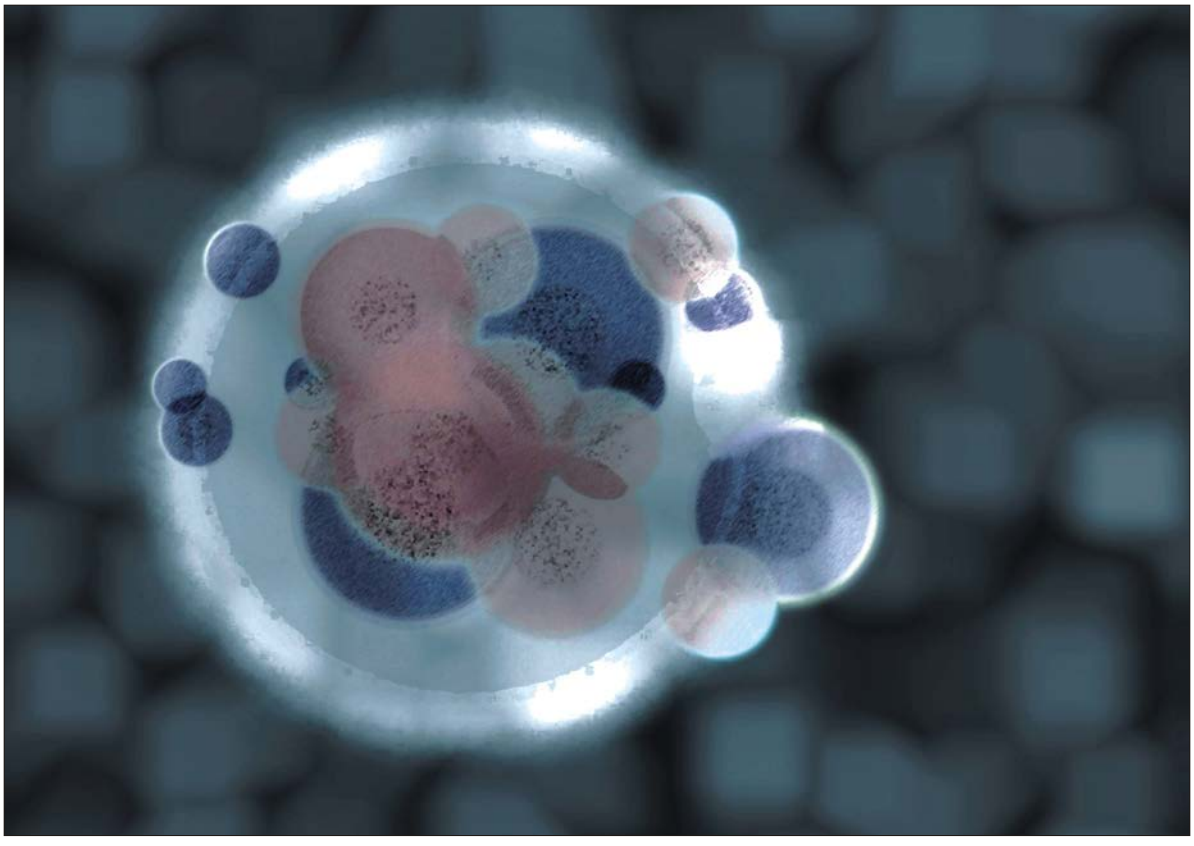

Journal of Biological Sciences

ISSN 1727-3048

science alert

ANSInet
an open access publisher
http://ansinet.com

Journal of Biological Sciences 4 (1): 62-71, 2004
ISSN 1727-3048

Genetic Analysis for Two Components of Field Resistance: Lesion Size and Number, to Rice Blast

[1]M.S. Ali, [2]S.Khatun, [3]M.K. Bashar, [2]M.S. Alam, D. Purba, [4]M. Kawase, [4]K. Okuno and S. Kiyosawa
Tsukuba International Agricultural Training Centre, Tsukuba 305, Japan
[1]Biotechnology Division, [2]Plant Breeding Division, BRRI, Gazipur 1701, Bangladesh
[3]Genetic Resources and Seed Division, BRRI, Gazipur 1701, Bangladesh
[4]National Institute of Agrobiological Resources, Tsukuba 305, Japan

Abstract: In two tests, 108 and 96 F_3 lines derived from a cross of rice varieties, Nipponbare (*japonica*) and Juma (*indica*) were used for gene analysis of lesion size and lesion number as components of field resistance to blast, respectively. Blast isolate, Ken 54-20 was used in evaluating disease resistance of the hybrid population. Nipponbare showed a small number of large lesions and Juma showed a large number of small lesions in one of the two tests. F_3 plants with higher levels of resistance (evaluated as; highly resistant) than their parents were observed in some lines. Resistances were evaluated on individual plant basis and divided into four reaction types, R, N (Nipponbare type, small lesion number), J (Juma type, small lesion size) and S (susceptible). Resistances in three classes, R, R+N and R+N+J, were analyzed by the cumulative frequency distribution curve method. To explain these three types of segregations, three genes (controlling inhibition of lesion size and number) with minor effect were assumed: AACC in Nipponbare and BB in Juma. Additive effect of these three genes, AABBCC, was considered for explaining R type resistance, AACC for N type field resistance and BB for J type field resistance. But in another test (Test 2) with different F_3 segregating lines derived from the same F_1, resistance was explained by BBDD genes in Juma and EE gene in Nipponbare. This suggests that at least one gene in Nipponbare was not expressed in Test 2, indicating of epistatic change of gene action between the tests. There was no association between seed sterility and blast resistance, although there was an association between color of the basal leaf sheath and seed sterility. This means that genes responsible for seed sterility are not linked with blast resistance genes.

