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MULTISTAGE TESTING OF BLAST RESISTANCE IN RICE

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RINGKASAN

Artikal ini cuba memberi satu gambaran lengkap berkenaan dengan penanaman pokok menentang penyakit karah mengikut sistem kajian tertentu pada beberapa peringkat hidupan pokok. Kajian 'specific' dan 'non-specific resistance' yang menghasilkan dua jenis yang tahan, RC6 dan RC7, dalam tempuh empat tahun, diterangkan.

INTRODUCTION

During the past decade, blast resistance breeding programmes in many countries have relied on sources of resistance identified by the International Blast Nursery (IBN) method of screening seedlings for disease resistance (OU & JENNINGS, 1969). Work on field or non-specific resistance has also been conducted almost exclusively on seedlings (SAKURAI & TORIYAMA, 1967; OU *et al.*, 1971).

The blast organism, *Pyricularia oryzae*, is however known to attack the rice plant at all stages of growth. Recently there has been increasing controversy on the ability of a single stage testing method to measure disease resistance throughout the life cycle of the plant. For example it has been reported that the susceptibility of certain varieties to panicle blast is not evident from their reaction to seedling blast (WILLIS *et al.*, 1968; BUDDENHAGEN, 1974). Other varieties appear to be highly susceptible in the nursery but rarely suffer severe attack in the field (KHUSH, 1973). Similar differences in seedling and adult plant reaction have been observed in other crops (HOOKER & SAXENA, 1971).

Improvements to the IBN method of screening at Bumbong Lima have resulted in nursery reactions which are better correlated with field reactions (CHIN, 1974a). The modified technique however, whilst invaluable for use in the elimination of susceptible materials in early generation progenies, requires to be complemented by the testing of more advanced lines at other stages of plant growth.

This paper reports on the systematic evaluation of blast resistance at different stages of plant growth and its contribution towards the development of resistant lines of rice. The term specific (vertical) resistance employed in this paper has been defined elsewhere (ANON, 1972). The term non-specific (horizontal) resistance as used here however, does not conform strictly to ROBINSON'S (1969) definition, but implies resistance in a quantitative sense as described originally by OU (1972).

MATERIALS AND METHODS

In 1970, crosses between Jaya and either Tetep or Tadukan were effected by the Breeding Section of the Rice Branch, MARDI. The objective was to incorporate the broad spectrum of resistance present in the latter two varieties (OU *et al.*, 1971; CHIN 1975), into the highly susceptible variety Jaya. Two successive backcrosses to Jaya were made to recover the agronomic/plant type characteristics of Jaya.

A schematic illustration of the assessment tests conducted during various progeny generations is presented in *Figure 1*.

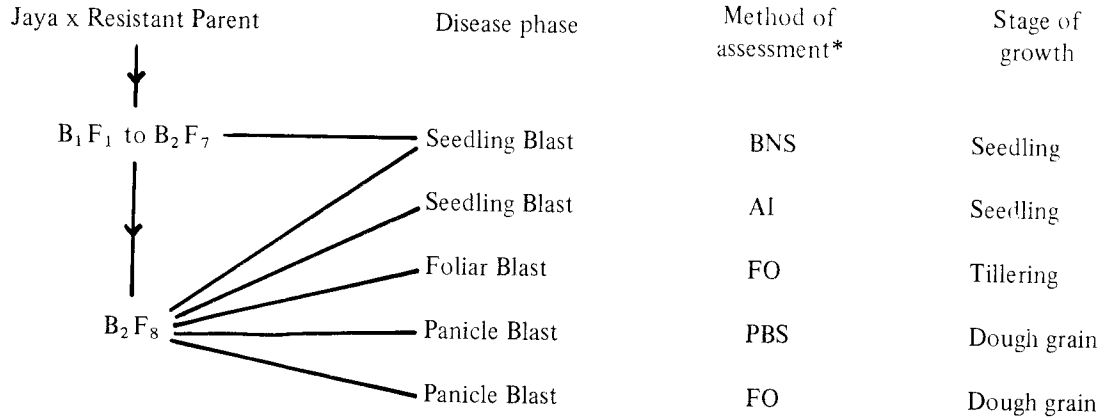
Due to the large volume of materials handled in the early generations, assessment of blast resistance was based mainly on the improved IBN method (CHIN, 1974a).

