotypes and virulence of selected isolates against selected advance varieties and breeding lines were reported.

Materials and methods

Collection, isolation and maintainance of Xoo isolates

Rice leaves with typical lesions were collected from rice fields in the major growing areas. More than 346 isolates/ specimens were collected from farmers' fields in various localities in Perlis, Kedah, Pulau Pinang, Perak, Selangor and Kelantan. Infected leaves were washed with distilled water, placed on glass rods in petri dishes and incubated at room temperature (28 °C) for 24 h. The developed bacterial ooze was then streaked on peptone sucrose agar (PSA). All pure culture isolates were maintained on PSA and stored at -10 °C.

For inoculation, the inoculum of each isolate was prepared by suspending the bacteria in sterile distilled water prior to the inoculation period. The absorbance value (590 μ m) was adjusted to 1 to give a bacterial suspension with a concentration of approximately 5.0 x 10⁹ cfu/ml.

Test plants, inoculation and virulence evaluation

Seedlings of each test variety, at the age of 21 days after sowing (DAS), were transplanted in the greenhouse, single plant per point at a 25 cm spacing, in a completely randomised design with three replications. Each replication was represented by five plants. Plants were given fertilization of nitrogen:phosphorus:potassium (NPK) at the rate of 135:80:40 kg/ha respectively.

Test plants were inoculated at 65 DAS, by using shearing method as previously described (Saad 1990). The length of the infected leaf tissues (lesion length) and the length of two healthy leaves/plant were also determined at 21 days after inoculation (DAI). Percentage lesion length was calculated. An isolate capable of giving >31% lesion length on a particular variety is considered as virulent on that particular variety, and vice versa (Saad 1995).

Pathotype designation and their distribution

Six varieties representing two subsets of three varieties each were used as the differential varieties for the pathotype designation (*Table 1*). Plants of these varieties were inoculated by respective *X. oryzae* isolates as previously described. Percentage lesion length was then calculated and classified into virulent or avirulent.

Each isolate of *X. oryzae* was classified into a pathotype designation. Pathotypes were designated according to the coded triplet nomenclature system of Limpert and Muller (1994). This system used host differential varieties ordered in subsets of three (*Table 1*). Reaction of each isolate on a differential variety first described in a binary values of (1) for virulent or (0) for avirulent. Binary values of each component were further converted to decenary values by the conversion factors 2^0 , 2^1 and 2^2 for the first, second and third members of the respective subset of differential varieties

| Variety | Gene* | Percentage lesion length | | | | |
|---------------------|-------|--------------------------|--------------------|--|--|--|
| | | Highest | Lowest | | | |
| First subset | | | | | | |
| IR 8 | Xa-11 | 100% high, MXO 200 | 0% low, MXO 252 | | | |
| Cempo Selak Unknown | | 93.7% high, MXO 530 | 2.7% low, MXO 371c | | | |
| Zenith Xa-6 | | 98.4% high, MXO 66 | 1.9% low, MXO 595b | | | |
| Second subset | | | | | | |
| IR 20 | Xa-4 | 75.5% high, MX0 534b | 1.6% low, MXO 252 | | | |
| IR 1545-339 | Xa-5 | 92.1% high, MXO 92 | 1.5% low, MXO 252 | | | |
| DV 85 Xa-7 | | 89.8% high, MXO 362e | 0.6% low, MXO 84 | | | |

Table 1. Percentage lesion length as observed on the inoculated differential varieties after inoculation with several representatives of *Xanthomanas oryzae* isolates

MXO = isolate number

*Source: Ogawa and Khush (1989)

respectively. Decenary values were summed to derive a single number for each subset. Since only six differential varieties were used and divided into two subsets, two digit numbers were used as the pathotype code of *X. oryzae* in the current study.