Key words: Field resistance, genetics, rice blast, lesion size, lesion number and functional values

INTRODUCTION

Disease resistance was divided into true resistance and field resistance (Müller and Haigh, 1953). The latter has been used for describing resistance of potato late blight and rice blast diseases. In rust of wheat (Broers, 1989) resistance has been divided into seedling resistance and adult-plant resistance, which are similar to true and field resistance, respectively. Partial resistance and quantitative resistance also have been used for low levels of resistance (reviewed by Kiyosawa *et al*., 1986). Genes for true resistance to rice blast, *Pi-a* and *Pi-I* were found in *japonica* varieties and in some *indica* varieties, *Pi-k* and *Pi-k*^{m} were found in Chinese varieties, *Pi-ta* and *Pi-ta*^{2} were in Philippine (*indica*) variety, *Pi-z* in United States variety and *Pi-k*^{p}, *Pi-z*^{t}, *Pi-b* and *Pi-t* were in *indica* varieties (Kiyosawa, 1974; 1976). *Pi-sh* was identified in Japanese varieties (Inbe and Matsumoto, 1985). *Pi-k*^{s} has been traced in Japanese varieties when the test was carried out only with a Philippine fungus strain (Kiyosawa, 1969). Nine quantitative trait loci, *Pi-24(t)* to *Pi-32(t)* have been reported from IR64, a highly resistant *indica* variety. Five of these resistance loci were mapped at chromosomal locations where no resistance gene was previously reported, defining new resistance genes (Sallaud *et al*., 2003). However, all the genes controlling true resistance were found of specific in nature and major in type.

Genetic studies on field resistance have been conducted since 1967. Genes controlling field resistance are minor genes. In some varieties, two or more minor genes were found by the Mendelian method of gene analysis and many minor genes or polygenes were found by a method of statistical genetics (Purba *et al*., 1994). Purba *et al*. (1994) estimated the functional values of genes controlling field resistance by the cumulative frequency distribution curve method (He *et al*., 1989). In almost all cases, accumulation of minor genes resulted in additive effect. In a few cases, complementary gene action was observed where resistance was not expressed by

single genes but by a combination of genes. Particularly, some *japonica* and *indica* have shown complementary gene effect relating to minor genes for blast resistance (Purba *et al*., 1994). In general, field resistance of Japanese varieties is low, whereas there are many *indica* varieties having high level of field resistance (Kiyosawa *et al*., 1986).

From the pathological or epidemiological point, field resistance has been divided into three/four components of resistance, for example: infection frequency, latent period and sporulation capacity (Asher and Thomas, 1987); latent period, disease severity and sporulation (Cunfer *et al*., 1988), or infection frequency (uredinium density); latent period and urediospore (uredinium) size (Broers, 1989; Pretorius *et al*., 1994); lesion number, the percentages of sporulating lesions, lesion diameters and conidial production per sporulating lesions (Shew *et al*., 1989). Nevertheless, the genetic basis of resistance remains poorly understood for most rice varieties and new resistance genes remain to be identified (Berruyer *et al*., 2003). So it is of due interest to determine the type of genes responsible for different components of field resistance. In the present paper, the results of gene analysis for genes controlling lesion size and lesion number in a F_3 population of Nipponbare (*japonica*), having field resistance that inhibits the increase of lesion size, with Juma (*indica*), with field resistance to control lesion number, are reported.

MATERIALS AND METHODS

All tests were carried out in the greenhouse with temperature range 20-22°C, except daily and seasonal changes of natural environmental conditions. F_3 lines of Nipponabare x Juma were used for genetic analysis. Tests were replicated twice. Inoculation was performed on 15 June and 27 October, respectively. In Test 1, one hundred and 12 F_3 lines (17 plants in each line) were sown in 12 wooden boxes (45 x 15 x 10 cm). Other than hybrid population, F_1 plants and two rows for each parent were planted for Test 1. Except F_1, all other materials were also sown for Test 2. At about 6.0 and 4.0 plant stage as measured by leaf number counted from incomplete leaf in Test 1 and 2, respectively, test plants were inoculated with blast fungus strain, Ken 54-20 (race number 003.0) (Kiyosawa, 1984). Inoculated plants were kept in inoculation chamber at 28°C and relative humidity (RH) 100% for 24 hours. Then the boxes were put in a growth chamber. The temperature and RH of the growth chamber was 28°C and about 82%, respectively. Disease evaluation was conducted 7 days after inoculation on individual plant basis following Kiyosawa (Kiyosawa *et al*., 1981).

The distribution of parental reactions was overlapped as shown in Table 1 (for number of lesions) and Table 2 (for type of lesions), which were divided into four groups:

b: brown spot;
bg: small lesion having gray center and brown margin,
bG: large lesion having gray center and brown margin; and
pG: large lesion having gray center and no or purple margin.

In our method, 112 lines with 17 plants per line have generally been grown for gene analysis. When only 17 Plants per line are used, it is impossible to differentiate 1 : 0 segregation ratios from 15 : 1 and even to differentiate 3 : 1 from 15 : 1, if there are only major genes. Therefore, the cumulative frequency distribution curve method (Kiyosawa, 1970; 1976), which can be used without differentiating these segregation ratios, was used for gene analysis. In this method at first, observed frequencies (Table 5) of resistant plants per 17 plants were plotted against the number of resistant plants per line to get the frequency distribution curve as shown in Fig. 1. These frequencies were added from left to right to obtain a cumulative frequency distribution curve.

