The Inheritance of Blast Resistance of an IRRI's Rice Variety, IR 1905-81-3-1

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Three crosses of IRRI's varieties including IR 1905-81-3-1 as a common parent were genetically analyzed in the third generation for their blast resistance with Philippine and Japanese fungus strains. When two Philippine fungus strains were employed for inoculation, the segregations were very complicated. In the cross, IR 1905-81-3-1 $\times$ IR 32, the F_3 segregation was explained by two minor genes in P_1 , one of which had a major-genic action under some conditions, and one minor gene in P_2 controlling a weak resistance. In the second cross, IR 1905-81-3-1 $\times$ IR 2071-586-5-6-3, and the third, IR 1905-81-3-1 $\times$ IR 36, segregations differing from the first cross were observed although a common parent in the cross and a common fungus strain for inoculation were used. In the second and third crosses, recessive complementary genes were suggested to control resistance of a more resistant variety, IR 1905-81-3-1. When the second Philippine fungus strain was used, the observed segregation was different from one for the first strain in the first cross. This difference was considered to be due to environmental variation rather than the difference of fungus strains used. After all, it was thought that the resistance of IR 1905-81-3-1 was controlled by three or more genes of which the action greatly varied with the change of environmental conditions and that other varieties used as parents of the crosses had one gene controlling a weak resistance. KEY WORDS: *Oryza sativa*, IRRI's varieties, blast resistance, gene analysis.

Introduction

The inheritance of disease resistance of crop plants has been investigated in many crops and many genes have been found (KIYOSAWA 1980). In rice plant, the inheritance studies on disease resistance have been performed on blast and bacterial leaf blight (KIYOSAWA 1974, OGAWA *et al.* 1978, SIDHU and KHUSH 1978). Particularly, on blast resistance, extensive studies have been carried out in Japan using Japanese rice varieties. Up to date, thirteen genes were found. Among them, three, $Pi-a$, $Pi-i$ and $Pi-k^s$, were found in Japanese native varieties, and eight, $Pi-k$, $Pi-k^m$, $Pi-ta$, $Pi-ta^2$, $Pi-z$, $Pi-z^t$, $Pi-b$ and $Pi-t$, were in Japanese varieties which were improved by introducing high resistance in foreign varieties. The other two, $Pi-k^h$ and $Pi-k^p$, were found in an Indian and a Pakistan variety, respectively. Of known resistance genes, $Pi-z^t$, $Pi-ta$, $Pi-ta^2$, $Pi-b$, $Pi-t$ and $Pi-k^h$ were derived from *indica* type varieties. However, resistance of some *indica* type varieties cannot be explained only by these genes (KIYOSAWA *et al.* 1981). It is considered that *indica* type varieties may have many unknown genes. In this study, some rice varieties developed at the International Rice Research Institute (IRRI) were aimed to analyze genetically.

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Materials and Methods

Seeds in F_3 generation of three crosses of IRRI's varieties harvested at IRRI were employed exclusively; IR 1905-81-3-1 $\times$ IR 32, IR 1905-81-3-1 $\times$ IR 2071-586-5-6-3, and IR 1905-81-3-1 $\times$ IR 36*. Ten F_3 lines were sown in a wooden box which contained field soil and 20 g of granular fertilizer. One row included 17 plants, as a rule. Two parents distributed in one or two places in total twelve boxes, except seven boxes used for the first cross that only 66 F_3 lines were available. The plants were grown in a greenhouse controlled between 22° and 35°C.

As fungal materials, two Philippine fungus strains, Ph 80-50 and Ph 80-64, and one Japanese fungus strain, Ken 53-33, were used. These fungus strains were cultured on plates of an oatmeal decoction sucrose agar medium in 20 petri dishes to prepare spores for inoculation. After incubation for ten days in a dark chamber at 28°C, aerial mycelia were rubbed off with a paintbrush and then placed under fluorescent lamps in 28°C chamber for three days. For the first two days, the lid of petri dish was uncovered and then covered for the last one day to protect the culture from desiccation. Spore suspension was prepared by rubbing off spores from the surface of the cultures with a paintbrush after adding deionized water in 50 ml per seedling box to 20 petri dishes in which the fungus strain was cultured. The spore suspension obtained was sprayed using spray-gun with a compressor.

Inoculated plants were kept in a moist chamber at 28°C, and then transferred to the greenhouse.

One week after inoculation, the lesions developed were divided into four types as described by Kiyosawa (1974) and the number of lesions of each type was counted.