Frequency of virulence

Frequency of virulence (FV) was a parameter indicating the cumulative virulence of all isolates present in a location against a particular variety or gene of resistance. It might indicate whether the variety was resistant or susceptible against a collection of isolates within a locality tested against it. Frequency of virulence was calculated as follows:

 $\frac{\text{Frequency of}}{\text{virulence (FV)}} = \frac{\text{The number of virulent isolates x 100}}{\text{Total number of isolates tested}}$

Methods of plant management, inoculation and scoring against infection were similar to those previously described. Percentage lesion length on the inoculated varieties was computed.

Virulence of Xanthomonas oryzae against MR 84 and other MARDI varieties

Several rice varieties including MR 1, MR 77, MR 84, MR 103, MR 106, MR 159, MR 167, Seribu Gantang, Patong 32 and IR 42 were planted in the field. Rice variety MR 84 was a popular variety, which was widely grown in major granary areas during the 90s. The leaves of these plants were inoculated with 27 selected isolates/ pathotypes of $P_{1.0}$ – $P_{7.7}$ (*Table 2*).

Results and discussion *Lesion length on the inoculated rice varieties*

Disease induced lesions on the inoculated leaves were recorded at about 21 DAI. The percentage lesion length varied among varieties and was influenced by the isolates used for the inoculation. The calculated percentage lesion length ranged from 0% to 100%. For example, percentage lesion length on IR 8 was as high as 100% when the variety was inoculated with isolate number MXO 200 and to as low as 0% when it was inoculated with the isolate number MXO 252 (*Table 1*). On the other hand for the rice variety DV 85, the highest percentage lesion length was about 90% (very virulent) when it was inoculated with isolate number MXO 362e, and to as low as 1% with isolate number MXO 84 (avirulent) (*Table 1*).

Pathotype designation and classification

When a cut point at 31% lesion length was made, isolates were divided into the virulent and avirulent isolates (with a binary values of 1 and 0 respectively) against any differential varieties. These binary values were used to derive pathotype designation based on the coded triplet nomenclature system of Limpert and Muller (1994). For example, an isolate which exhibited high virulence on IR 8 (1 x $2^0 = 1$), but showed avirulence on both Cempo Selak (0 x $2^1 = 0$) and Zenith (0 x $2^2 = 0$) in the first set (1 + 0 + 0 = 1); and demonstrated avirulence on IR 20 (0 x $2^0 = 0$), IR1545-339 (0 x 2^1 = 0) and DV 85 (0 x $2^2 = 0$) respectively in the second set (0 + 0 + 0 = 0), would be coded as the pathotype $P_{1,0}$ (*Table 2*).

In short, pathotype $P_{1,0}$ was virulent against IR 8 (with Xa-11), but avirulent against other varieties. Similarly calculated, an isolate which is virulent against all differential varieties was designated as pathotype P_{77} , while pathotype P_{00} was avirulent against all differential varieties. On the other hand, pathotype $P_{7,0}$ was virulent against all the three varieties in set one but avirulent against the other three in set two. Based on the present result, all the 346 isolates could be classified into 28 pathotypes. A summary of designated pathotypes is given in *Table 2*. Twenty seven pathotypes were virulent against IR 8 and only 11 and 14 pathotypes were virulent against DV 85 and Cempo Selak respectively. All isolates collected from major granary areas of Peninsular Malaysia, which are presently kept at