To get expected frequency and cumulative frequency distribution curves, one gene was first assumed in each of the parents: AA in Nipponbare and BB in Juma. Frequencies of resistant plants (functional values) in parents (9/34=0.2647 and 0/33 = 0; Table 5) were given to genotypes, AA++ and ++BB, respectively, for R (r) in the R : (N+J+S) segregation ratio. For other genotypes, various functional values were assigned in a range with the following restrictions on dominance and epistasis:

For A gene,	AA ≥ A+ ≥ ++	Functional values
For B gene,	BB ≥ B + ≥ ++	/resistance are assigned
For A and B genes,	AABB ≥ AA++ or ≥ ++BB	according to frequency
For all genotypes,	R ≤ R+N ≤ R+N+J	of genes

(Here, AA means functional value of genotype AA)

These functional values were given to all genotypes in F_2 and F_3 generations and frequencies of r plants in F_3 generation for individual F_2 plants were calculated accordingly. Binomial distribution of resistant plants in 17 F_3 plants sown for each F_2 genotype was calculated based on following equation:

$$f_{17}c_e\, p^r\, (1-p)^{(17-r)}$$

(f is frequency in F_2, C is combination, r is the number of resistant plants in 17 plants and p is frequency of resistant plants in F_3 generation per F_2 genotype)

By adding frequencies in the distribution curves for each genotype, an expected frequency distribution curve was drawn as shown in Fig. 1. An expected cumulative frequency distribution curve was obtained by adding frequencies in the frequency distribution curve from left to right. Among cumulative frequency distribution curves obtained by giving various functional values to various genotypes, a curve with minimum difference between observed and expected curves was finally selected. Significance of the difference was tested followed by Kiyosawa (1974; 1976). When the difference was statistically significant for assumption of two genes, the possibility of three genes was tested. In this case, two options were considered: AACC in Nipponabare and BB in Juma and AA in Nipponbare and AACC in Juma (hereafter, AACC/BB and AA/BBCC were used, respectively). In these cases, the functional values/resistance are assigned mostly according to frequency of genes as before. Finally, a set of functional values, which can commonly explain all three segregations, was selected for each F_2 plant. In test 2, we could not differentiate Nipponbare from Juma by the number of lesions, due to overlapping sizes of lesions as showed by Nipponbare. Therefore, responses were divided into three group: r (more resistant than Juma), m (Juma type) and s (Nipponbare type) and r: (m+s) and (r+m): s segregations were analyzed by cumulative frequency distribution curve method.

RESULTS

The nature of field resistance for its components (lesion size and number) was studied from F_3 population derived from two rice varieties, namely, Nipponbare and Juma. An investigation on association of blast resistance genes and genes conferring purple base and seed sterility was also followed. There are significant differences in the number of lesions (Table 1) and predominant lesion type (Table 2) between the parents. Therefore, individual plants in the F_3 lines were first divided into two groups based on the number of lesions: plant having a) less than five lesions and b) five or more lesions. The former group was again divided into two classes based on the number of lesions: R (no lesions) and N (Nipponbare type, with one to four lesions). The latter was divided into two classes based on predominant lesions: J (Juma type, having bg as predominant lesions) and S (susceptible type, having bG as predominant lesions). Individual plants of parents and the F_3 lines were classified into four groups: R, N, J and S (Table 3). Then, the ratio of R : N : J : S was obtained for each F_3 lines and parents. F_3 analyses were, then, carried out on three ratios, R: (N+J+S), (R+N): (J+S) and (R+N+J) : S. In each analysis, these segregations were analyzed as r : s ratio. Here r means resistant and s means susceptibility. Accordingly, the number and functional values of genes controlling R, R+N

Table 1: Number of lesions formed on two parents

		Number of plants with number of lesions described										
		Number of lesions (range)										
Variety		0-5	6-10	11-15	16-20	21-25	25-30	31-35	36-40	41-45	Mean	Resistance[1)]
Nipponbare	a*:	17	17								1.18	M
	b:	15	15	1							1.82	MR
Juma	a:	3	3	2	6	2	3				15.78	M
	b:	1	1	8	1	4	2		1		10.88	M
Nipponbare	c:	4	3	3	4	3		1			10.00	S
Juma	c:	2	2	2	3	7				1	16.71	MR

*: a and b are the result of this experiment and c, in other experiment

1): The resistance evaluated R^h by Kiyosawa *et al.* (1981) and evaluated M on the basis of predominant lesions

Table 2: Different types of lesions developed on Nipponbare and Juma

		Mean number of lesions					
Test 1		b	bg	bG	pG	Total	Reaction
Nipponbare	a	0.66	0.12	0.89		1.18	M
	b	0.19	0.81	0.75	0.06	1.71	M
Juma	a	5.59	4.47	5.71		15.76	M
	b	0.81	6.57	3.50		10.88	M
Susceptible	a	0.20	3.12	5.47	3.65	12.53	MS
Line in F_3	b	0.76	3.94	13.12	1.12	18.94	MS
Test 2							
Nipponbare	a				7.67	7.67	S
	b		0.78	1.11	9.30	11.19	S
Juma	a	1.33	11.67	9.77		22.77	M
	b	5.00	11.30	3.33		19.63	M
Susceptible	a		1.24	2.53	9.88	13.60	S
F_3 line	b	0.35	0.41	1.65	8.29	10.70	S

a and b mean average of varieties in different boxes for Nipponbare and Juma and average of different lines in different boxes for susceptible line in F_3.

Table 3: Ratio of four response types of two parents in Test 1

	Response types[1)]					
	Expt.	R	N	J	S	Total
Nipponbare	a	5	12			17
Nipponbare	b	4	11	1	1	17
Juma	a		2	15		17
Juma	b		3	11	2	16
Nipponbare	c	1	1	1	12	15
Juma	c	2	1	14		17

1): Classification into R, N, J and S is not always suitable for Expt. c. Concentrations of spores of suspension inoculated are 100,000/ml for a and b and 380,000/ml for c

Table 4: Association among blast resistance, color of plant base and seed sterility in Test 1 and Test 2

Character	χ^2 in uniformity test		
Test 1	Character	D. purple base	E. seed sterility
A	R type resistance	1.60	0.59
B	R + N type resistance	5.38*	1.48
C	R+N+J type resistance	0.21	1.28
D	Purple base	-	1.11
Test 2			
A.	r : (m + s) type resistance	1.41	0.37
B	(r + m) : s type resistance	0.69	0.69
C	Purple base	-	3.75*

* Means significance at the 5% level

and R+N+J responses were estimated, respectively, for each of the test.