Plants at the B_2F_8 generation were subjected to multistage testing for resistance. At the seedling stage, screening was conducted in the dryland blast nursery as in earlier generations. However, in addition to the main nursery at Bumbong Lima, tests were conducted in nurseries at three other widely separated locations in the country (viz. Telok Chengai, Lundang and Tanjong Karang) in order to expose the material to a wide spectrum of blast races. Selected lines were tested for non-specific resistance by artificial inoculation of the seedlings in the greenhouse (see Non-specific Resistance Test below).

In the field, plants at the tillering stage were assessed visually for foliar blast severity according to the standard area diagrams developed by CHIN & HO (1973). At maturity all entries were screened for panicle blast resistance. Field observations on panicle blast severity at adaptability trial sites were also conducted.

Panicle Blast Screening (PBS)

Entries for testing were transplanted in micro plots of 0.8m x 1.6m at a spacing of 20cm x 20cm (i.e. 4 x 8 = 32 hills) with 2–3 seedlings per hill. Control plots of the susceptible variety Jaya were located after every tenth testing plot.



*BNS : Blast Nursery Screening (Dryland Nursery/Bumbong Lima and other locations. See text).
PBS : Panicle Blast Screening (Field/Bumbong Lima).
AI : Artificial Inoculation (Greenhouse/Bumbong Lima).
FO : Field Observations (Field/Bumbong Lima and adaptability trial sites).

Figure 1. Steps in the assessment of blast disease resistance at different stages of plant growth.

Fertilizer was applied at the rate of 120 : 40 : 30, NPK. Nitrogen was applied as 1/3 basal and 1/3 each for the first and second top dressings. The other nutrients were applied as basal dressings only.

At the dough grain stage 10 hills were harvested at random from the centre of each test plot. A random selection of 100 tillers was made from each sample of 10 hills and the number of panicles infected by blast was counted. Field techniques developed previously (CHIN, 1974b) were used to identify lesions caused by blast.

Tests conducted on lines RC6 (RU370–54–1–5–1–2), RC7 (RU370–54–1–5–3–1), RC8 (RU370–33–2–2–2) and RC9 (RU371–41–3–5–2) were replicated three times. Other entries were not replicated.

Non-specific Resistance Test (Artificial Inoculation)

As a result of the above tests and other agronomic criteria like yield and earliness of maturation, three of the above four lines RC6, RC7 and RC9, were subjected to further tests for non-specific resistance.

Single conidial isolates of *P. oryzae* were obtained from IBN type '4' (susceptible) lesions occurring on each of the three lines in the blast nursery. Inoculum was multiplied by the following method:-

The isolates were cultured on liquid potato sucrose medium for five days. The culture solution was then decanted and the mycelial mat rinsed with sterile distilled water. Abundant conidia were produced on the mycelial mats after a further incubation period of three days under fluorescent light.

Plants used for inoculation were grown in the greenhouse in wooden nursery boxes (30cm x 30cm x 12cm) under upland conditions. Each box contained ten 'varieties' viz, eight International Differential varieties (ANON, 1967), Jaya and one of the three progeny lines RC6, RC7 or RC9.

One row of each 'variety' was sown. The rows (12cm x 3cm) were spaced 3cm apart. Seeding rate was 36 seeds per row. Fertilizer was applied at the rate of 3 g. Ammonium Sulphate and 1 g. of each of Muriate of Potash and Christmas Island Rock Phosphate.

A spore suspension (adjusted to 3–4 x 10^4 spore/ml) of each isolate was prepared in sterile distilled water and inoculated by spraying with an atomiser onto plants at the 3–4 leaf stage. Each isolate was only inoculated on plants in boxes containing the progeny line from which it was obtained. After inoculation, the plants were incubated in a moist chamber without light for 24 hours. They were then transferred to the greenhouse and disease reactions on the youngest fully expanded leaf were taken seven days after inoculation. Lesions of the IBN type '1–3' (OU, 1965), were considered resistant (R) and those of type '4' and above were classified as susceptible (S). In each box the total number of 'susceptible' lesions on twenty plants selected at random from the progeny row and from Jaya were also recorded.

