

## Small differential interactions for partial resistance in rice cultivars to virulent isolates of the blast pathogen

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### Summary

Six rice genotypes, differing in partial resistance, were exposed to three isolates of the blast pathogen. Of the variance due to host and pathogen genotypes, 39% was due to host genotype effects, 60% was due to isolate effects, and only 1% was due to host genotype  $\times$  isolate interactions. Although small, this interaction variance was highly significant and mainly due to the IR50  $\times$  W6-1 and IR37704  $\times$  JMB8401-1 combinations. Although behaving largely as race-non-specific (large main effects only), the partial resistance cannot be classified as race-non-specific. The results suggest that minor genes for partial resistance operate in a gene for gene relationship with minor genes in the pathogen.

### Introduction

Blast disease of rice, caused by *Magnaporthe grisea* (anamorph *Pyricularia oryzae*) is an important disease in most of the world's rice growing areas. A high degree of control against the disease can be obtained by planting resistant cultivars. However, in only a few cases breeders have been successful in breeding cultivars with resistance that is durable. The complete resistance that is usually selected for, is controlled by hypersensitivity genes. This type of resistance is highly race-specific, and the pathogen population appeared to be able to adapt to such cultivars quite easily, often causing a quick break down of the resistance (Ezuka, 1972; Jeanguyot, 1983). Partial resistance (PR), on the other hand, is reported to be associated with durability (Ezuka, 1979; Yeh & Bonman, 1986). Unfortunately, selection for PR to blast is difficult. In the field, a mixture of races is usually present and this compli-

cates screening for PR due to epistatic effects of hypersensitivity genes on the expression of PR (Ezuka, 1979; Notteghem, 1993). As an alternative, Niizeki (1967) and Sakurai & Toriyama (1967) recommended to screen for PR in the greenhouse using a single isolate with as many virulence factors as possible. Since partial resistance in the temperate Japonica cultivars appeared to be largely race-non-specific (Ezuka, 1972; Yunoki et al., 1970), the same approach was followed for tropical Indica cultivars in a study by Roumen (1992).

However, what may be true for temperate Japonica cultivars is not necessarily true for tropical Indica cultivars. It is by no means certain that using a single virulent race for screening of partial resistance is representative for all virulent races. Therefore, the research described in this paper aimed at detecting interactions between virulent pathogen races and rice genotypes for PR to leaf blast.

## Material and methods

The rice genotypes CO39, IR36, IR37704-98-3-3-2, IR50, IR64 and IR66 were used, representing a range of PR to leaf blast (Roumen, 1992). In the remainder of this paper, IR37704-98-3-3-2 is abbreviated to IR37704. The pathogen isolates used were Po6-6, JMB8401-1 and W6-1. These isolates can be considered distinct races since they induce a differential reaction on certain rice genotypes (Table 1). However, on the rice genotypes used in the present study, these isolates produced a susceptible infection type.

Three consecutive experiments were planted, with an interval of three weeks between each experiment. Plants were grown in plastic trays (24 × 30 cm) in a greenhouse. In total, 18 trays were sown per experiment. Per tray, six rows of 10 plants were sown with the six rice genotypes randomized over the rows.

Plants were grown under non-flooded conditions. When the plants reached the second leaf stage, nitrogen was applied at an equivalent of 5 g/m<sup>2</sup> by adding an ammoniumsulfate solution to each tray. The same amount of nitrogen was applied when the plants reached the fourth leaf stage and again one day before inoculation. The plants were inoculated when most of the plants had reached the sixth leaf stage. In the morning of the day of inoculation, the point of emergence of the youngest leaf was marked with a felt-tip pen. Six (random) trays were inoculated per isolate.

Spores of each isolate were produced by culturing the isolates on a medium in Petri dishes, similar as described by Bonman et al. (1986). JMB8401-1 and W6-1 were grown on rice polish agar (20 g rice polish, 5 g saccharose and 18 g bacto-agar per litre distilled water, with a pH set to 6), but Po6-6 was grown on prune agar, since this isolate sporulates much better on this medium (3 pieces of prunes, 1 g yeast extract, 5 g bacto-agar per litre distilled water, with a pH set to 6). Inoculation was done shortly before sunset inside a plastic cage (one cage per isolate). Per batch of six trays, 400 ml of a solution with  $5 \times 10^4$  spores/ml in 0.5% gelatine was sprayed over the plants as a fine mist using an electric sprayer. After inoculation, all trays were

placed together in another plastic cage to ensure a high humidity until the next morning. The trays were then returned to the greenhouse.

Six days after inoculation, the number of sporulating lesions on the leaves of the main culm was counted, and the total number of sporulating lesions and the number of leaves with at least one sporulating lesion were calculated for each plant.