Generally, a hybrid between *indica* and *japonica* show a high degree of hybrid sterility in F_1 and F_2 plants. There was a cool spell in 1998 when F_2 plants were grown and consequently increased seed sterility was observed. It ranged from 2 to 95%, respectively. Color of basal leaf sheath was examined to determine the influence of sterility on segregation ratios for resistance and plant color (Table 4). No relationship was found between resistance and sterility, although statistically significant deviation (χ^2= 5.38*) from random association was found between sheath color and resistance (R+N type resistance). Segregation for blast resistance was not skewed by seed sterility.

Test 1: The observed number of resistant plants per line are shown in Table 5 for R : (N+J+S), (R+N): (J+S) and (R+N+J): S segregations. When two genes model, AA in Nipponbare and BB in Juma, are tested, maximum of differences between observed and expected cumulative frequency distribution curve was found statistically significant for (R+N) : (J+S) (Fig. 1), although not significant for the other two segregations, (R + N + J) : S and R : (N + J + S) (Table 7). Therefore, a three gene model was tested. For AACC/BB, the maximum of differences between expected curve selected and observed curve were: 0.0599, 0.0641 and 0.0679 for the three segregations, respectively. But for the model, AA/BBCC, 0.0575, 0.2037 and 0.0980, respectively was obtained. The latter does not show agreement between observed and expected cumulative frequency distribution curves. Therefore, the former showing good agreement was considered. The frequency and cumulative frequency distribution curves are shown in Fig. 2, 3 and 4. For these cases the functional values for the three segregation ratios are shown in Table 7.

Table 5: Distribution of the number of plants per line in Test 1 and Test 2

	Number of lines in segregation ratio of				
Number of resistant	Test 1			Test 2	
plants per line	R : (N + J + S)	(R + N) : (J + S)	(R + N + S) : S	r : (m +s)	(r + m) : s
0	44 Δ 0	9	0	45 OΔ□0	4 O 0
1	15	7	1	18	9
2	20	9	0	13	9
3	12	11Δ 0.1515	1	4	9 □ 0.17
4	5	9	1	5	6
5	3 O 0.2647	8	2	4	5
6	1	4	2	4	3
7	2	8	3	1	5
8	3	3	3	0	6
9	1	8	2	0	5
10	2	11	5	1	6
11	0	8	4	0	7
12	0	3	8	1	9
13	0	4	11	0	3
14	0	2	9	0	3
15	0	2	17	0	3
16	0	1 O 0.9411	24 Δ 0.9394	0	2
17	0	1	15 O 0.9706	0	2 Δ 1
Total	108	108	108	96	96

:O, Δ and □ are the frequencies of resistant plants, Nipponbare, Juma and F_1, respectively for different segregation ratios and the figures following them are the functional values

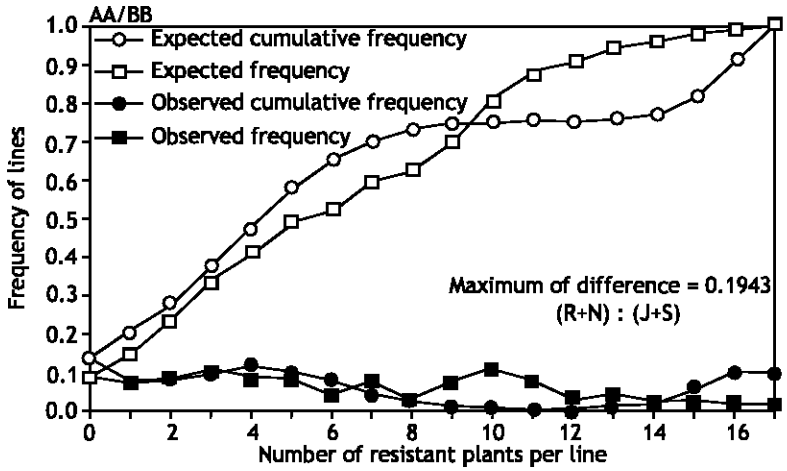

Fig. 1: Frequency and cumulative frequency distribution curves of resistant segregrants in F_3 of Nipponbare x Juma in Test 1

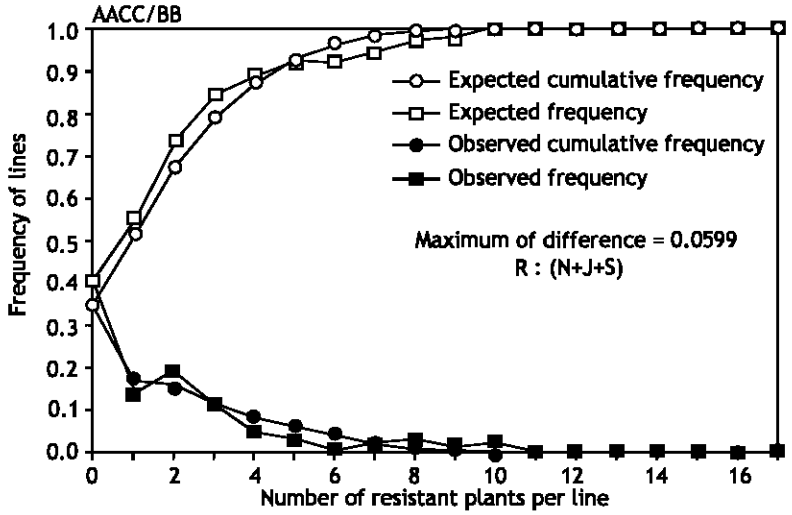

Fig. 2: Frequency and cumulative frequency distribution curves of resistant segregrants in F_3 of Nipponbare x Juma in Test 2

