Estimation of Functional Values of Field-Resistance Genes to Blast Disease in Some Rice Varieties

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Summary

F₃ lines of the hybrids of some varieties and Japanese susceptible varieties were tested with two Japanese blast fungus strains, Ken 54-20 (race number, 003.0) and Ao 14-20 (337.3), to learn the mode of inheritance of blast resistance. Varieties used were as follows : Chinese varieties (Sifeng 43, Quan-wei-ai and Nan Jing 11), Korean varieties (Milyang 23, Raekyeong, Ir 338), IRRI's varieties (IR 24, IR 36 and IR 2061), Indian varieties (RP 9-3 and CR 44), and Hokuriku 129 (Habataki). Analyses after tests were carried out by cumulative frequency distribution curve method by Kiyosawa. In this study, plants were divided into three groups, r, m and s, based on the degree of resistance, and resistance genes were divided into two. One was the major gene which had a functional value of 1 and the other was the minor gene which had a functional value lower than 1 in a segregation of r : (m+s). The functional value of 1 means that plants which had its genotype show resistant reaction in 100 % of plants. The resistance of these varieties was explained by assuming some major genes and minor genes. Sifeng 43 has one major gene and one minor gene. Quan-wei-ai and Nan Jing 11 have two minor genes with their additive (or complementary) effect. It was suggested that there are minor genes controlling resistance in intermediate type (m) in Milyang 23, Raekyeong, Iri 338, IR 24 and IR 36. IR 2061 has two or more major genes and some minor genes. Resistance of Hokuriku 129 was explained by two major genes. At the least, Milyang 23, Raekyeong, Iri 338, IR 24, IR 36 and CR 44 were suggested to have *Pi-b*.

Key Words : *Pyricularia*, *Oryza sativa*, blast resistance, rice, inheritance, field resistance.

Introduction

Disease resistance of plants has been divided into two groups, true (seedling, or major genic) resistance and field (partial, adult, or minor genic) resistance. As far as blast resistance genes in Japanese rice varieties, this classification is very useful for expressing degrees of resistance, because there is no or little intermediate re-

sistance. Classification of varieties in other countries than Japan, especially indica-type varieties is, however, very difficult to divide into two groups, because there are many varieties showing intermediate resistance to various fungus strains (Kiyosawa *et al.* 1986). These facts indicate that there are many varieties with resistance in the field resistance type in varieties in countries other than Japan.

Since the breakdown of varieties with true resistance, the importance of field (partial, quantitative) resistance is being reconsidered, and the number of papers concerning field resistance has been greatly increasing during this decade (Geiger and Heun 1989). In recent studies, genetic analyses are carried out by various methods : conventional method of gene analysis (Steffenson 1992, Reifschneider *et al.* 1992, Miklas and Grafton 1992), diallel analysis (Balkema-Boomstra and Mastebroek 1993, Berg *et al.* 1992), partitioning of components (Baayen and van der Plas 1992, Roumen 1992, Stegmark 1991), selection (Koch and Parlevliet 1991, Stegmark 1991, Roderick and Jones 1991), the use of reciprocal translocation (Brewster *et al.* 1992), two generation mean analysis (Wilkinson *et al.* 1991), etc.

By these methods, combining ability (Berg *et al.* 1992, Balkema-Boomstra and Mastebroek 1993), heritability (Miklas and Grafton 1992), durability (Bonman 1992, Steffenson 1992), possibility of selection (Koch and Parlevliet 1991, Roderick and Jones 1991, Reifschneider *et al.* 1992), effect of environment (Steffenson 1992, Bonman 1992), influence of leaf age on resistance (Koch and Mew 1991, Roumen 1992), mode of inheritance (Reifschneider *et al.* 1992, Sawhney *et al.* 1992) and mapping (Wilkinson *et al.* 1991) on various disease of various crop plants were examined. Especially, studies on durability of resistance increase (Steffenson 1992, Johnson 1984).

On field resistance against blast disease in rice plants, estimates of heritability (Abe *et al.* 1974), the number of resistance genes (Kiyosawa 1970, Abe *et al.* 1974, Higashi and Kushibuchi 1978, Goto 1970), mode of inheritance (Horisue *et al.* 1978, Higashi and Kushibuchi 1978, Higashi and Saito 1985, Goto 1970, 1976, 1978, Kiyosawa *et al.* 1971, He *et al.* 1989a, b), and linkage relationships (Higashi and Saito 1985) have to date been carried out in Japan.

For example, by the general Mendelian method, field resistance of Norin 22 was analyzed with a fungus strain, Ken 54-04, which is not very aggressive with re-

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sults indicating that the resistance is controlled by one major gene and two or more minor genes (Kiyosawa *et al.* 1967). Kiyosawa (1970) furthermore analyzed field resistance of Homare Nishiki and Ginga with Ken 54-04; the resistance was explained by one major gene and two minor genes. The same F_3 lines of the hybrid of Homare Nishiki and Aichi Asahi were twice tested with the same fungus strain. Although tests were carried out at a two month interval, clearly different segregation ratios were obtained between them. This indicates that the expression of field resistance is greatly influenced by environmental conditions.