In order to avoid erroneus readings due to contamination by airborne spores during incubation, the results of each inoculation was checked against an uninoculated control.

The international race numbers of the isolates were determined by their reactions on the International Differential Varieties (ANON, 1968).

RESULTS AND DISCUSSIONS

IBN Screening at Bumbong Lima

The seedling reactions of some B_2F_5 to B_2F_8 generation lines in the dryland blast nursery are listed in *Table I.* Data on the amount of leaf blast on transplanted plants at the tillering stage in the field are included for comparison.

In the blast nursery, resistant reactions (type '1 to 3' in IBN scale) were recorded for many lines e.g. RU370 54–1–5–1 up to B_2F_7. At B_2F_8 however susceptible reactions (type 4) were observed on these lines. This apparent breakdown of specific resistance was accompanied by similar changes in the reactions of Tadukan and Tetep (the resistant donor parents) in the nursery. These changes indicate that the race population in the nursery at this generation included race(s) which are pathogenic to the plants.

However few lesions of type '4' occurred on the leaves. In contrast to the highly susceptible parent 'Jaya' on which numerous lesions occurred killing the plants (scale '7'), the lesions remained few on the resistant progeny lines. Numerous hypersensitive or resistant spots (type '1' or '2') however occurred on their leaves suggesting that these lines possessed specific resistance to most of the races present in the nursery.

Two reasons may be advanced to explain the relative paucity of lesions on the resistant lines. As these lines were susceptible in the qualitative sense (showing 'susceptible' or IBN type '4' lesions), but produced few lesions they appeared to possess a quantitative or non-specific form of resistance. Alternatively the few lesions present on the lines could be due to a low concentration of conidia of the virulent race(s) present in the blast nursery.

Non-specific Resistance Test

Results from the greenhouse inoculation tests support the former hypothesis that the lines possess non-specific resistance. When each of the progeny liens, RC6, 7 or 9 were inoculated together with Jaya, using heavy conidial concentrations of virulent races originally isolated from the lines, fewer lesions appeared on the lines than on Jaya.

As an example, race ID–11 produced 8 lesions per seedling on Jaya but only 2 per seedling on RC6. Similarly race ID–13 produced 12.4 lesions per seedling on Jaya but only 4.7 per seedling on RC7. The differences in numbers of susceptible lesions between Jaya and each of the progeny lines are illustrated in *Figure 2.*

The reactions of the isolates on the International Differential Varieties and their International Race designations are listed in *Table II.*

IBN Screening at Other Locations

Results of the screening of four progeny lines, RC6, RC7, RC8 and RC9 at Bumbong Lima and three other locations, Telok Chengai, Tanjong Karang and Lundang are presented in *Table III.*