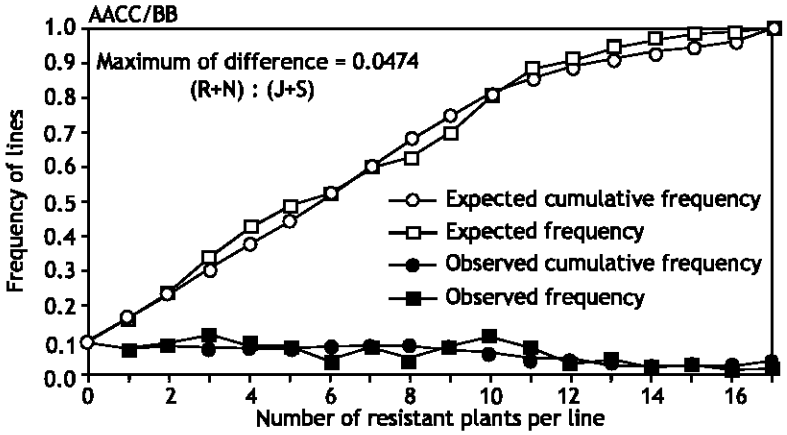

Fig. 3: Frequency and cumulative frequency distribution curves of resistant segregrants in F_3 of Nipponbare x Juma in Test 1

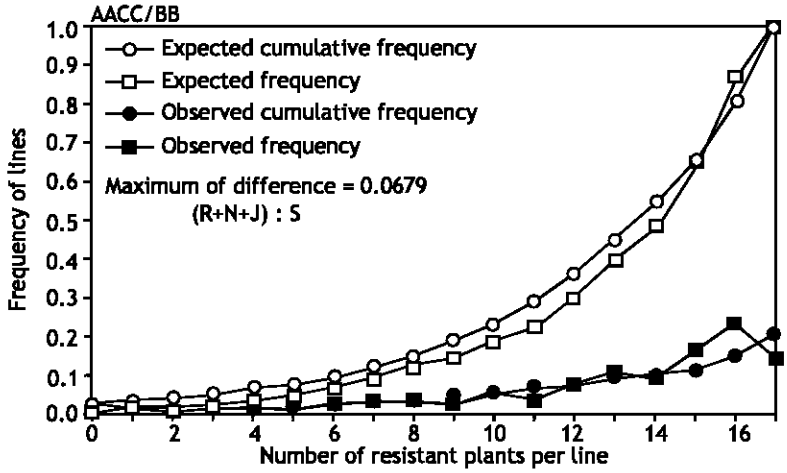

Fig. 4: Frequency and cumulative frequency distribution curves of resistant segregrants in F_3 of Nipponbare x Juma in Test 1

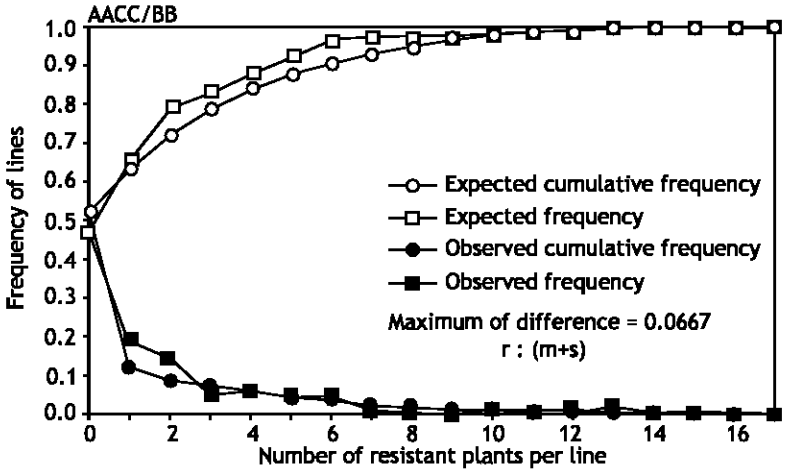

Fig. 5: Frequency and cumulative frequency distribution curves of resistant segregrants in F_3 of Nipponbare x Juma in Test 2

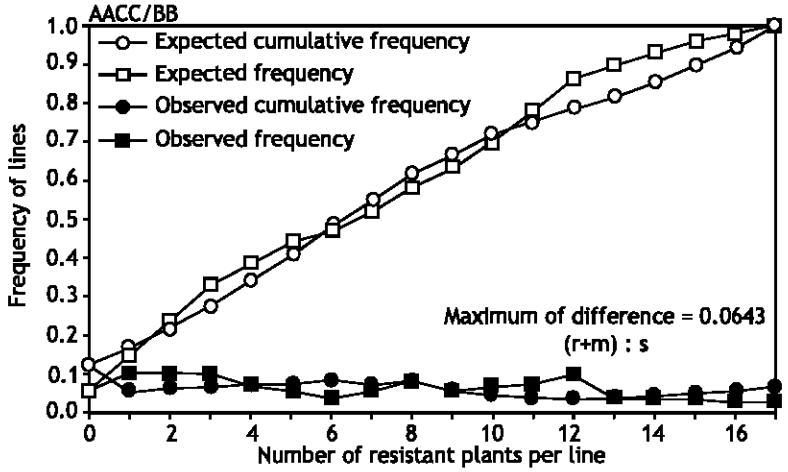

Fig. 6: Frequency and cumulative frequency distribution curves of resistant segregrants in F_3 of Nipponbare x Juma in Test 2

Table 6: Maximum difference between expected and observed curves when two or three genes in Test 1 explained each segregation ratio

	Genotypes assumed for Nipponbare and Juma		
Segregation	AA/BB	AACC/BB	AA/BBCC
R : (N + J + S)	0.0470	0.0599	0.0575
(R + N) : (J + S)	0.1943*	0.0641	0.2037*
(R + N + J) : S	0.0856	0.0679	0.0980

** Means that the difference is significantly larger from 0 at 1% level

The results suggest that resistance of hybrids of Nipponbare x Juma is controlled by three minor genes: two in Nipponbare and one in Juma. These three genes have a complementary effect, which is defined as the effect making resistance [with functional value ranging from 0.05888 (1/17) to 1] in all possible combinations of three genes (functional value of individual genes is near 0) in R: (N + J + S) segregation and have additive effect in other segregations (Table 7).