An analysis mentioned above was performed by a cumulative frequency distribution curve method developed by Kiyosawa (1970). Population size of plants which can be used in an experiment is largely limited by the size of the inoculation chamber and greenhouse. In Kiyosawa's experiments, 17×120 plants is the limited population size. Seventeen is the number of plants per line and 120 is the number of lines including two parents. By the use of 17 plants, a 3 : 1 segregation ratio can be differentiated from a 1 : 0 segregation ratio. However, a 15 : 1 segregation ratio cannot be differentiated from a 16 : 0 or even from a 3 : 1 segregation ratio in a digenic hybrid. The cumulative frequency distribution curve method was devised as a method in which differentiation of these segregation ratios is not necessary. The method was detailed by Kiyosawa (1970, 1976). By many tests, including the above examples, it has been suggested that this method can be used for approximate estimation of the ability of minor genes.

He *et al.* (1989 a, b) applied this method to the field nursery tests, and recognized the presence of two minor genes which had additive or complementary effects in Chinese upland japonica-type varieties, Haonaihuan, Zhalulong, Mowangu and Mongwangu.

Very recently, Roumen (1993) investigated the field (partial) resistance of rice plants to blast disease from many points of this problem.

In the present paper, we attempted to estimate functional values of field resistance genes existing in some varieties in countries other than Japan.

Materials and Methods

Korean varieties [Milyang 23, Raekyeong and Iri 338 (Sam-seong)], Chinese varieties (Sifeng 43, Quan-wei-ai and Nan Jing 11), varieties of IRRI (IR 24, IR 36 and IR 2061), Indian varieties (RP 9-3 and CR 44) and a Japanese variety Hokuriku 129 (Habataki) developed from Korean varieties (Fig. 1) were used as resistant varieties. These varieties were crossed with Japanese varieties, Reimei ("Pi-a"), Norin 22, and Norin 29. The F_3 populations of these hybrids were tested for resistance by spraying them at 5 to 6 leaf stage with fungus strains Ken 54-20 (003.0) and often Ao 14-20 (337.3) by Kiyosawa's method (Kiyosawa 1969, Kiyosawa *et al.* 1981).

Resistance was divided into three groups, r, m and s. The r is a type which a resistant variety shows, and the s is type of susceptible variety. The m is an intermediate type between resistant and susceptible varieties. Under the test conditions, Shin 2 which has intermediate field resistance showed r or m symptoms in 0 to 30 % of plants, when Aichi Asahi with the highest field susceptibility showed s type symptoms in 100 % of plants. Accordingly, conditions used for tests were suitable for detecting field resistance, but more or less not for differentiating field resistance and escape from infection.

An observed frequency distribution curve in which frequencies of F_3 lines were plotted against the number

Fig. 1. Genealogical relationships of varieties used :

of resistant plants per 17 plants from experimental data, and then a cumulative frequency distribution curve was drawn by adding obtained frequencies from left to right, based on the method shown by Kiyosawa (1970, 1976). An expected frequency distribution curve was drawn as shown on a digenic hybrid in Fig. 2. In F_3 lines of the hybrid with two major genes, 1 : 0, 15 : 1, 3 : 1 and 0 : 1 segregating lines segregate in a ratio of 7 : 4 : 4 : 1. Among them, the 15 : 1 segregating lines show a binomial distribution having a center at the point of $(15/16) \times 17$, and the 3 : 1 segregating lines draw a binomial distribution at the point of $(3/4) \times 17$. Areas of both are 4/16, together. The frequencies of 1 : 0 and 0 : 1 segregating lines are 7/16 and 1/16, respectively. These frequencies were added to get the frequency distribution curve. By adding these frequencies from left to right, an expected cumulative frequency distribution curve was obtained. In such a case with two major genes, to express concretely, calculation for getting frequencies of individual genotypes were conducted by giving 1 to eight genotypes consisting of two genes, and 0 to a genotype, + + + +, respectively.

For instance, when a more resistant parent shows weak resistance, or when frequencies of lines in which all plants show resistant reaction (right side of Fig. 2) was significantly less than 1/4 which was expected by the contribution of only one major gene, the additive effect of minor genes is expected. In such a case, various values of less than 1 were given to all genotypes except + + + + and frequencies were calculated and selected to get an expected cumulative frequency distribution curve in agreement with the observed cumulative frequency distribution curve.

When m type plants were present, one datum was analyzed twice, the first was for $(r+m) : s$ segregation and the second was for $r : (m+s)$ segregation. This came into force for estimating the function of each gene. Plants showing intermediate reactions of two parents were considered to occur due to two reasons : one is the incomplete dominance of resistance genes and the other

Fig. 2. Segregation of F_3 lines of Norin 29 $\times$ Hokuriku 129.