| Pathotype code | Set 1 | | Set 2 | | | |
|------------------|-------|-------------|--------|-------|-------------|-------|
| | IR 8 | Cempo Selak | Zenith | IR 20 | IR 1545-339 | DV 85 |
| P _{0.0} | 0 | 0 | 0 | 0 | 0 | 0 |
| P _{1.0} | 1 | 0 | 0 | 0 | 0 | 0 |
| P ₁₁ | 1 | 0 | 0 | 1 | 0 | 0 |
| P ₁₂ | 1 | 0 | 0 | 0 | 1 | 0 |
| P ₁₃ | 1 | 0 | 0 | 1 | 1 | 0 |
| P ₁₄ | 1 | 0 | 0 | 0 | 0 | 1 |
| P ₁₇ | 1 | 0 | 0 | 1 | 1 | 1 |
| $P_{30}^{1.7}$ | 1 | 1 | 0 | 0 | 0 | 0 |
| P ₃₁ | 1 | 1 | 0 | 1 | 0 | 0 |
| P ₃₂ | 1 | 1 | 0 | 0 | 1 | 0 |
| P ₃₃ | 1 | 1 | 0 | 1 | 1 | 0 |
| $P_{34}^{3.5}$ | 1 | 1 | 0 | 0 | 0 | 1 |
| P ₃₇ | 1 | 1 | 0 | 1 | 1 | 1 |
| P ₅₀ | 1 | 0 | 1 | 0 | 0 | 0 |
| P ₅₁ | 1 | 0 | 1 | 1 | 0 | 0 |
| P ₅₂ | 1 | 0 | 1 | 0 | 1 | 0 |
| P ₅₃ | 1 | 0 | 1 | 1 | 1 | 0 |
| P ₅₄ | 1 | 0 | 1 | 0 | 0 | 1 |
| P ₅₆ | 1 | 0 | 1 | 0 | 1 | 1 |
| P _{5.7} | 1 | 0 | 1 | 1 | 1 | 1 |
| P ₇₀ | 1 | 1 | 1 | 0 | 0 | 0 |
| P ₇₁ | 1 | 1 | 1 | 1 | 0 | 0 |
| P ₇₂ | 1 | 1 | 1 | 0 | 1 | 0 |
| P ₇₃ | 1 | 1 | 1 | 1 | 1 | 0 |
| P ₇₄ | 1 | 1 | 1 | 0 | 0 | 1 |
| P ₇₅ | 1 | 1 | 1 | 1 | 0 | 1 |
| P ₇₆ | 1 | 1 | 1 | 0 | 1 | 1 |
| P _{7.7} | 1 | 1 | 1 | 1 | 1 | 1 |
| No. of virulent | 27 | 14 | 15 | 13 | 14 | 11 |

Table 2. Classification of *Xanthomonas oryzae* pathotypes based on their virulence against 2 sets of differential varieties in Malaysia

1 = virulent; 0 = avirulent

MARDI Seberang Perai, were classified into respective pathotypes and tabulated (*Table 3*).

Distribution of Xoo pathotypes

Out of 346 isolates collected, 50 were from Perlis, 126 from Kedah and 17 from Selangor (*Table 4*). Twenty one isolates were grouped as pathotype $P_{0,0}$, and 102 isolates were classified as pathotype $P_{1,0}$. Some pathotypes occurred in a higher frequency and distributed in all rice states, while some others had low frequency and thinly distributed. The most prevalent pathotype was the pathotype $P_{1.0}$ (frequency = 29.5%), and was distributed in all states. This was followed by pathotype $P_{7.0}$ (15.3%) and $P_{5.0}$ (11.9%). Pathotype $P_{0.0}$ (6.1%) could also be obtained from all the states and so are the pathotypes $P_{1.1}$, $P_{3.0}$, $P_{5.0}$ and $P_{7.0}$. Several other pathotypes, such as pathotypes $P_{3.7}$, $P_{5.4}$, $P_{5.5}$, $P_{5.6}$, $P_{7.3}$ and $P_{7.7}$ were represented by only one isolate each respectively, and their occurrence was localised in certain states only.

| Table 3. Designation of Xanthomanas oryz | ae isolates into pathotypes |
|--|-----------------------------|
|--|-----------------------------|

