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SHORT NOTES:

PATHOGENIC VARIATION AMONG MALAYSIAN ISOLATES OF *XANTHOMONAS ORYZAE*

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Keywords: Xanthomonas oryzae, pathogenic variation, Malaysian isolates, pathotypes

RINGKASAN

Satu kajian ke atas pelbagaian dua puluh isolat *Xanthomonas oryzae* telah menghasilkan keujudan lima pathotip. Sungguh pun pathotip 2 lebih predominan, pathotip 3 dan 4 adalah isolat yang paling virulen. Kaitan hasil kajian ini dengan program pembaikbaka padi juga dibincangkan.

INTRODUCTION

Several varieties of rice resistant to the bacterial blight disease have been developed in recent years. The resistance of some of these varieties have, however, 'broken down' with the evolution of new virulent isolates of the pathogen. In 1957, bacterial blight unexpectedly broke out on a resistant variety 'Asakaze' in Kyushu district of Japan and was attributed to a new strain of *Xanthomonas oryzae* (KUHURA *et. al.*, 1958). Since then, extensive studies on strains of *X. oryzae* have been made in Japan (EZUKA and HORINO, 1974; YAMAMOTO *et. al.*, 1977). In tropical Asia, wide variation in pathogenicity of *X. oryzae* has also been reported from India (RAO *et. al.*, 1971) and the Philippines (OU *et. al.*, 1971; MEW & CRUZ, 1979). An understanding of the pathogenic variation and specialization of the organism is, therefore, crucial to the success of the breeding programme for resistance against the disease.

This study attempts to provide information, hither to unavailable, on the pathogenicity of isolates of *X. oryzae* obtained from different areas of Peninsular Malaysia. It is intended that differential isolates, developed in the present study be used to classify the rice varieties in our germplasm collection.

MATERIALS AND METHODS

Six rice varieties IR8, IR20, IR2545-33, DV85, Chempo Selak and TN1 were used as differential varieties (*Table 1*) in the study of pathogenic variation among 20 isolates of *X. oryzae* (*Table 2*).

The experiment was conducted during two cropping seasons in 1979/80.

(a) Main Season (1979–80) Test:

Initially, fourteen isolates of the pathogen, collected from a number of rice growing regions of the country were tested on five varieties of rice. As a routine procedure before each test, the isolates were inoculated on TN1 plants and re-isolated.

The rice seeds were sown in 12 inches clay pots and the seedlings were thinned to three per pot. The plants were grown under uniform fertility conditions receiving NPK at the rate of 80:40:30. All the potassic and phosphatic fertilizers and half of the nitrogen

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TABLE 1. DIFFERENTIAL HOST VARIETIES USED IN PRESENT STUDY

Variety	*Genes for resistance	Source
IR8	None	IRRI
IR20	Xa 4	IRRI
IR1545-339	Xa 5	IRRI
DV85	Xa 5 + Xa 7	IRRI
Chempo Selak	Not known	IRRI
TN1	None	Bumbong Lima

*Mew & Cruz (1979).

fertilizer were applied basally. The rest of the nitrogen was applied at the tillering stage and before inoculation in equal proportions.

The bacterial isolates were cultured on Wakimoto's medium (Ou, 1972) for 72 hours at 28°C. The bacterial growth was then suspended in distilled water and the concentration of bacterial cells was adjusted to 0.9 optical density at 625 nm for use as inoculum.

TABLE 2. ISOLATES OF *XANTHOMONAS ORYZAE* TESTED AGAINST DIFFERENTIALS

Isolate	Season of collection	Place of collection
MXO 1	M/S 1978–79	Permatang Rambai, Seberang Perai
MXO 2	M/S 1978–79	Kampong Lembah, Seberang Perai
MXO 3	M/S 1978–79	Permatang Manggis, Seberang Perai
MXO 4	M/S 1978–79	Batu 18, Kedah
MXO 5	M/S 1978–79	Bumbong Lima, Seberang Perai
MXO 6	M/S 1978–79	Batu 4, Kerian
MXO 7	M/S 1978–79	Permatang Kuang, Seberang Perai
MXO 8	M/S 1978–79	Parit 300, Sungai Kota, Kerian
MXO 9	M/S 1978–79	Padang Halban, Salor, Kota Bharu
MXO 10	M/S 1978–79	Pasir Mas, Kota Bharu
MXO 11	M/S 1978–79	Titi Serong, Parit Buntar
MXO 12	M/S 1978–79	Hutan Kampong, Kedah
MXO 13	M/S 1978–79	Batu 5, Kedah
MXO 14	M/S 1978–79	Telok Chengai, Kedah
MXO 16	M/S 1979–80	Simpang Empat, Perlis
MXO 21	M/S 1979–80	Padang Serai, Kedah
MXO 23	M/S 1979–80	Kampong Serangas, Kuala Trengganu
MXO 27	M/S 1979–80	Kampong Jeram, Kuala Trengganu
MXO 28	M/S 1979–80	Kampong Gaung, Besut
MXO 29	M/S 1979–80	Kampong Bukit Beliti, Kuala Trengganu