Note : Figures were obtained by experiments different from those in tables. For example, 2. means functional value of genotype AAB+.

is the presence of minor genes in the resistant parent or additive or complementary effect of minor genes in both parents, if a susceptible parent has no weak resistance. In Fig. 3, curve a is a distribution of homozygous plants for resistance genes, and curve b is a distribution of heterozygous plants for resistance genes. If the resistance gene is completely dominant, i. e. two curves a and b duplicate, although the frequency of b is two times that of a. The function of minor genes should appear naturally at intermediate positions of both parents. This method makes differentiation of incompletely dominant major genes and minor genes possible. In this figure, functional values of about 1 and 0.8 were given to heterozygous plants (genotype A+) in $(r+m) : s$ and $r : (m+s)$ segregations, respectively. For the minor gene shown in Fig. 3, functional values of 0.9 and 0.03 were given in $(r+m) : s$ and $r : (m+s)$ segregations, respectively. Here, a functional value means the frequency of plants included in a resistant range in a plant population of a genotype, and it was given to each genotype in F_3 .

In the present paper, a gene controlling resistance with functional value of 1 was defined as a major gene, and others as a minor gene(s).

Results

Chinese varieties : Sifeng 43, Quan-wei-ai and Nan Jing 11
 F_3 lines of Norin 22 $\times$ Sifeng 43 were inoculated with Ken 54-20 (Table 1). This result indicates that Sifeng 43 has one major gene and one minor gene with functional value 0.35 in $(r+m) : s$ segregation. In an $r : (m+s)$ segregation, the functional value was 0.12 and is not largely different from $(r+m) : s$ segregation (Table 1). This is thought to depend upon a small number of m plants. This also indicates that this resistance is almost completely dominant.

Fig. 3. Distribution of plants having various genotypes for disease resistance.

a : Distribution of plants having a genotype. AA. Ratio of the plants right side of line Y' is 1.0.

b : Distribution of plants having a genotype. A+. Ratio of the plants right side of line Y' is 0.8.

c : Distribution of plants having a genotype. ++.

d : Distribution of plants having homozygously minor genes. Ratio of the plants right side of line Y and line Y' are 0.9 and 0.03, respectively.

Fig. 4. Segregation of F_3 lines of Shin 2 $\times$ Quan-wei-ai. (See Fig. 2 for explanatory notes)

Fig. 5. Segregation of F_3 lines of Shin 2 $\times$ Quan-wei-ai. (See Fig. 2 for explanatory notes)

Two cumulative frequency distribution curves for $r : (m+s)$ and $(r+m) : s$ segregation in F_3 lines of Norin 22 $\times$ Quan-wei-ai are shown in Figs. 4 and 5. In this case, a large difference was found between the two curves.

A functional value of 1 was given to only one genotype, AABB. When the same values were given to AAB+ and A+BB; A+B+, AA++ and ++BB; and A+++ and ++B+, respectively, obtained cumulative frequency distribution curve could be explained well. This suggests that the genes A and B are duplicate genes. In the $r : (m+s)$ curve, when 1 was given to AABB, and 0 was given to all other genotypes, a good agreement between the expected and observed curves was found, indicating that the function of both genes was complementary: both are minor genes and the effect is additive (Table 1). The difference in the two curves indicates that there is a dominant effect. It is thought that one of minor genes come from Japanese variety, Norin 22, because parts of their F_3 lines showed higher resistance than Quan-wei-ai.

In F_3 lines of Nan Jing 11 $\times$ Reimei, $(r+m) : s$ ratio was explained by the additive effect of two minor genes, which have functional values of 0.53, alone. The $r : (m+s)$ segregation was explained by the additive effect of two genes having functional values, 0 and 0, alone, to make 1, together (Table 1). It is considered that one of two minor genes come from Reimei, as highly resistant lines were observed in F_3 lines, but not in both parents.

Hokuriku 129 (Habataki)

Norin 29 $\times$ Hokuriku 129 was also inoculated with Ken 54-20 in F_3 generation. Two segregations were explained by two major genes which were completely dominant (Fig. 2, Table 1).