Test 2: In Test 2, 96 F_3 lines were used for analysis. Reactions of parents, particularly Nipponbare and segregation ratio in the F_3 generation mostly differed from Test 1. The number of lesions on Nipponbare was fewer than Juma (Table 2). It could not be differentiated from most susceptible lines in the F_3 generation. There was no association between seed sterility (6~99% in F_2 plants) and blast resistance, although there was an association between color of the basal leaf sheath and seed sterility (Table 4). This at least means that genes responsible for seed sterility did not affect segregations for blast resistance. The frequencies of lines with various numbers of resistant plants in 17 plants of F_3 lines are shown in Table 5. Only two lines were homogeneously resistant out of the 96 lines tested. Therefore, the results could not explain by the two-gene model. Three genes in resistant parent, Juma, were required to explain the segregation. Functional values shown in Table 7 are given to the hybrid genotypes and grouped in r : (m + s) and (r + m) : s rations to minimize the maximum difference between expected and observed cumulative frequency distribution curves (Fig. 5 and 6). Three genes in Juma and no gene in Nipponbare were considered to explain the segregations. This is very different from the conclusion made in case of Test 1 where two genes in Nipponbare and one in Juma explained the data.

In Test 2, Nipponbare did not show any resistance. Two genes (BBDD) in Juma and one gene (EE) in Nipponbare were considered to explain resistance/susceptibility reaction of parents. However, a few numbers of plants was more resistant than the parents. For (r + m) type resistance, the assumption of

Table 7: Functional values assigned to hybrid genotypes of Nipponbare andJuma in two tests

	Segregation ratio						
	Test 1			Test 2			
Genotype[1)]	R : (N + J + S)	(R + N) : (J + S)	(R + N + S):S	Genotype[2)]	r : (m + s)	Genotype[2)]	(r + m):s
AACCBB	0.3529	0.9411	1.0000	BBDDEE	0.7059	AADDFF	1.0000
AACCB+	0.2647	0.9411	1.0000	BBDDE+	0.6041	AADDF+	1.0000
AACC++	0.2647 O	0.9411	0.9706 O	BBDD++	0.0000 ▼	AADD++	0.9412
AAC+BB	0.2647	0.8235	0.9412	BBD+EE	0.5882	AAD+FF	0.9412
AAC+B+	0.2353	0.7647	0.8235	BBD+E+	0.2353	AAD+F+	0.9412
AAC+++	0.1765	0.6471	0.7647	BBD+++	0.0000	AAD+++	0.7647
AA++BB	0.1765	0.7059	0.9412	BB++EE	0.0588	AA++FF	0.7647
AA++B+	0.1765	0.5882	0.8235	BB++E+	0.0000	AA++F+	0.7047
AA+++	0.0588	0.4706	0.7647	BB+++	0.0000	AA+++	0.7049
A+CCBB	0.2647	0.5294	1.0000	B+DDEE	0.2353	A+DDFF	0.5294
A+CCB+	0.1765	0.4706	0.9412	B+DDE+	0.1765	A+DDF+	0.5294
A+CCB+	0.1765	0.2941	0.9394	B+DD++	0.0000	A+DD++	0.4118
A+CC++	0.1765	0.4706	0.9394	B+DD++	0.0588	A+D+FF	0.4706
A+C+BB	0.0588	0.2353	0.7647	B+D+EE	0.0000 □	A+D+FF	0.1700 □
A+C+B+	0.0000	0.1175	0.7059	B+D+E+	0.0000	A+D+F+	0.1176
A+C+++	0.0000	0.3529	0.9394	B+D+++	0.0000	A+D+++	0.3529
A+++B+	0.0000	0.0000	0.7647	B+++E+	0.0000	A+++F+	0.3529
A+++++	0.0000	0.0000	0.7059	B+++++	0.0000	A+++++	0.0588
++CCBB	0.0000	0.1515	1.0000	++DDEE	0.1176	++DDFF	0.3529
++CCB+	0.0000	0.0000	0.7394	++DDE+	0.0588	++DDF+	0.1176
++CC++	0.0000	0.0000	0.2941	++DD++	0.0000	++DD++	0.0588
++C+BB	0.0000	0.1515	0.9394	++D+EE	0.0000	++D+FF	0.2353
++C+B+	0.0000	0.0000	0.2941	++D+E+	0.0000	++D+F+	0.0588
++C+++	0.0000	0.0000	0.1176	++D+++	0.0000	++D+++	0.0000
++++BB	0.0000 ▼	0.1515 ▼	0.9394 ▼	++++EE	0.0000 O	++++FF	0.0000
++++B+	0.0000	0.0000	0.2941	++++E+	0.0000	++++F+	0.0000
++++++	0.0000	0.0000	0.0000	++++++	0.0000	++++++	0.0000 O
Maximum difference	0.0599	0.0641	0.0679	-	0.0667	-	0.0844

Test 1: O and ▼ are the functional values assigned to Nipponbare and Juma, respectively in Test 1

1): Among AACCBB genes AACC genes are assumed in Nipponbare BB in Juma, respectively.

Test 2: O,▼ and □ are the functional values of hybrid genotypes of Nipponbare, Juma and F_1, respectively

2): In AAD+E+, AAD+ and E+ show segregation of genes from Nipponbare and Juma, respectively.

three genes (AADDFF) in Juma was required to explain only two homozygously resistant F_3 lines out of 96 F_3 lines and resistance of Juma. Functional values given to all genotypes for each segregation is shown in Table 7.

Additive or complementary effects appeared in some lines of F_3 in (r+m) : s segregation, namely, BB (0.7049) + DD (0.04588) → BBDD (0.9412) or DD (0.0588) + FF (0.0000) → DDFF (0.3529).

The apparently contradictory results of Tests 1 and 2 are explained as follows. Two genes in Nipponbare assumed from result in Test 1 were not expressed under the conditions of Test 2 and two additional genes, which were found in Juma in Test 2, were nonfunctional or not detected under the conditions in Test 1. Conditions were different in greenhouse between June and October and in plant stages in leaf number, 6.0 and 4.0. Conditions in Test 2 are considered to be more favorable for disease development than those for Test 1. The difference between two tests indicates that the two genes, which were functional in Test 1, were not functional in Test 2 and two minor genes in Juma, which were not found under epistatic function of Nippponbare's genes, were detected in Test 2.