TABLE 3. VARIATION IN PATHOGENICITY OF 14 ISOLATES OF *XANTHOMONAS ORYZAE* TO 5 IRRI VARIETIES (MAIN SEASON 1979–80)

	Varieties										
	IR8		IR20		IR1545–339		DV85		Chempo Selak		
Isolate	lesion length* (cm)	Disease reaction*	lesion length (cm)	Disease reaction	lesion length (cm)	Disease reaction	lesion length (cm)	Disease reaction	lesion length (cm)	Disease reaction	Virulence index
MXO 1	2.86	R	1.45	R	1.69	R	1.30	R	1.71	R	1.80
MXO 2	2.81	R	1.29	R	1.38	R	1.67	R	1.75	R	1.80
MXO 3	3.21	R	1.18	R	1.50	R	1.77	R	2.11	R	1.95
MXO 4	3.03	R	1.26	R	1.44	R	1.37	R	1.69	R	1.76
MXO 5	11.39	S	4.82	R	1.97	R	1.80	R	2.00	R	4.40
MXO 6	3.01	R	1.29	R	1.54	R	1.69	R	1.98	R	1.90
MXO 7	4.85	R	3.49	R	1.88	R	1.57	R	1.68	R	2.69
MXO 8	1.65	R	1.64	R	1.19	R	1.52	R	1.59	R	1.52
MXO 9	2.79	R	1.41	R	1.42	R	1.42	R	1.57	R	1.72
MXO 10	14.09	S	3.58	R	1.90	R	2.96	R	2.30	R	4.97
MXO 11	1.83	R	1.31	R	1.67	R	1.68	R	1.79	R	1.66
MXO 12	2.15	R	1.33	R	1.68	R	1.98	R	2.03	R	1.83
MXO 13	6.44	S	1.83	R	1.98	R	2.98	R	3.70	R	3.39
MXO 14	3.06	R	1.52	R	1.79	R	2.54	R	2.68	R	2.31

*Average of 15 leaves +R: resistant; S: susceptible (SES Scale, Anon, 1975)

Plants were inoculated at the 45 days old stage by the clipping method (KAUFFMAN *et al.*, 1973) on the upper two to three completely expanded leaves. The leaves were cut at about two to three cm from the tip with a pair of scissors previously dipped in bacterial suspension. Twenty days after inoculation, the overall nature of infection was scored according to the Standard Evaluation System (ANON, 1975). The extent of lesion development below the point of inoculation on 15 leaves selected at random from three hills was also measured.

A 'Virulence Index' was prepared by averaging the lesion lengths produced by a particular isolate on all varieties.

(b) Off Season (1980) Test:

An additional number of samples of bacterial leaf blight were collected during the off-season (1980). The pathogenic variability of 6 isolates were studied. Two virulent isolates, MXO5 and MXO13, from the previous season's test, were included for comparison. In addition to IR8, another susceptible variety (TN1) was used in this test.

Similar procedures as in the previous experiment were followed.

RESULTS AND DISCUSSION

(a) Main Season (1979–80) Test:

The average lesion length of 15 leaves and the reaction of each variety-isolate combination are presented in *Table 3.* The isolates varied in their virulence on the same variety and on different varieties. For example, IR8 appeared to be resistant to some isolates but susceptible to others. The other varieties appeared to be more resistant to all isolates.

Isolates MXO5, MXO10 and MXO13 were found to have the highest 'virulence indices'. There was no evidence of differential reactions among the isolates. However, the isolates could be classified into two groups, a higher virulence group consisting of MXO5, MXO10 and MXO13 and a lower virulence group consisting of the rest of the isolates.

(b) Off Season (1980) Test:

The reaction of six varieties of rice to eight bacterial isolates recorded 20 days after inoculation, are presented in *Table 4.* The isolates varied significantly ($P < 0.01$) in their virulence. Isolates MXO16 and MXO28

TABLE 4. VARIATION IN PATHOGENICITY OF 8 ISOLATES OF *X. ORYZAE* ON 6 DIFFERENTIAL VARIETIES (OFF-SEASON 1980)

	Lesion length (cm)*						
Isolate	IR8	IR20	IR1545–339	DV85	Chempo Selak	TN1	Virulence index
MXO 5	24.60	2.12	2.07	1.27	1.27	28.20	9.92
MXO 13	5.18	1.44	1.90	2.20	2.71	8.94	3.78
MXO 16	23.50	4.65	4.40	3.70	9.60	28.30	12.53
MXO 21	20.20	3.93	3.83	2.09	1.69	28.40	8.36
MXO 23	2.99	1.24	2.12	2.25	1.36	7.18	2.86
MXO 27	1.90	0.83	1.20	1.42	0.82	4.64	1.80
MXO 28	24.95	3.27	5.21	6.30	4.20	26.60	11.76
MXO 29	2.92	0.82	1.40	1.78	1.37	3.98	2.05
						LSD ($P < 0.01$)	5.67

Interaction effect (isolate × variety) significant at $P < 0.01$.