Korean varieties; Milyang 23, Raekyeong and Iri 338

Korean varieties, Milyang 23, Raekyeong and Iri 338 (Table 2), were analyzed. These Korean varieties were inoculated with two fungus strains in F_3 generation of hybrid with Japanese varieties. One fungus strain was Ken 54-20 (003.0) and the other was Ao 14-20 (337.3) which was collected from the Japanese variety, Hama

Table 1. Functional value given to each genotype in gene analysis of Chinese varieties and Hokuriku 129

Cross combination	Norin 22 $\times$ Sifeng 43				Norin 22 $\times$ Quan-wei-ai				Nan Jing 11 $\times$ Reimei				Norin 29 $\times$ Hokuriku 129			
Reaction of parents	r	m	s	Total	r	m	s	Total	r	m	s	Total	r	m	s	Total
No. of plants P_1	3		14	17			17	17	7	10		17	2		32	34
P_2	16			16	6	9	1	16			14	14	33			33
Segregation	$(r+m) : s$		$r : (m+s)$		$(r+m) : s$		$r : (m+s)$		$(r+m) : s$		$r : (m+s)$		$(r+m) : s$		$r : (m+s)$	
Maximum difference	0.0445		0.0753		0.0625		0.0665		0.0795		0.0625		0.0625		0.0625	
Genotype	AABB	1.0000		1.0000	1.0000		1.0000	1.0000	1.0000		1.0000	1.0000	1.0000		1.0000	1.0000
	AAB+	1.0000		1.0000	0.8824		0.1176	0.8235	0.0000		0.8235	0.0000	1.0000		1.0000	1.0000
	AA++	1.0000		1.0000	0.8235		0.1176	0.5299	0.0000		0.5299	0.0000	1.0000		1.0000	1.0000
	A+BB	1.0000		1.0000	0.8824		0.0000	0.8235	0.0000		0.8235	0.0000	1.0000		1.0000	1.0000
	A+B+	1.0000		1.0000	0.8235		0.0000	0.5294	0.0000		0.5294	0.0000	1.0000		1.0000	1.0000
	A+++	1.0000		1.0000	0.5294		0.0000	0.1176	0.0000		0.0000	0.0000	1.0000		1.0000	1.0000
	++BB	0.3529		0.1176	0.8235		0.0000	0.5294	0.0000		0.5294	0.0000	1.0000		1.0000	1.0000
	++B+	0.3529		0.1176	0.5294		0.0000	0.1174	0.0000		0.0000	0.0000	1.0000		1.0000	1.0000
	++++	0.0000		0.0000	0.0000		0.0000	0.0000	0.0000		0.0000	0.0000	0.0000		0.0000	0.0000

Inoculated with Ken 54-20.

Table 2. Functional value given to each genotype in gene analysis of Korean varieties

Cross combination	Reimei × Milyang 23 ¹⁾				Reimei × Milyang 23 ²⁾				Raekyeong × Reimei ¹⁾				Raekyeong × Reimei ²⁾				Iri 338 × Reimei ¹⁾				Iri 338 × Reimei ²⁾			
Reaction of parents	r	m	s	Total	r	m	s	Total	r	m	s	Total	r	m	s	Total	r	m	s	Total	r	m	s	Total
No. of plants P ₁	17 17				17 17				17 17				16 1 17				17 17				12 3 2 17			
P ₂	15 15				14 1 15				1 1 14 16				15 15				17 17				17 17			
Segregation	(r+m) : s		r : (m+s)		(r+m) : s		r : (m+s)		(r+m) : s		r : (m+s)		(r+m) : s		r : (m+s)		(r+m) : s		r : (m+s)		(r+m) : s		r : (m+s)	
Maximum difference	0.0611		0.0696		0.0795		0.0953		0.0774		0.0900		0.0631		0.0895		0.0753		0.0453		0.0477		0.0545	
Genotype	A A B B C C	1.0000						1.0000									1.0000							
	A A B B C +	1.0000						1.0000									1.0000							
	A A B B + +	1.0000						1.0000									1.0000							
	A A B + C C	1.0000						1.0000									1.0000							
	A A B + C +	1.0000						1.0000									1.0000							
	A A B + + +	1.0000						1.0000									1.0000							
	A A + + C C	1.0000						1.0000									1.0000							
	A A + + C +	1.0000						1.0000									1.0000							
	A A + + + +	1.0000						1.0000									1.0000							
	A + B B C C	1.0000						1.0000									1.0000							
	A + B B C +	1.0000						1.0000									1.0000							
	A + B B + +	1.0000						1.0000									1.0000							
	A + B + C C	1.0000						1.0000									1.0000							
	A + B + C +	1.0000						1.0000									1.0000							
	A + B + + +	1.0000						1.0000									1.0000							
	A + + + C C	1.0000						1.0000									1.0000							
	A + + + C +	1.0000						1.0000									1.0000							
	A + + + + +	1.0000						1.0000									1.0000							
	+ + B B C C	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
	+ + B B C +	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.3529	1.0000	1.0000	0.3529	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
	+ + B B + +	1.0000	1.0000	1.0000	0.6471	1.0000	0.8235	1.0000	0.8235	1.0000	0.1765	1.0000	1.0000	0.1765	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.9412	1.0000	0.9412	1.0000
	+ + B + C C	1.0000	1.0000	1.0000	0.8235	1.0000	0.8824	1.0000	0.8824	1.0000	0.2353	1.0000	1.0000	0.2353	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
	+ + B + C +	1.0000	1.0000	1.0000	0.8235	1.0000	0.8824	1.0000	0.8824	1.0000	0.2353	1.0000	1.0000	0.2353	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
	+ + B + + +	0.9412	1.0000	0.3529	0.2353	1.0000	0.8235	1.0000	0.8235	1.0000	0.7059	1.0000	1.0000	0.1176	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.4706	1.0000	0.4706	1.0000
	+ + + + C C	0.8235	0.2353	0.3529	0.3529	0.4706	0.2353	1.0000	0.1176	1.0000	0.0588	1.0000	1.0000	0.6471	0.9412	1.0000	0.7049	1.0000	1.0000	1.0000	0.4118	1.0000	0.4118	1.0000
	+ + + + C +	0.8235	0.2353	0.3529	0.3529	0.4706	0.2353	1.0000	0.1176	1.0000	0.0588	1.0000	1.0000	0.6471	0.9412	1.0000	0.7049	1.0000	1.0000	1.0000	0.4118	1.0000	0.4118	1.0000
	+ + + + + +	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	1.0000	0.0000	1.0000	0.0000	1.0000	1.0000	0.0000	0.0000	1.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	1.0000