DISCUSSION

Components of field resistance: Field resistance is divided into three components by more or less different methods by a number of investigators (Asher and Thomas, 1983; 1987; Denissen, 1993; Broers, 1989; Pretorius et al., 1994). Shew *et al.* (1989) divided it into four: lesion number, the percentages of lesions sporulating, lesions diameters and conidial production per sporulating lesions. Bruno and Nelson (1990) divided partial resistance of wheat to septoria glume blotch into six components: incubation period, latent period, percentage of diseased leaf tissue, initial spore production at the end of latent period, total spore production at 100% necrosis and maturation period.

In the present paper, it is suggested that different minor genes controlled lesion size and lesion number. These studies indicate that field resistance includes at least four components: lesion number (infection frequency), latent period, lesion size and sporulation capacity. Among them, different genes control lesions size and number. The present studies showed that one or two genes controlling lesion number in Nipponbare were very sensitive to environmental conditions and /or plant stage. At least some partial resistance of rice blast is expressed as fewer and smaller lesions in the leaf blade but latent period does not appear to be an important component (Roumen and de Boef, 1993).

Genetic relationships among four components: The present paper describes that two minor genes controlling field resistance (lesion size and lesion number), which are present in different varieties, are independent of each other. Transgressive segregation was found in the F_3 generation. Roumen (1993) showed that transgressive segregations were found in rice blast resistance in *indica* hybrids. In our case at least one gene in each parent controlled the reduction of number of lesions in the hybrid. According to Roumen (1993), the number of genes reducing the lesion number was five or more in *indica* varieties. Some other reports indicate that there are complicated genetic relationships among genes controlling these components (Cunfer *et al.*, 1988; Denissen, 1993; Broers,1989). However, the latency period appeared as the most important component in wheat and found to be controlled by one to three genes in wheat (Jacobs and Broers, 1989).

From the epidemiological standpoint, sporulation capacity is very important component of field resistance and there is not always a positive correlation between sporulation on the medium and lesion formation on host leaves (Kiyosawa and Cho, 1973; Shew *et al.*, 1989). This information indicates that there are genes acting commonly and also genes acting separately to control these components. Also fungus strains are considered to have different strategies to face the struggle for existence.

Field resistance of indica and japonica varieties and time of gene expression: In the present test, Nipponbare and Juma have shown different field resistance genes in operation, which may indicate that there are many different field resistance genes in *indica* and japonica varieties. In tests with many *indica* varieties conducted worldwide, many intermediate resistances were identified with Japanese and Philippines fungus strains. This suggested that these varieties have many field resistance genes. Purba *et al.* (1994) investigated the blast resistance of some hybrids of *indica* and *japonica* and found additive or complementary gene action in a few hybrids.

Regarding time of expression of field resistance genes, a gene (A) in Nipponbare was epistatic over a gene (B) in Juma in Test 1. We assume that the gene (A) is functional at the penetration stage of the fungus and the gene (B) is functional at the elongation stage of the fungus in host cell. From the literature (Asher and Thomas, 1987; Cunfer *et al.*, 1988; Broers, 1989; Shew *et al.*, 1989; Pretorius *et al.*, 1994) it is evident that there are at least four components of field resistance. Some of these components are thought to express at different stages during penetration of fungus.

Epistasis change: In the present study, epistasis change in gene action was observed. In Test 1, the additive effect of AA and CC assumed in Nipponbare was epistatic over BB in Juma, but in Test 2, BB was epistatic over AA and CC, as AA and CC was not expressed in Test 2. Change

in dominance of resistance genes has been found between plants and between pathogens (Kiyosawa, 1970; Kolmer and Dyck, 1994). For rice blast, change from complete dominance to complete recessiveness has been reported for *Pi-i* gene against plant stage, method of inoculations and fungus strains (Kiyosawa, 1970). Usually, the test plant size in disease resistance experiments is limited by greenhouse space and size of inoculation chamber. Particularly, when evaluating field resistance, the same results are very difficult to secure by repeating tests, even when the same F_3 lines are used. As a result, the number of resistance genes that can be detected may frequently give differential reaction from test to test (Kiyosawa, 1970).

Molecular approaches: There are at least 40 blast genes so far identified and mapped, eight of them are mapped on chromosome no 11. Four of the genes are on each of chromosome no 12 and 6. However, only a few are cloned (Salluad *et al*., 2003). The broad spectrum of resistance exhibited by some gene, for example, *Pi-ta*, is suggested due to a tightly linked cluster of at least several *R* genes that map in the same region as *Pi-ta* and *Pi-ta*^{2}, located on centromeric region of chromosome 12 (Chao *et al*., 1999). However, complete genome sequencing will facilitate characterization of this complex chromosomal region (Valent *et al*., 2001). Some molecular works are providing information on allelic relationship of previously identified genes after mapping and sequencing (Salluad *et al*., 2003; Jeon *et al*., 2003).

At present, genomic era is highly advanced. Sequencing of *Arabidopsis* and rice is completed and available for public use. On the other hand, gene expression profiles are now analyzed on a lot basis (micro array). Isolation, characterization and transformation of genes are routine works of many laboratories. Despite of all these achievements at the molecular level, classical genetic analysis studies will be continued as an important activity in order to determine the nature (dominance/recessiveness or polygenic) of gene action. Consequently more resistance genes/sources will be available providing wider scopes of gene exploitation for developing varieties with broaden genetic backgrounds.

REFERENCES

Asher, M.J.C. and C.E. Thomas, 1987. The inheritance of mechanisms of partial resistance to *Erysiphe graminis* in spring barley. Plant Pathology, 36: 66-72.