| Pathotypes | Isolates |
|------------------|--|
| P _{0.0} | MXO 40, MXO 87, MXO 159, MXO 252, MXO 258, MXO 309, MXO 346, MXO 350, MXO 351d, MXO 352a, MXO 352c, MXO 353a, MXO 353b, MXO 353c, MXO 353c, MXO 358c, MXO 358d, MXO 369a, MXO 371a, MXO 371c, MXO 507 b, MXO 615a, MXO 624. |
| P _{1.0} | MXO 5, MXO 72, MXO 84, MXO 95, MXO 104, MXO 118, MXO 120, MXO 171, MXO 173, MXO 174, MXO 178, MXO 179, MXO 218, MXO 236, MXO 239, MXO 241, MXO 242, MXO 243, MXO 245, MXO 246, MXO 249, MXO 250, MXO 251, MXO 257, MXO 261, MXO 264, MXO 267, MXO 269, MXO 270, MXO 271, MXO 272, MXO 273, MXO 277, MXO 280, MXO 284, MXO 285, MXO 286, MXO 287, MXO 289, MXO 290, MXO 293, MXO 300, MXO 304, MXO 311, MXO 313, MXO 322, MXO 323, MXO 325, MXO 330, MXO 334, MXO 335, MXO 336, MXO 338, MXO 339, MXO 341, MXO 342, MXO 343, MXO 351b, MXO 351c, MXO 351c, MXO 352e, MXO 355a, MXO 355c, MXO 355d, MXO 355e, MXO 359b, MXO 359e, MXO 361a, MXO 362a, MXO 365, MXO 365, MXO 365d, MXO 365e, MXO 366, MXO 367, MXO 368, MXO 368a, MXO 369, MXO 370a, MXO 398, MXO 400, MXO 487b, MXO 493, MXO 495, MXO 496b, MXO 518a, MXO 531, MXO 534b, MXO 545, MXO 554, MXO 563c, MXO 563d, MXO 595b, MXO 599a, MXO 603a, MXO 615c, MXO 735, MXO 745, MXO 748 |
| P _{1.1} | MXO 155, MXO 162, MXO 320, MXO 321, MXO 345, MXO 352d, MXO 291, MXO 295, MXO 312, MXO 549, MXO 551, MXO 436, MXO 441, MXO 442, MXO 461, MXO 570, MXO 401, MXO 402, MXO 519a, MXO 541, MXO 542 |
| P _{1.2} | MXO 260, MXO 359a, MXO 359c, MXO 359d, MXO 360b, MXO 360c, MXO 362c, MXO 371b, MXO 371d, MXO 491, MXO 504b, MXO 509a, MXO 511b, MXO 519, MXO 369d, MXO 324, MXO 506a, MXO 543, MXO 546, MXO 600b |
| P _{1.3} | MXO 281, MXO 354c, MXO 429, MXO 492, MXO 510b, MXO 511a, MXO 522 |
| P _{1.4} | MXO 176, MXO 240, MXO 248, MXO 255, MXO 256, MXO 263, MXO 361b, MXO 604d, MXO 623 |
| P _{1.7} | MXO 437, MXO 514 |
| P _{3.0} | MXO 17, MXO 145, MXO 184, MXO 188, MXO 191, MXO 193, MXO 195, MXO 231, MXO 288, MXO 361c, MXO 362d, MXO 368e, MXO 374, MXO 487a, MXO 505, MXO 507a, MXO 520, MXO 600c, MXO 604d |
| P _{3.1} | MXO 348, MXO 360e, MXO 371d, MXO 371f, MXO 372, MXO 467, MXO 469, MXO 537, MXO 538, MXO 594d |
| P _{3.2} | MXO 276, MXO 361e, MXO 362b, MXO 370c, MXO 463, MXO 530 |
| P _{3.3} | MXO 354b, MXO 354d |
| P _{3.4} | MXO 234, MXO 370e, MXO 502a, MXO 600a |
| P _{3.7} | MXO 599d |
| P _{5.0} | MXO 12, MXO 14, MXO 34, MXO 46, MXO 54, MXO 55, MXO 66, MXO 71, MXO 86, MXO 90, MXO 91, MXO 94, MXO 97, MXO 99, MXO 101, MXO 105, MXO 106, MXO 111, MXO 112, MXO 114, MXO 116, MXO 119, MXO 121, MXO 122, MXO 124, MXO 137, MXO 156, MXO 187, MXO 192, MXO 196, MXO 253, MXO 275, MXO 278, MXO 319, MXO 358b, MXO 358e, MXO 364, MXO 550b, MXO 360a MXO 578 |
| P _{5.1} | MXO 294, MXO 347, MXO 534a, MXO 539 |
| P ₅₂ | MXO 186, MXO 486a, MXO 555 |
| P _{5.3} | MXO 522a, MXO 562c |
| P _{5.4} | MXO 274 |
| P _{5.5} | MXO 362e |
| P _{5.6} | MXO 354a |
| P _{7.0} | MXO 39, MXO 62, MXO 64, MXO 89, MXO 93, MXO 100, MXO 103, MXO 107, MXO 110, MXO 117, MXO 123, MXO 125, MXO 126, MXO 148, MXO 151, MXO 157, MXO 158, MXO 161, MXO 163, MXO 169, MXO 177, MXO 183, MXO 189, MXO 190, MXO 194, MXO 198, MXO 199, MXO 200, MXO 205, MXO 207, MXO 208, MXO 209, MXO 210, MXO 212, MXO 217, MXO 233, MXO 235, MXO 236, MXO 244, MXO 349, MXO 259, MXO 370b, MXO 220, MXO 221, MXO 222, MXO 223, MXO 224, MXO 225, MXO 360d, MXO 486b, MXO 550a, MXO 591c, MXO 592c, |
| P _{7.1} | MXO 226, MXO 440, MXO 517a |
| P _{7.2} | MXO 108, MXO 216, |
| P _{7.3} | MXO 352b, |
| P _{7.4} | MXO 229, MXO 283, MXO 361d |
| P _{7.5} | MXO 262, MXO 615b |
| P _{7.6} | MXO 57, MXO 536 |
| P _{7.7} | MXO 92 |