¹⁾ and ²⁾ : Inoculated with Ken 54-20 (003.0) and Ao 14-20 (337.3), respectively.

Asahi ("*Pi-a*, *Pi-i*, *Pi-k*, *Pi-b*") (Kiyosawa *et al.* 1984). In this case, when the same F₃ lines show different cumulative frequency distribution curves to different fungus strains, it indicates that the resistant variety contains one in resistance genes, which correspond to virulence genes different between both fungus strains. The resistance genes which can be included are *Pi-i*, *Pi-k* or *Pi-k^m*, *Pi-ta* or *Pi-ta²*, or *Pi-b*. By this method, we cannot determine the location of the genes on chromosomes. Accordingly, genes included are ones with the same or similar specificity, but cannot always conclude to be the gene on the same locus.

In Iri 338 × Reimei and Raekyeong × Reimei, similar results were obtained except the following (Table 2): in r : (m+s) segregation, the second gene B controls resistance under the homozygous and heterozygous conditions, so the gene is a major gene and completely dominant in Iri 338 × Reimei and Raekyeong × Reimei. The other genes are minor ones. Raekyeong and Iri 338 also has one in genes, *Pi-i*, *Pi-k*, *Pi-k^m*, *Pi-ta*, *Pi-ta²* and *Pi-b*.

In analysis of Reimei × Milyang 23 (Table 2), the segregation was similar to Raekyeong × Reimei, but the

second major gene was incompletely dominant. A major gene is naturally epistatic in interaction with minor genes in all cases.

IRRI's varieties ; IR 24, IR 36 and IR 2061

Some IRRI's varieties were analyzed when crossed with the Japanese variety Reimei, and their F₃ lines were inoculated with fungus strains, Ken 54-20 and Ao 14-20 (Table 3). The differences between (r+m) : s and r : (m+s) segregations found in Reimei × IR 24 and IR 36 × Reimei indicate that there is one minor gene controlling intermediate resistance, and the difference by fungus strains inoculated indicates the presence of one in *Pi-i*, *Pi-k*, *Pi-k^m*, *Pi-ta*, *Pi-ta²* and *Pi-b* resistance genes. In contrast to these two varieties, in a hybrid of IR 2061 × Reimei there is no or little difference between (r+m) : s and r : (m+s) segregations or also between fungus strains inoculated. This suggests that there are no resistance genes, *Pi-i*, *Pi-k*, *Pi-k^m*, *Pi-ta*, *Pi-ta²* and *Pi-b*, if there are no resistance genes over three, because this cumulative frequency distribution curve method cannot recognize four or more genes.

Table 3. Functional value given to each genotype in gene analysis of IRR1's varieties