TABLE I. BLAST REACTIONS OF PROGENY LINES B_2F_5 TO B_2F_8 AT SEEDLING AND TILLERING STAGES OF GROWTH

Cross & Designation	IBN (Seedling) Reaction							*Foliar Blast (Tillering)	**Adapt Trial
Jaya^3/Tadukan	B_2F_5	Line No.	B_2F_6	Line No.	B_2F_7	Line No.	B_2F_8		
RU370–14–2	1 to 3	–1	1 to 3	–4	1		4	–	
RU370–14–3	1 to 3	–2	1 to 4	–5	1				
RU370–33–2	1 to 2	–2	1 to 3	–2	1		4	–	S
RU370–34–3	1 to 2	–3	1 to 3	–7	1	–2	4/5	–	
						–4	4/5	–	
RU370–54–1	1 to 2	–5	1	–1	1	–2	4	–	S
				–3	1	–1	4	+	S
				–5	1	–5	4	+	
RU370–54–3	4 to 6	–4	4 to 7	–1	4	–4	6	++	
				–3	4	–4	6	+++	
				–4	4	–5	6	+++	
				–5	4	–5	6	++	
RU370–58–1	2 to 5	–4	5 to 7	–5	5	–5	6	++	
RU370–75–1	2 to 5	–1	3 to 4	–3	4				
		–2	4 to 7	–5	5				
Check varieties									
Jaya	6		6		7		7	++	
Tadukan	1		1		1		3/4	–	
Jaya^3/Tetep									
RU371–21–1	1 to 2	–3	4 to 7	–5	4	–4	7	+++	
RU371–28–1	1 to 2	–4	2 to 4	–2	3	–3	4	+	
		–5	2 to 4	–1	3	–1	4	–	
						5	4	–	
				2	3	–1	5	++	
				–4	3	–1	5	++	
						–4	5	+	
				–5	3	–1	4	+	
RU371–28–2	1 to 2	–5	1 to 3	–2	1		4	+	
				–4	1	–2	4	+	
RU371–41–3	1 to 2	–5	1	–2	4		4	–	S
Check varieties									
Jaya	6		6		7		7	+++	
Tetep	1		1		1		3/4	–	

*The symbols –, +, ++, +++ correspond to the 0, 1, 5 and 25% scales of disease severity developed by CHIN & HO, (1973).
**"S" denotes selection for yield/adaptability trials based on agronomic and disease resistance criteria.

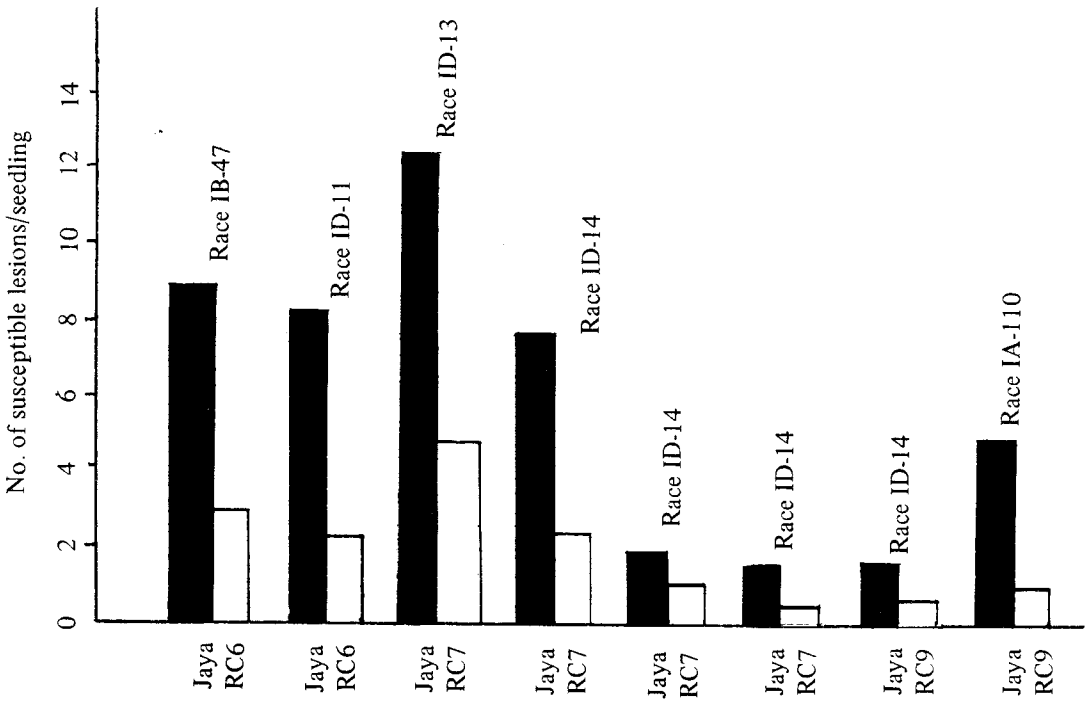


Figure 2. Numbers of susceptible lesions per seedling on Jaya and progeny lines RC6, RC7 & RC9 inoculated with virulent races of P. oryzae.