| Pathotypes | Perlis | Kedah | P. Pinang | Perak | Selangor | Kelantan | Total |
|------------------|--------|-------|-----------|-------|----------|----------|-------|
| P _{0.0} | 9 | 4 | 2 | 1 | 1 | 4 | 21 |
| P ₁₀ | 16 | 34 | 19 | 21 | 7 | 5 | 102 |
| P ₁₁ | 4 | 10 | _ | 5 | _ | 1 | 20 |
| P ₁₂ | 1 | 3 | 2 | 5 | 3 | 6 | 20 |
| P ₁₃ | 1 | 3 | 0 | 3 | 0 | 0 | 7 |
| P _{1.4} | 1 | 3 | 3 | 0 | 1 | 1 | 9 |
| P _{1.7} | 0 | 1 | 0 | 0 | 0 | 1 | 2 |
| P _{3.0} | 1 | 9 | 4 | 3 | 1 | 2 | 20 |
| P _{3.1} | 1 | 7 | 0 | 2 | 0 | 1 | 11 |
| P _{3.2} | 0 | 3 | 0 | 2 | 0 | 1 | 6 |
| P _{3.3} | 2 | 0 | 0 | 0 | 0 | 0 | 2 |
| P _{3.4} | 0 | 2 | 1 | 0 | 0 | 1 | 4 |
| P _{3.7} | 1 | 0 | 0 | 0 | 0 | 0 | 1 |
| P _{5.0} | 3 | 17 | 8 | 7 | 2 | 4 | 41 |
| P _{5.1} | 1 | 2 | 0 | 1 | 0 | 0 | 4 |
| P _{5.2} | 0 | 1 | 2 | 0 | 0 | 0 | 3 |
| P _{5.3} | 0 | 2 | 0 | 0 | 0 | 0 | 2 |
| P _{5.4} | 0 | 1 | 0 | 0 | 0 | 0 | 1 |
| P _{5.6} | 0 | 0 | 0 | 1 | 0 | 0 | 1 |
| P _{5.7} | 1 | 0 | 0 | 0 | 0 | 0 | 1 |
| P _{7.0} | 5 | 19 | 14 | 10 | 2 | 3 | 53 |
| P _{7.1} | 0 | 1 | 0 | 1 | 0 | 1 | 3 |
| P _{7.2} | 0 | 0 | 1 | 1 | 0 | 0 | 2 |
| P _{7.3} | 1 | 0 | 0 | 0 | 0 | 0 | 1 |
| P _{7.4} | 0 | 2 | 0 | 0 | 0 | 1 | 3 |
| P _{7.5} | 1 | 1 | 0 | 0 | 0 | 1 | 3 |
| P _{7.6} | 1 | 0 | 0 | 1 | 0 | 0 | 2 |
| P _{7.7} | 0 | 1 | 0 | 0 | 0 | 0 | 1 |
| Total | 50 | 126 | 56 | 64 | 17 | 33 | 346 |