Cross combination	Reimei × IR 24 ¹⁾				Reimei × IR 24 ²⁾				IR 36 × Reimei ¹⁾				IR 36 × Reimei ²⁾				IR 2061 × Reimei ¹⁾				Iri 2061 × Reimei ²⁾				
Reaction of parents	r	m	s	Total	r	m	s	Total	r	m	s	Total	r	m	s	Total	r	m	s	Total	r	m	s	Total	
No. of plants P ₁	1	2	31	34	4	25	29		31	1		32	16	16		32	23		23		32		32		
P ₂	35			35	28	5		33			33	33			34	34			32	32		32	3	31	34
Segregation	(r+m) : s		r : (m+s)		(r+m) : s		r : (m+s)		(r+m) : s		r : (m+s)		(r+m) : s		r : (m+s)		(r+m) : s		r : (m+s)		(r+m) : s		r : (m+s)		
Maximum difference	0.0428		0.0561		0.0373		0.0468		0.0200		0.0539		0.0823		0.0626		0.0560		0.0184		0.0774		0.0423		
Genotype	A A B B C C	1.0000							1.0000								1.0000	1.0000			1.0000			1.0000	
	A A B + C C	1.0000							1.0000								1.0000	1.0000			1.0000			1.0000	
	A A + + C C	1.0000							1.0000								1.0000	1.0000			1.0000			1.0000	
	A + B B C C	1.0000							1.0000								1.0000	1.0000			1.0000			1.0000	
	A + B + C C	1.0000							1.0000								1.0000	1.0000			1.0000			1.0000	
	A + + + C C	1.0000							1.0000								1.0000	1.0000			1.0000			1.0000	
	+ + B B C C	1.0000							1.0000								1.0000	1.0000			1.0000			1.0000	
	+ + B + C C	1.0000							1.0000								1.0000	1.0000			1.0000			1.0000	
	+ + + + C C	1.0000							1.0000								1.0000	1.0000			1.0000			1.0000	
	A A B B C +	1.0000							1.0000								1.0000	1.0000			1.0000			1.0000	
	A A B + C +	1.0000							1.0000								1.0000	1.0000			1.0000			1.0000	
	A A + + C +	1.0000							1.0000								1.0000	1.0000			1.0000			1.0000	
	A + B B C +	1.0000							1.0000								1.0000	1.0000			1.0000			1.0000	
	A + B + C +	1.0000							1.0000								1.0000	1.0000			1.0000			1.0000	
	A + + + C +	1.0000							1.0000								1.0000	1.0000			1.0000			1.0000	
	+ + B B C +	1.0000							1.0000								1.0000	1.0000			1.0000			1.0000	
	+ + B + C +	1.0000							1.0000								1.0000	1.0000			1.0000			1.0000	
	+ + + + C +	1.0000							1.0000								1.0000	1.0000			1.0000			1.0000	
	A A B B + +	1.0000	1.0000		1.0000	1.0000			1.0000	1.0000			1.0000	1.0000			1.0000	1.0000			1.0000	1.0000			
	A A B + + +	1.0000	1.0000		1.0000	0.8624			1.0000	1.0000			1.0000	1.0000			1.0000	1.0000			1.0000	1.0000			
	A A + + + +	1.0000	1.0000		0.6471	0.4118			1.0000	1.0000			0.8235	0.8235			1.0000	1.0000			1.0000	1.0000			
	A + B B + +	1.0000	1.0000		0.9412	0.6471			1.0000	1.0000			1.0000	0.4706			1.0000	1.0000			1.0000	1.0000			
	A + B + + +	1.0000	1.0000		0.9412	0.4118			1.0000	1.0000			1.0000	0.4706			1.0000	1.0000			1.0000	1.0000			
	A + + + + +	1.0000	1.0000		0.4706	0.1176			1.0000	1.0000			0.3529	0.2941			1.0000	1.0000			1.0000	1.0000			
	+ + B B + +	0.4118	1.0000		0.7059	0.1176			0.8235	0.9412			0.5882	0.1766			1.0000	0.9412			0.8235	0.4706			
	+ + B + + +	0.4118	0.8235		0.7059	0.0000			0.8235	0.5882			0.5882	0.1766			1.0000	0.9412			0.8235	0.4706			
	+ + + + + +	0.0000	0.0000		0.0000	0.0000			0.0000	0.0000			0.0000	0.0000			0.0000	0.0000			0.0000	0.0000			

¹⁾ and ²⁾: Inoculated with Ken 54-20 (003.0) and Ao 14-20 (337.3), respectively.

Table 4. Functional value given to each genotype in gene analysis of Indian varieties

Cross combination	RP 9-3 × Reimei ¹⁾				RP 9-3 × Reimei ²⁾				CR 44 × Reimei ¹⁾				CR 44 × Reimei ²⁾			
Reaction of parents	r	m	s	Total	r	m	s	Total	r	m	s	Total	r	m	s	Total
No. of plants P ₁	14			14	17			17				17				17
P ₂	1	11		12			16	16	15			15	14	1		15
Segregation	(r+m) : s		r : (m+s)		(r+m) : s		r : (m+s)		(r+m) : s		r : (m+s)		(r+m) : s		r : (m+s)	
Maximum difference	0.0887		0.0830		0.0642		0.0660		0.0865		0.0843		0.0651		0.0850	
Genotype	A A B B	1.0000		1.0000	1.0000		0.9412		1.0000		1.0000		1.0000		1.0000	
	A A B +	1.0000		1.0000	1.0000		0.9412		1.0000		1.0000		0.9412		0.8824	
	A A + +	0.9412		0.9412	1.0000		0.7647		0.9412		0.9412		0.4118		0.2353	
	A + B B	1.0000		0.8235	1.0000		0.6471		1.0000		0.8824		0.5294		0.4118	
	A + B +	0.9412		0.8235	1.0000		0.6471		1.0000		0.8824		0.4706		0.3529	
	A + + +	0.6471		0.4706	1.0000		0.4118		0.8235		0.4706		0.2941		0.0588	
	+ + B B	0.5882		0.5882	0.5294		0.2353		0.3529		0.4118		0.1765		0.1176	
	+ + B +	0.4706		0.5882	0.5294		0.2353		0.3529		0.4118		0.0000		0.0000	
	+ + + +	0.0000		0.0000	0.0000		0.0000		0.0000		0.0000		0.0000		0.0000	

¹⁾ and ²⁾: Inoculated with Ken 54-20 (003.0) and Ao 14-20 (337.3), respectively.