The lines were generally more resistant in the other locations than at Bumbong Lima. Only one line RC9 was susceptible at Telok Chengai, none were susceptible at Tanjong Karang and three RC6, 7 and 8 were susceptible at Lundang. However, at no location did any line exceed reaction '4'. Since the race populations in the four locations are different (CHIN, unpublished), the four lines appear to have a broad spectrum of resistance to the disease.

Foliar Blast Observations

The data on leaf blast severity in the field show good correlation with that on seedling blast in the nursery (see *Table I*). Lines with type '4' reaction in the nursery showed few to no lesions (less than 1% of leaf area infected) in the field. Those with reactions exceeding scale '4' showed more severe (5 to 25% infection) leaf blast in the field. Generally, since the disease pressure is usually higher in the blast nursery than in the field, lines with type '4' reactions are highly resistant in the field.

Panicle Blast Screening

Results from the panicle blast screening of twenty two entries are listed in *Table IV*. Blast nursery reactions of these entries at the B_2F_8 generation are included for comparison.

TABLE II. INTERNATIONAL RACE DESIGNATIONS OF ISOLATES USED IN THE STUDY OF NON-SPECIFIC RESISTANCE OF RC6, RC7 AND RC9

Isolate No.	Source	*Blast Reaction A	B	C	D	E	F	G	H	International Race Designation
Py 1.1	RC6	R	S	R	S	R	R	R	S	IB – 47
Py 1.2	RC6	R	R	R	S	R	S	R	S	ID – 11
Py 2.1	RC7	R	R	R	S	R	R	S	S	ID – 13
Py 2.2	RC7	R	R	R	S	R	R	S	R	ID – 14
Py 2.3	RC7	R	R	R	S	R	R	S	R	ID – 14
Py 2.5	RC7	R	R	R	S	R	R	S	R	ID – 14
Py 4.2	RC9	R	R	R	S	R	R	S	R	ID – 14
Py 4.3	RC9	S	R	R	S	R	R	S	R	IA – 110

International Differential Varieties:

A : Raminad Str. 3
B : Zenith
C : NP – 125
D : Usen
E : Dular
F : Kanto 51
G : Sha-tiao-tsao
H : Caloro

*R – Resistant
S – Susceptible

TABLE III. IBN REACTIONS OF RC6, 7, 8 & 9 AT FOUR SCREENING LOCATIONS

Cross & Designation	Bumbong Lima	Telok Chengai	Tanjong Karang	Lundang
Jaya[3]/Tadukan				
RC6 (RU–370–54–1–5–1)	4	3	1	4
RC7 (RU–370–54–1–5–3)	4	3	2	4
RC8 (RU–370–33–2–2–2)	4	3	3	4
Jaya[3]/Tetep				
RC9 (RU–371–41–3–5)	4	4	2	3

Many lines which had type '4' reactions in the blast nursery also showed good resistance to panicle blast e.g. RC6, RC7 and RC8, all showed less than 10% panicle infection. In comparison the susceptible check 'Jaya' showed a mean of over 80% infection.

In general there appears to be good correlation between blast nursery reactions and the severity of panicle blast (correlation coefficient = 0.87 at P = 0.001).

However, the nursery reactions of some lines did not indicate their susceptibility to panicle blast e.g. RU371–28–1–4–2–3 gave a type '4' reaction in the nursery but was highly susceptible to panicle blast (73.5% infection). RU371–28–1–5–1–1 also showed a type '4' reaction but was moderately susceptible (27.2% infection) to panicle blast.

Replicated Panicle Blast Screening

Data on the replicated tests conducted on RC6, RC7, RC8 and RC9 are presented in *Table V*. Although the lines gave identical reactions in the blast nursery, significant (P = 0.05) differences in panicle blast severity among the lines were obtained. Among the four lines RC9 was most susceptible to panicle blast (16.2% infection). RC6, RC7 and 8 were less susceptible with 6.7%, 7.9% and 1.2% panicles infected respectively.