Table 4. Distribution of *Xanthomonas oryzae* isolates according to pathotypes in major rice growing states in Peninsular Malaysia

Virulence analysis

Almost all isolates collected from Perlis, Kedah, Pulau Pinang, Perak, Selangor and Kelantan were virulent against IR 8 (Xa-11) when based on the frequency of virulence among the evaluated isolates (Table 5). About 91% of these isolates were virulent and capable of inflicting severe lesion length on IR 8. Similarly, very high percentages of isolates were also capable of inflicting severe symptom and virulent against a local, previously popular variety MR 84 (85%) and a universal susceptible TN 1 (87%). A relatively moderate number of isolates were also virulent against Zenith (Xa-6: 43%) and Cempo Selak (Unknown gene: 39%). However, most isolates were avirulent against IR 20 (Xa-4), IR 1545-339 (xa-5) and DV 85 (xa-5 and Xa-7).

Virulence against MR 84 and other MARDI varieties

Inoculating X. oryzae inoculum on rice variety MR 84 showed that the inoculum had caused a disease lesion of about 60% of the leaf length (*Figure 1*). This may suggest that MR 84 was susceptible to the challenged inoculum, which was virulent to the variety. This was followed by Seribu Gantang and MR 167. Rice variety MR 77 suffered a lesion length of slightly above 30% of the leaf length, indicating that it was on the border of moderately susceptible, and the inoculum used had reached the status

| | IR 8 | C. Selak | Zenith | IR 20 | IR 1545-339 | DV 85 | MR 84 | TN 1 |
|----------|-------|----------|--------|-------|-------------|-------|-------|------|
| Perlis | 80.0 | 40.0 | 40.0 | 32.5 | 22.5 | 15.0 | 55.7 | 90.0 |
| Kedah | 93.0 | 45.2 | 52.6 | 14.7 | 11.6 | 15.0 | 91.3 | 82.0 |
| S/Perai | 100.0 | 45.1 | 51.0 | 2.0 | 11.8 | 3.9 | 90.0 | 90.0 |
| Perak | 96.0 | 40.0 | 44.0 | 18.0 | 32.0 | 6.0 | 97.9 | 87.0 |
| Selangor | 85.7 | 27.7 | 33.3 | 0 | 6.7 | 13.3 | 86.7 | 86.0 |
| Kelantan | 89.7 | 34.5 | 34.5 | 13.8 | 27.6 | 10.3 | 89.7 | 89.0 |
| Means | 90.9 | 38.8 | 42.6 | 13.5 | 18.7 | 10.6 | 85.2 | 87.3 |

Table 5. Frequency of virulence (%) of *Xanthomonas oryzae* pv. *oryzae* isolates from different states against several rice varieties



Figure 1. Mean reaction of selected varieties against 27 isolates of Xoo

of virulent against it. On the other hand, rice varieties such as MR 106 and MR 159 recorded percentage lesion length of slightly less than 30%, suggesting that they were moderately resistant, and the isolates used as moderately avirulent.

Utilisation of resistant varieties as a management option to control pest and diseases is widely accepted. It is environmentally acceptable and relatively cheaper than using chemicals. However, deploying of resistant virieties as a mean to suppress pest and disease populations need a good management system. As with other bacterial pathogen, *X. oryzae* is represented by variable individuals with specialisation in their specificity of infection to differential varieties (Mew and Vera Cruz 1979; Eamchit and Mew 1982). Consequently, a variety might be resistant when it was first introduced into a locality inhibited by a majority of avirulent individuals. However, a wider and prolong cultivation may lead it to succumb to infection due to pathogen adaptation.