Indian varieties; RP 9-3 and CR 44

Hybrids of RP 9-3 and CR 44 with Reimei were inoculated with Ken 54-20 and Ao 14-20 in the F₃ generation (Table 4). These hybrids have a very distinctive difference. The segregation ratios were explained by additive effect of two minor genes in both hybrids. A large difference between the two hybrids was found in cumulative frequency distribution curves, when they were inoculated with two fungus strains (Figs. 6 and 7). It was shown that CR 44 had one gene in *Pi-i*, *Pi-k*, *Pi-k^m*, *Pi-ta*, *Pi-ta²* and *Pi-b*, and RP 9-3 had none of these genes.

Discussion

In the genetic studies on rice varieties including Chinese, Korean, IRRI's and Indian varieties for blast resistance, some minor genes were found. Additive effects of minor genes were recognized in hybrids of Japanese varieties with Iri 338, IR 36, Quan-wei-ai, Nan Jing 11, RP 9-3, and CR 44. As a special case of additive effect of minor genes, complementary action of minor genes which are not functional alone (Nan Jing

11) were found. In the cases of the presence of two or more minor genes, functions of the two genes are similar in some varieties (Quan-wei-ai and Nan Jing 11), and are quite different in other cases (Milyang 23, Raekyeong, IR 24 and IR 36). In Hokuriku 129, minor gene(s) were not found. One minor gene is thought to come from Japanese varieties used for crossing, at the least in hybrids of Quan-wei-ai and Nan Jing 11.

Major genes which showed resistant reactions also in $r : (m+s)$ segregation were found in some varieties. Only one major gene was found in Milyang 23, IR 24, IR 36, and Sifeng 43. Two major genes were found in IR 2061 and Hokuriku 129.

To date, this cumulative frequency distribution curve method was applied to some varieties, Japanese varieties (Rikuto Norin Mochi 4, Homare Nishiki and Ginga), IRRI's variety (IR 1905-81-3-1), and a Korean variety (Tongil). In Rikuto Norin Mochi 4, Homare Nishiki and Ginga, their resistance was explained by one major gene (minor gene with high functional value according to the definition in this paper) and one or more minor gene(s) (Abe *et al.* 1974). The resistance in IR 1905-81-3-1 was shown to be controlled by the additive effect of two minor genes and one gene which is nonfunctional alone (Kiyosawa *et al.* 1983). Resistance of Tongil was shown to be controlled by at least two genes, *Pi-a* and *P-b*. Response of this variety to some fungus strains suggests that another gene controlling high resistance is present (Kiyosawa and Cho 1980).

In the present analyses, Milyang 23, Raekyeong, Iri 338, IR 24, IR 36 and CR 44 showed clearly different cumulative frequency distribution curves against the two fungus strains Ken 54-20 and Ao 14-20. This suggests that these varieties have a gene in resistance genes, *Pi-i*, *Pi-k*, *Pi-k^m*, *Pi-ta*, *Pi-ta²* and *Pi-b*, which correspond to virulence genes differing between two fungus strains.

It is considered that resistance of Tongil and the above five varieties except IR 36 and CR 44 originated from IR 8, T.N. 1, Suweon 232, DZ 192 and/or IR 127 according to the genealogical relationship in Fig. 1. Analyses of these varieties furnish a key to identifying the resistance genes in derivatives of these varieties.

IR 36 was developed using 13 rice varieties from six different countries and a wild species of rice, *Oryza nivara* (Innes 1992). It is known that the variety is susceptible in the Philippines and has minor gene(s) to reduce the number and size of lesions (Bonman 1992). In the present paper, it was clarified that IR 36 had one gene in major genes, *Pi-i*, *Pi-k*, *Pi-k^m*, *Pi-ta*, *Pi-ta²* and *Pi-b* and at least two minor genes functional to Japanese fungus strains.

In Table 5, reactions of some varieties to Japanese differential fungus strains and some Philippine fungus strains are shown. This was extracted from Kiyosawa *et al.* (1986). These reactions were observed by an injection method, which was a better method than a spray method, to differentiate higher resistance of indica type

Fig. 6. Segregation of F₃ lines of RP 9-3 × Reimei.

Note: The same 58 F₃ lines were divided into two and inoculated separately with Ken 54-20 and Ao 14-20 under the same conditions.