Berruyer, R., H. Adreit, J. Milazzo, S. Gaillard, A. Berger, W. Dioh, M.H. Lebrun and D. Tharreau, 2003. Identification and fine mapping of *Pi33*, the rice resistance gene corresponding to the Magnaporthe grisea avirulence gene ACE1. Theor Appl Genet. (in press).

Broers, L.H.M., 1989. Influence of development stage and host genotypes on three components of partial resistance to leaf rust in spring wheat. Euphytica, 44: 187-195.

Bruno, H.H. and L.R. Nelson, 1990. Partial resistance to *Septoria* blotch analyzed in winter wheat seedlings. Crop Sci., 30: 54-59.

Cunfer, B.M., D.E., Stooksbury and J.W. Johnson, 1988. Components of partial resistance to *Leptosphaeria nodorum* among seven soft red winter wheats. Euphytica, 37:129-140.

Denissen, C.J.M., 1993. Components of adult plant resistance to leaf rust in wheat. Euphytica, 70: 131-140.

He, Y., S. Kiyosawa, Y. Wang, J. Li, C. Li and T. Higashi, 1989. Inheritance of blast resistance in Chinese upland rice varieties, Zhalulong, Mowanggu and Mongwangu. Oryza, 26: 288-298.

Inbe, T. and S. Matsumoto, 1985. Inheritance of resistance of rice varieties to the blast fungus strains virulent to the variety "Reiho". Japan. J. Breed., 35:332-339 (in Japanese).

Jacobs, Th. and L.H.M. Broers, 1989. The inheritance of host plant effect on latency period of wheat leaf rust in spring wheat. I. Estimation of gene action and number of effective factors in F_1, F_2 and backcross generations. Euphytica, 44: 197-206.

Jeon, J.S., D. Chen, G.H. Yi, G.L. Wang and P.C. Ronald, 2003. Genetic and physical mapping of *Pi5(t)*, a locus associated with broad-spectrum resistance to rice blast. Mol Genet Genomics, 269: 280-289.

Kiyosawa, S., 1969. Inheritance of resistance of rice varieties to a Philippine fungus strain of *Pyricularia oryzae*. Japan J. Breed., 19: 61-73.

Kiyosawa, S., 1970. Inheritance of blast resistance of the rice varieties Homare Nishiki and Ginga. I. Resistance of Homare Nishiki and Ginga to the fungus strain Ken 54-04. Bull. Natl. Inst. Agr. Sci., D21: 73-105.

Kiyosawa, S., 1974. Studies on genetics and breeding of blast resistance in rice. Misc. Publ,. Natl. Inst. Agr. Sci. D1:1-58 (in Japanese).

Kiyosawa, S., 1976. Methods for tests and gene analysis of blast resistance of rice varieties. Oryza, 13:1-32.

Kiyosawa, S., 1984. Establishment of differential varieties for pathogenicity tests of rice blast fungus. Rice Genet. Newsl., 1: 95-97.

Kiyosawa, S. and C.I. Cho, 1973. Relation of the sporulating ability on the rice plant to some other characters in blast fungus strains. Japan J. Breed., 23: 239-244.

Kiyosawa, S., H. Ikehashi, H. Kato and Z.Z., Ling, 1981. Pathogenicity tests of Philippine isolates of blast fungus using two sets of rice varieties. Japan. J. Breed., 31: 367-376.

S. Kiyosawa, D.J. Mackill, J.M. Bonman, T. Tanaka and Z.Z. Ling, 1986. An attempt of classification of world's rice varieties based on reaction pattern to blast fungus strains. Bull. Natl. Inst. Agrobiol. Resour., 2: 41-59.

Kolmer, J.A. and P.L. Dyck, 1994. Gene expression in the *Triticum aestivum* - *Puccinia recondita* f. sp. *tritici* gene–for–gene system. Phytopathol., 84: 437-440.

Müller, K.O. and J.C. Haigh, 1953. Nature of 'field resistance' of the potato to *Phytophthora infestans* de Bary. Nature, 171: 781-783.

Pretorius, Z.A., F.J. Kloppers and S.C. Drijepondt, 1994. Effects of inoculum density and temperature on three components of leaf rust resistance controlled by *Lr34* in wheat. Euphytica, 74: 91-96.

Purba, D., S. Kiyosawa, I. Ando and T. Furutani, 1994. Estimation of functional values of field-resistance genes to blast disease in some rice varieties. Breeding Science, 44: 285-293.

Roumen, E.C., 1993. Inheritance of host plant effect on the relative infection efficiency of *Magnaporthe grisea* in rice cultivars. In: E.C. Roumen. Partial, Resistance in Rice to Blast and How to select for it. pp: 61-72.

Roumen, E.C. and W.S de Boef, 1993. Latent period to leaf blast in rice and its importance as a component of partial resistance. Euphytica, 69: 185-190.

Sallaud, C., M. Lorieux, E. Roumen, D. Tharreau, R. Berruyer, P, Svestasrani, O. Garsmeur, A. Ghesquiere and J.L. Notteghem, 2003. Identification of five new blast resistance genes in the highly blast-resistant rice variety IR64 using a QTL mapping strategy. Theor Appl. Genet., 106: 794-803.

Shew, B.B., T. Sommartya and M.K. Beute, 1989. Components of partial resistance in peanut genotypes to isolates of *Cercopsoridium personatum* from the United States and Thailand. *Phytopathol.*, 79: 136-142.

Valent, B., G.T. Bryan, Y. Jia, L. Farrall, S.A. McAdams, K.N. Faulk and M. Lev, 2001. Enhancing of deployment of genes for blast resistance: opportunities from cloning a resistance gene/avirulence gene pair. In Rice Genetics IV. G. S. Khush, D.S. Brar and B. Hardy (Ed.). Proc. Fourth Int. Rice Genet. Symp., 22-27 October, 1997, Los Baños, Philippines. New Delhi, India: Science Publishers, Inc. and Los Baños (Philippines), Int. Natl. Rice Res. Inst., pp: 309-321.