Statistical analysis (ANOVA) was done using the mean values of each plant row as experimental unit. The experiments were analyzed as a split plot design with trays as main plots and rows as sub plots. For the analysis, GENSTAT was used on a VAX mainframe computer. Since the number of sporulating lesions that is formed was previously shown to be the most important component of PR in these rice genotypes (Roumen, 1992), interactions for the number of lesions were assumed to be representative for interactions for PR.

## Results

The capacity to induce sporulating lesions on the tested rice genotypes differed remarkably between the pathogen isolates. Isolate W6-1 caused the

Table 1. Infection type<sup>1</sup> of seven selected rice genotypes after inoculation at the sixth leaf stage with three *Magnaporthe grisea* isolates

Rice genotype	Isolate		
	JMB8401-1	W6-1	Po6-6
Surjamkuhi	6	0	0
Azucena	3	3	5
Lubang	2	1	5
Tres Meses	0	1	5
Malos	0	3	5
Kuroka	0	5	6
C22	0	6	6

<sup>1</sup> 6: large spindle shaped sporulating lesions without dark margin

5: large spindle shaped sporulating lesions with dark margin

4: small spindle shaped sporulating lesions with dark margin

3: more or less round, small sporulating lesions with dark margin

2: more or less round, brown, non-sporulating lesions

1: tiny pinpoint size dark non-sporulating lesions

0: no visible symptoms

largest number of lesions on the genotypes in all three experiments, which was about five times more than the number of lesions resulting from inoculation with isolate JMB8401-1 (Table 2).

The host genotype effects on lesion number were very similar for the three isolates, despite the large difference for their aggressiveness (Table 3). Nearly all variation associated with host-pathogen effects was explained by main effects of isolates and genotypes (60 and 39%, respectively), while only 1% was due to isolate × host genotype interactions (Table 4). Most of the small, but highly significant isolate × genotype interaction was traced back to the W6-1 × IR50 and JMB8401-1 × IR37704 combinations (Table 3).

Furthermore, the ANOVA indicated a very small genotype × experiment interaction. This interaction appeared to be entirely derived from a significantly ( $P \leq 0.95$ ) lower number of sporulating lesions on IR50 in series 2 than in series 1 and 3, regardless of isolate.

The number of sporulating lesions that developed on the genotypes appeared to be closely associated to the average number of leaves on the main culm (from the top) with at least a single sporulating lesion. Regardless of isolate, the rank correlation coefficient between the two parameters was 0.92.

The average number of leaves per plant (from the top) with at least one sporulating lesion signif-

Table 2. The average number of sporulating lesions on leaves of the main culm for three *Magnaporthe grisea* isolates in three experiments, relative (%) to the mean value of isolate W6-1

Isolate	Experiments			Mean
	1	2	3	
W6-1	100 <sup>1</sup> a	100 a	100 a	100 a
Po6-6	24 b	78 a	31 a	44 b
JMB8401-1	17 b	23 b	23 b	21 c

<sup>1</sup> Within experiments, each value is the mean over six genotypes. 100% = 8.4, 9.3, and 11.4 lesions/plant for experiment 1, 2, and 3, respectively

Within columns, significant differences between isolates are indicated with different letters (Bonferroni's test for inequalities;  $\alpha = 0.05$ )

icantly differed between the isolates ( $P \leq 0.95$ ), indicating that some isolates have a greater capacity to successfully infect relatively old leaf tissue than others (Table 5). Although treatment differences were less clear when expressed in number of leaves than in number of lesions, counting the number of leaves with lesions still detected the relatively small interaction between isolate JMB8401-1 and rice line IR37704 (Table 5).

**Discussion**

The result that most of the variation for PR be-

Table 3. Average number of sporulating lesions in leaves of the main culm of six rice genotypes, relative to the value for CO39 for three isolates of *Magnaporthe grisea* in three experiments (Exp.)

Genotype	Isolate Po6-6				Isolate W6-1				Isolate JMB8401-1				Mean over isolates
	Exp.				Exp.				Exp.				
	1	2	3	Mean	1	2	3	Mean	1	2	3	Mean	
CO39	100	100	100	100	100	100	100	100	100	100	100	100	100 a <sup>3</sup>
IR50	40	31	44	38	55	38	52	48 <sup>1</sup>	38	22	39	33	40 b
IR37704	38	36	41	38	30	40	40	37	22	16	25	21 <sup>2</sup>	32 b
IR66	19	23	19	20	30	24	19	24	21	14	19	18	21 c
IR36	10	11	16	12	14	14	9	12	13	11	17	14	14 d
IR64	7	8	7	7	10	10	6	9	5	4	10	6	7 e

<sup>1</sup> Significantly higher ( $P \leq 0.01$ ) than the expected value of 37 without genetic interaction

<sup>2</sup> Significantly lower ( $P \leq 0.01$ ) than the expected value of 37 without genetic interaction

<sup>3</sup> Values followed by different letters are significantly different at  $\alpha = 0.05$  (Bonferroni's test for inequalities)