Fig. 7. Segregation of F₃ lines of CR 44 × Reimei. (See Fig. 6 for explanatory note)

Table 5. Reactions of varieties related to the varieties used in the present paper.
(Kiyosawa *et al.* 1986)

Variety	Fungus strain and race number											
	P-2b	Ken 53-33	Ina 72	Hoku 1	Ken 54-20	Ken 54-04	Ina 168	Ken 53-33 -zt ⁺	Ken 54-20 -b ⁺ -z ⁺	Ph 80-32	Ph 80-22	Ph 80-50
	303.0	137.1	031.1	007.0	003.0	003.0	101.0	537.1	043.2	433.5	433.5	103.4
KOREA												
Honan Joseng	R ^h	MR	R ^h	R	R	R	R ^h	M	R ^h	S	S	S
Iri 338	R	M	M	MR	MR	M	MR	MR	R ^h	S	S	S
Milyang 22	M	M	MR	M	M	M	M	M	R ^h	S	S	S
Milyang 23	MR	M	R ^h	M	M	M	MR	M	M	S	S	S
Milyang 25	M	M	R ^h	M	M	M	R ^h	M	R	S	S	S
Nopung	M	M	MR	M	M	M	R ^h	M	MR	S	S	S
Raekyeong	MR	M	MR	M	MR	M	MR	M	MR	S	S	S
Suweon 251	M	M	R ^h	M	M	M	MR	M	M	S	S	S
Suweon 258	M	M	R ^h	MR	M	M	R ^h	M	M	S	S	S
Suweon 262	MR	MR	R	MR	MR	M	R ^h	MR	R ^h	S	S	S
Suweon 264	MR	MR	R	M	M	M	R	MR	R ^h	S	S	S
Suweon 284	R ^h	R ^h	R ^h	R ^h	MR	R ^h	R ^h	MR	R ^h	S	S	S
Tong II	M	M	MR	R	M	M	M	M	R ^h	S	S	S
Tong II Chall Bea	R	MR	R ^h	M	R	R	R ^h	M	MR	S	S	S
Yung Nam Joseng	R	R	MR	MR	M	M	MR	R	R ^h	S	S	S
CHINA												
Nan Jing 11	M	MS	MR	M	M	M	M	S	M	M	M	M
IRRI												
IR 8	M	M	R ^h	M	M	M	R ^h	M	M	S	MS	S
IR 24	M	M	M	MR	M	M	R ^h	M	MR	S	S	S
IR 26	R ^h	M	MR	R	M	MR	M	MS	MR	S	S	S
IR 30	R ^h	MR	R	MR	MR	MR	MR	MR	R ^h	R	MR	S
IR 38	MR	R	M	R ^h	MR	MR	R ^h	R ^h	MR	R ^h	R	S
IR 44	MR	R	MR	R ^h	MR	M	M	M	R	R	R	S
IR 1561-228	M	S	MR	M	M	M	MR	S	M	M	M	M
IR 2061-465	R ^h	MR	R ^h	R	MR	MR	MR	R ^h	R ^h	S	S	S
IR 2071-625	MR	M	M	R ^h	M	M	M	M	M	R ^h	MS	S
INDIA												
RP 9-3	M	M	MR	R	M	M	R	M	R	S	S	S
CR 44	MR	M	M	R	M	M	M	M	M	M	MS	MS

Inoculated by injection method.

varieties from each other. When plants having *Pi-k* or *Pi-k^m* genes were injected with the four fungus strains, Hoku 1, Ken 54-20, Ken 54-04 and Ina 168, the R^h reactions were generally expressed. Accordingly, varieties Milyang 22, Milyang 23, Milyang 25 and Suweon 258 have at least no *Pi-k* or *Pi-k^m*.

The responses of varieties showing intermediate resistance by the injection method cannot be explained by the function of a single gene of *Pi-i*, *Pi-ta* or *Pi-ta²*. Furthermore, if one of these genes is included in these varieties, the responses to Philippine fungus strains, Ph 80-32 (433.5), Ph 80-22 (433.5) and Ph 80-50 (103.4), should be resistant. Accordingly, in varieties which were estimated to have a single major resistance gene, the reaction patterns in Table 5 cannot be explained by these genes. A remaining possibility is the presence of the *Pi-b* gene. When thinking of the possibility of this gene, a discrepancy exists in response to the Philippine fungus strains. These fungus strains do not attack BL 1 having *Pi-b*; therefore, the response to these fun-

gus strains should be resistant. A study carried out to elucidate this point showed that a segregation in F₂ speculated from F₃ analysis was a 15 : 1 ratio with a Philippine fungus strain and 3 : 1 segregations with Japanese seven differential fungus strains. This indicates that BL 1 has an additional gene which is effective with some Philippine fungus strains and which was derived from a Japanese variety, Norin 25. Thus, *Pi-b* is the only gene which can explain the intermediate resistance by the injection method against fungus strains Ken 53-33, Hoku 1, Ken 54-20 and Ken 54-04.

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