Development of new virulent strains, biotypes or pathotypes resulted from the selection processes, whereby avirulent individuals would die and diminish when they were forced to survive on a particular variety carrying a specific gene for resistance. However, within a population, there exist a small portion of virulent individuals, previously unnoticed, that would start to express, develop and slowly taking over, and becoming the majority members within that population. Survival of these individuals on the resistant plant may lead to resistance breakdown and possible death of the plant varieties that was originally resistant to the population. As such, the making of this resistance variety

approach was not always stable (Mew et al. 1992). The duration taken for the development of a new virulent pathotypes and the breakdown of a resistance variety varies. All this is partly due to the levels of resistance conferred by the operational gene of resistance and the virulence nature of the organism.

Because of that, a continuous monitoring process on the status of pathogen population in a each locality is needed. Once a new virulent pathotype developed, it should be replaced with other variety carrying different gene of resistance, where the common or majority of individual pathogens in the locality remain avirulent to that specific gene.

Results from the present study on collections of Xanthomonas oryzae isolates from major granary areas of Peninsular Malaysia indicated that about 6% of the isolates were avirulent against all the tested varieties. Their presence and infection would seldom cause damage to the crop. Another 30% from the 346 isolates could be grouped into pathotype $P_{1,0}$. Pathotype P₁₀ was only virulent against rice variety IR 8 which carried resistant gene Xa-11, but remains avirulent against other differential varieties used. Pathotype $P_{1,0}$ could be detected in all states. The presence and widely dispersal of this pathotype in all granary areas are expected since all varieties planted in the region carried the IR 8 gene. IR 8 is the first variety developed that has high yield potential in tropical Asia due to its non-lodging shorter plant and nitrogen responsive. It was widely used as a parent in the breeding programme that produced MR 84 (Alias et al. 2001).

The most virulent pathotype is $P_{7.7}$ which is capable of causing severe lesion on all differential varieties used. This pathotype is represented by an isolate MXO 92 which was collected from Kedah. However, most virulent pathotypes were only represented by a small number of isolates and limited to certain localities only. However, the virulent pathotype might develop and become

widespread if the susceptible varieties are introduced, widely accepted and planted in several consecutive years.

Conclusion

Rice variety MR 84, which was popular during the 90s, was found to be susceptible to most of X. oryzae isolates in all the localities. This was due to its prolong planting and, at the peak of its popularity, the variety covers more that 80% of the rice granary areas in the country. Because of that, other resistant rice varieties such as MR 219 and MR 220 were introduced to the farmers. These newly introduced varieties were able to suppress the disease incidences and reduced the potential crop loss. Other differential resistant varieties such as Cempo Selak, Zenith, IR 20, DV 85 and Patong 32 remain resistant to most isolates. These varieties could be utilised as the breeding donor for resistance against bacterial blight disease and should be released in a proper manner, so that their usefulness could be maximised.

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Abstrak

Satu kajian telah dijalankan terhadap himpunan pencilan *Xanthomonas oryzae* dari kawasan utama tanaman padi di Semenanjung Malaysia. Kajian terhadap 346 pencilan menunjukkan kebanyakannya (29.5%) dikelompokkan dalam patotip $P_{1,0}$. Patotip $P_{1,0}$ hanya virulen terhadap varieti IR 8 yang membawa gen rintang Xa-11, tetapi masih tak virulen terhadap beberapa varieti pembeza lainnya. Patotip ini boleh dijumpai di semua kawasan utama tanaman padi. Patotip paling virulen ialah $P_{7,7}$ yang menyebabkan bintik kerosakan teruk ke atas semua varieti pembeza yang telah dikaji. Namun demikian, kebanyakan patotip virulen hanya diwakili oleh sebahagian kecil daripada pencilan terkumpul dan terhad di beberapa kawasan sahaja. Varieti padi MR 84 yang popular pada tahun 90-an telah menjadi rentan terhadap pencilan utama. Namun demikian, varieti padi seperti Cempo Selak, Zenith, IR 20, DV 85 dan Patong 32 adalah rintang dan kebanyakan pencilan yang dikaji masih lagi tak virulen terhadap mereka.