All four lines were however relatively resistant in comparison to the susceptible check Jaya' which had an average of 88.6% infection.

Panicle Blast Observation at Adaptability Trial Sites

Little to no panicle blast was observed on RC6, 7, 8 and 9 at the adaptability trial sites. Mean disease severity from five sites ranged from 1.4% to 1.9% of panicles infected. In comparison a susceptible line RC2, showed a mean of 9.2% infection at the five sites.*

These observations are in agreement with the panicle blast screening results conducted at Bumbong Lima.

CONCLUSIONS

The studies reported in this paper suggest that, whilst the IBN method of disease screening is invaluable in the evaluation of large volumes of early generation material in breeding programmes, disease assessment at different growth stages is necessary in advanced generations. A complete resistance profile of potential varieties should be developed through laboratory, nursery and field studies.

In general, the seedling test in the blast nursery gives a good indication of adult plant resistance. However a few lines showed very different adult and seedling reactions. Further, even among lines which appeared to give similar reactions at the seedling and adult stages of growth, smaller but significant differences occurred in panicle blast severity (see *Table V*). Tests on panicle blast resistance are therefore necessary, particularly on material which have already been selected for seedling blast resistance.

The seedling test also gives a good indication of foliar blast resistance at the tillering stage in the field. Infection is however generally lower in the field where disease pressure is usually less than in the blast nursery. Generally, material showing IBN type '4' reactions in the nursery are very resistant in the field.

TABLE IV. CORRELATION BETWEEN PANICLE BLAST SEVERITY AND SEEDLING BLAST REACTION

Cross & Designation	Adaptability Trial No.	% Panicle Infected	IBN Reaction
Jaya^3/Tadukan			
RU370–14–2–1–4	–	25.9	4
RU370–33–2–2–2	RC8	1.0	4
RU370–34–3–3–7–2	–	5.2	4/5
RU370–34–3–3–7–4	–	7.0	4/5
RU370–54–1–5–1–2	RC6	5.7	4
RU370–54–1–5–3–1	RC7	8.4	4
RU370–54–1–5–5–5	–	4.8	4
RU370–54–3–4–1–4	–	77.9	6
RU370–54–3–4–3–4	–	80.0	6
RU370–54–3–4–4–5	–	79.6	6
RU370–54–3–4–5–5	–	72.0	6
RU370–58–1–4–5–5	–	79.2	6
Jaya (susceptible check)		92.2	7
Jaya^3/Tetep			
RU371–21–1–3–5–4	–	75.3	7
RU371–28–1–4–2–3	–	73.5	4
RU371–28–1–5–1–1	–	27.2	4
RU371–28–1–5–1–5	–	35.1	4/5
RU371–28–1–5–2–1	–	40.3	5
RU371–28–1–5–4–1	–	53.0	5
RU371–28–1–5–4–4	–	30.9	5
RU371–41–3–5–2	RC9	12.7	4
Jaya (susceptible check)		85.2	7
		Correlation coefficient = 0.87	
		Significant level = 0.001	

*data courtesy of Y.H. Chen

TABLE V. DIFFERENCES IN PANICLE BLAST SEVERITY BETWEEN LINES WITH SIMILAR SEEDLING BLAST REACTIONS

Line	Designation	% Panicle Infected (mean of 3 replicates) +Transformed	Actual	IBN Reaction
RC6	RU370–54–1–5–1–2	15.0	6.7	4
RC7	RU370–54–1–5–3–1	16.3	7.9	4
RC8	RU370–33–2–2–2	6.4	1.2	4
RC9	RU371–41–3–5–2	23.7	16.2	4
	Jaya	70.3	88.6	7
		s.e. = 1.7		
		LSD (5%) = 4.0		

+In equivalent angles.

Discrepancies between seedling/foliar blast severity and the amount of panicle blast at maturity have also been previously reported (CHIN, 1974a). There is at the moment insufficient experimental results a draw any conclusions but it is suggested here that such consistent but small discrepancies could be due to different but closely linked genes controlling foliar and neck blast resistance. The situation is also complicated by the existence of numerous and highly variable races of the pathogen.