Table 4. Combined ANOVA over three experiments for the number of sporulating lesions per plant using the mean plant value per row as experimental unit after log (x + 1) transformation of the data

Source of variation	df	SS	MS	F	
Experiments	2	9.26	4.63		
Isolates	2	71.10	35.55	95.8	P ≤ 0.001
Main-plot residual	49	18.18	0.37		
Host genotype	5	113.71	22.74	300.3	P ≤ 0.001
Isolates × Host genotypes	10	5.29	0.53	7.0	P ≤ 0.01
Experiments × Host genotypes	10	1.84	0.18	2.4	P ≤ 0.05
Sub-plot Residual	242	18.33	0.08		
Grand Total	320	237.72			
Estimated grand mean	1.430				
Total df	324				
Number of missing values	3				

tween the rice genotypes was of a race-non-specific nature is in agreement with the general outcome of studies on interactions between rice genotypes and blast isolates for PR that were carried out in Japan (Sakurai & Toriyama, 1967; Kozaka, 1975; Ezuka, 1972, citing Asaga & Yoshimura, 1969; Toriyama, 1975, citing Niizeki, 1967 and Hirano & Matsumoto, 1971). Some exceptionally large genotype × isolate interactions for PR were observed by Yunoki et al. (1970), caused by the presence of a single gene, named Pi-f (Toriyama et al., 1968). Bonman et al. (1989) also observed a relatively large genotype × isolate interaction for PR, perhaps due to a gene similar to the Pi-f gene. In their study, Philippine isolates were far less aggressive on Korean rice cultivars than were Korean isolates, but all isolates were equally aggressive on the Indian cultivar CO39. In the present study, despite the considerable difference in aggressiveness between the isolates, the genotype ranking for PR did hardly change. Regardless of the race, PR was highest in IR64 and IR36, and screening for PR using just one of these three races would not have changed results of selection. The PR expressed in the rice genotypes was in good agreement with that in an earlier study (Roumen, 1992).

Considerable difference for aggressiveness among isolates was also observed in a field experiment where six defined isolates were tested to 15 rice genotypes in isolated field plots in Japan (Cen-

tral Agricultural Experiment Station, 1970; cited in: Ezuka, 1972). However, part of the difference in aggressiveness between the isolates in the present study could well be the result of some effect of the media on which the isolates were cultured. Some isolates are known to perform better on some media than on others (Otsuka et al., 1965) and this might influence the ability of the harvested spores to infect rice leaves.

The result that small, but significant interactions

Table 5. Average number of leaves on the main culm<sup>1</sup> with at least one sporulating lesion for six rice genotypes after inoculation with three isolates of *Magnaporthe grisea*, relative to the value of CO39 (= 100%)

Genotype	Isolate			Mean
	Po-6-6	W6-1	JMB8401-1	
CO39	100	100	100	100 a <sup>2</sup>
IR50	74	71	72	72 b
IR37704	78	78	56 <sup>3</sup>	71 b
IR 66	71	66	51	63 b
IR36	42	37	41	40 c
IR64	42	31	26	33 c
100% =	1.55 p <sup>2</sup>	2.02 q	1.23 r (leaves/plant)	

<sup>1</sup> Mean of three experiments

<sup>2</sup> Values followed by different letters are significantly different (Bonferroni's test for inequalities at  $\alpha = 0.05$ )

<sup>3</sup> Significantly lower ( $P \leq 0.05$ ) than the expected value of 72 in case of no genetic interaction

between rice genotypes and blast isolates were detected despite a relatively small sample size, indicates that such interactions are not uncommon. Genetic studies on other cultivars have shown PR to be oligo- or polygenically controlled with small effects for each gene (Higashi & Kushibuchi, 1978; Higashi & Saito, 1985; Notteghem, 1985), and among the cultivars used in the present study, evidence for oligo- or polygenic control of PR was found for IR36 and IR64 (Roumen, 1993). Therefore, the small interactions suggest that PR to leaf blast in these cultivars is controlled by minor genes which operate in a gene-for-gene relationship with minor genes in the isolates of the blast pathogen, similar as described by Parlevliet (1978) for PR genes to leaf rust in barley. As shown in a model study of Parlevliet & Zadoks (1977) and confirmed by an extensive study of this model using simulation (Jenns & Leonard, 1985), a gene-for-gene relationship in a polygenic system with relatively small effects of each gene would largely behave as race-non-specific. Assuming a polygene for polygene relationship, the significant interactions for IR50 and IR37704 suggest that these cultivars each have at least one PR gene that is not present in any of the other tested cultivars.

The high correlation between the number of sporulating lesions and the number of leaves on the main culm developing such lesions regardless of isolate and despite large differences in aggressiveness between the isolates, strongly supports earlier findings that a relatively high PR in a genotype is closely associated to a rapid increase of resistance with aging of the newly emerging leaves to high resistance levels (Roumen et al., 1992; Roumen, 1992).

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