*Average of 20 leaves from 2 replications.

TABLE 5. REACTION OF SIX RICE DIFFERENTIAL VARIETIES TO FIVE PATHOTYPES OF *X. ORYZAE*

Pathotype	Reaction					
	IR20	IR1545–339	DV85	Chempo Selak	IR8	TN1
0	R	R	R	R	R	R
1	R	R	R	R	R	S
2	R	R	R	R	S	S
3	R	R	R	S	S	S
4	R	S	S	R	S	S

R: resistant (lesion length < 5 cm)

S: susceptible (lesion length ⩾ 5 cm)

were the most virulent as indicated by their higher virulence indices. Isolates MXO27 and MXO29 appeared to be less virulent having average virulence indices of 1.80 and 2.05 respectively. The isolates produced very small lesions even on IR8 and TN1, the most susceptible of the varieties used. The reaction patterns of isolates MXO5 and MXO13, appeared to be similar to those obtained in the main season test. Longer lesions were however produced by MXO5 on IR8 presumably due to the better condition of plants in this test.

The interaction effect between isolates and varieties was significant at $P < 0.01$, indicating the existence of pathogenic races among the isolates. Based on these interactions the isolates could be classified into five pathotypes viz. 0:MXO27 and MXO29; 1:MXO23, 2:MXO5, MXO13 and MXO21; 3:MXO16; 4:MXO28 (*Table 5*).

Isolates MXO16, and MXO28 (representatives of pathotype 3 and 4 respectively) had the broadest spectrum of virulence among the isolates tested. Varieties resistant to both pathotypes were resistant without any known genes for resistance, however, appeared to be the predominant virulent pathotype. The present collection of isolates is inadequate for determining the frequencies of pathotypes in the country and further studies with more extensive collections need to be done. In the meantime it is proposed that pathotype 2 (MXO5) be used in the present mass screening programme for resistance to bacterial blight. Varieties resistant to pathotype 2 may be screened against isolates of pathotypes 3 and 4 to determine sources of broad spectrum resistance.

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SUMMARY

A study of pathogenic variability among twenty Malaysian isolates of *Xanthomonas oryzae* revealed the existence of five pathotypes. Pathotypes 3 and 4 were the most virulent of the isolates tested but pathotype 2 appeared to be predominant. The relevance of these findings to the rice breeding programme is discussed.

REFERENCES

ANONYMOUS, (1975). Standard Evaluation System for Rice. International Rice Research Institute, Los Banos, Philippines.

EKRA, A. and HORINO, O. (1974). Classification of rice varieties and *Xanthomonas oryzae* strains on the basis of their differential interaction. *Bulletin of the Tokai-Kinki National Agriculture Experiment Station 27:* 1-19.

KUHARA, S., SEKIYA, N. and TAGAMI, Y. (1958). On the pathogen of bacterial leaf blight of rice isolated from severely affected area where resistant variety was widely cultivated. *Annuals of the Phytopathological Society of Japan 23:* 9.

KAUFFMAN, H.E., REDDY, A.P.K., HSIEH, S.P.Y. and MERCA, S.D. (1973). An improved technique for evaluating resistance of rice varieties to *Xanthomonas oryzae. Plant Disease Reporter* 57(6): 537-541.

MEW, T.W., and VERA CRUZ, C.M. (1979). Variability of *Xanthomonas oryzae*. Specificity in infection of Rice Differentials. *Pytopathology, 69:* 152-155.

OU, S.H. (1972). Rice Diseases. Commonwealth Mycological Institute. 368 pp.

OU, S.H., NAQUE, F.G. and SILVA, J.P. Pathogenic variation among isolates of *Xanthomonas oryzae* of the Philippines. *Plant Disease Reporter 55:* 22-26.

RAO, Y.O., MOHAN, S.K. and REDDY, P.R. (1971). Pathogenic variability in *Xanthomonas oryzae. Disease Reporter 55:* 593-595.

YAMAMOTO, T., HARTINI, H.R., MACHMUD, NISHIZAWA, M.T. and TANTERA, D.M. (1977). Variation in pathogenicity of *Xanthomonas oryzae* (Uyeda et Ishiyama) Dowson and resistance of rice varieties to the pathogen. *Contributions of the Central Research Institute for Agriculture, Bogor 28:* 1-22.