Varieties/lines which are originally resistant (IBN scale '1–3') when first tested often breakdown to scale '4' (susceptible) in subsequent generations. It is important to test if such varieties will deteriorate further (to scale 5 and above) when planted widely in the field and conidia of virulent races increase in number. The inoculation test reported here (and originally proposed by OU, 1972b) suggests that the three lines RC6, 7 and 9 possess some non-specific resistance to the disease. Numbers of lesions on Jaya (susceptible check) exceeded those on the progeny lines from 1.5 to 5.9 times when similar high concentrations of conidial suspension were inoculated on the plants. However the level of quantitative resistance reported here is not as high as that reported by OU, *et al.*, (1971) for 'Tetep' and 'Carreon'.

The resistant reactions of the progeny lines in screening tests at locations with different race populations are a further indication of the broad spectrum of resistance present in these lines.

In conclusion the results indicate that RC6 or RC7 may be released as a resistant replacement for Jaya. Under field conditions little to no foliar blast is expected. Panicle blast occurrence is also likely to be limited. Further the studies indicate that these lines possess a quantitative or non-specific resistance to the disease and are therefore unlikely to deteriorate rapidly.

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SUMMARY

An attempt is made to provide a complete profile of plant resistance to the blast disease by systematic testing at different stages of plant growth. A five-year study on specific (vertical) and non-specific (horizontal) resistance leading to the selection of two resistant lines RC6 and RC7 is described.

REFERENCES

ANON. (1968). Standardization of the international race numbers of *P. oryzae* Cav. I.R.C. Newsletter *18*(2) : 1–6.

ANON. (1972). Genetic vulnerability of major crops. Natn. Acad. Sci. Washington, D.C. 307pp.

BUDDENHAGEN, I.W. (1974). An overview of rice pathology in India. Rice Path. Newsletter 1/74 : 19.

CHIN, K.M. & HO, B.L. (1973). Assessment keys for some rice disease, MARDI Report, *4*, 5pp.

CHIN, K.M. (1974a). Evaluating blast resistance. 9pp. Unpublished.

CHIN, K.M. (1974b). Comparative symptoms of ear blight and neck rot diseases of rice. *MARDI Res. Bull. 2*(2) : 7- 10.

CHIN, K.M. (1975). Recommended sources of blast resistance. 7pp. *MARDI Res. Bull.* 3,1: (71–74).

HOOKER, A.L. and K.M.S. SAXENA, (1971). Genetics of disease resistance in plants. Ann. Rev. Genetics *5* : 407–424.

KHUSH, G.S. (1973). Personal communication.

OU, S.H. (1965). A proposal for an international program of research on the rice blast disease. *In* The Rice Blast Disease, Proc. Symp. at IRRI, July 1963 : 441–6., John Hopkins Press, Baltimore, Maryland.

OU, S.H. and P.R. JENNINGS, (1969). Progress in the development of disease resistant rice. Ann. Rev. Phytopath. *7* : 383–410.

OU, S.H., NUQUE, F.L. EBRON, T.T. and V.A. AWODERU (1971). A type of stable resistance to blast disease of rice. Phytopathology *61*(6) : 703–706.

OU, S.H. (1972a). Studies on stable resistance to rice blast disease. *In* Rice Breeding : 227–236, International Rice Research Institute, Los Banos, Philippines.

OU, S.H. (1972b). Rice diseases. Kew Commonwealth Mycological Institute 1972, 368pp.

ROBINSON, R.A. (1969). Disease resistance terminology. Rev. Plant Path. *48* : 593–606.

SAKURAI, Y. and K. TORIYAMA (1967). Field resistance of the Rice plant to *Piricularia oryzae* and its testing method. Proc. Symp. on rice diseases and their control by growing resistant varieties and other measures. Agric. For. Res. Council, Min. Agric. For., Japan.

WILLIS, G.M., ALLOWITZ, R.D. and E.S. MENVIELLE, C.S. (1968). Differential susceptibility of rice leaves and panicles to *Piricularia oryzae*. Abs. in Phytopathology *58* : 1072.