Plants were divided into three groups, r, m and s, based on the number and type of lesions. The symbol of r was given to the response same as the more resistant parent and m was given to less resistant parent and s to susceptible plants, when two parents showed differentially resistant responses. When both parents showed a highly resistant type, s was given to susceptible plants and m to plants with intermediate reactions.

Based on these results, an observed frequency distribution curve was drawn for the number of susceptible plants in 17 plants per F_3 line. A cumulative distribution curve was drawn by adding the frequencies from left to right in the figure.

In order to explain the observed cumulative distribution curve, an expected cumulative distribution curve was drawn by giving one to three independent major genes.

In many cases of this study, observed distribution curves could not be explained by any major gene. Therefore expected curves were drawn by giving specific functional values, which were shown by the number of susceptible plants in 17 plants, to various genotypes consisting of one to three genes.

The difference between the observed and expected cumulative distribution curves was tested by the Kolmogorov-Smirnov's method as shown by Kiyosawa (1974, 1976).

* IR 1905 : [IR 8 $\times$ Te-tep] IR 32 : [BPI 76 $\times$ Tainan 3] IR 36 : [Siam 25 $\times$ Chianan 8]
IR 2071 : [(IR 1561-228-1-2 $\times$ IR 1737) $\times$ CR 94-13]

Table 1. The number of plants belonging to three classes in parents

Parent	Reaction to fungus strain									
	Expt. no.	Ph 80-50				Expt. no.	Ph 80-64			
		r	m	s	Total		r	m	s	Total
IR 1905-81-3-1	I	26			26	IV	26	1		27
IR 32		2	28	3	33				33	33
IR 1905-81-3-1	II	30			30					
IR 2071-586-5-6-3		2	4	23	29					
IR 1905-81-3-1	III	9	6		15					
IR 36 (IR 2071-625-1-252)		1	10	4	15					

Results

The F_3 lines of the first cross, IR 1905-81-3-1 $\times$ IR 32, were inoculated with Ph 80-50. The responses of the parents are shown in Table 1, and the frequency and cumulative distribution curves for F_3 lines are shown in Fig. 1. For these observed frequency and cumulative distribution curves (s curve), which were drawn from the numbers of plants belonging to class s, analyses were performed in the following order. The parents, P_1 and P_2 , showed s plants of 0/26 and 3/33, respectively. This indicates that both parents have different resistance genes. Therefore, AA and BB genes were at first given to both parents, respectively. AA and BB must make all plants in 26 plants and 30 (=33-3) in 33 plants resistant. These values, 0/26 and 3/33, were considered to be functional value of these genes for susceptibility.

A functional value, 0/17, was given to genotype AABB. The obtained cumulative distribution curve showed the difference of 0.167 from the observed one at the most different place (Table 2). This difference is statistically significant. To decrease the difference, 2/17 were given to A+ which was expected when gene A is incompletely dominant. The curve obtained thus was not significantly different from the observed s curve (Table 2, Fig. 1). When the genes in P_1 and P_2 parents were assumed to be dominant and recessive, the obtained curve was significantly different from the observed curve with the largest difference of 0.163. These indicate that each parent has one resistance gene, and the gene in IR 1905-81-3-1 makes all plants resistant and the gene in IR 32 makes 30 in 33 plants resistant. The gene in IR 1905-81-3-1 may be incompletely dominant, and the gene in IR 32 may be dominant.

In the F_3 population of the first cross, there were found some highly resistant plants (r class) (Table 1). In order

Fig. 1. Segregation in F_3 lines of the cross, IR 1905-81-3-1 $\times$ IR 32 when inoculated with Ph 80-50 (s curve).

Table 2. Functional value given to each genotype and the difference between the expected and observed cumulative distribution curves.

Expt. no.	Curve	Genotype								Difference
		AABBCC	AABB (AA) (A+)	AACC	BBCC	AA (AA) (A+)	BB	CC	++	
I	s		0/17			0/26 ^D (P ₁)	3/33 ^D (P ₂)		17/17	0.167**
I	s		0/17 2/17			0/26 (P ₁)	2/17 3/33 ^D (P ₂)		17/17	0.070
I	s		0/17			0/26 ^D (P ₁)	3/33 ^R (P ₂)		17/17	0.163**
I	m+s	0/17	31/33	0/26 (P ₁)	31/33	17/17 ^R	31/33 ^R (P ₂)	17/17 ^R	17/17	0.063
II	s	0/17	23/29	0/30 (P ₁)	23/29	17/17 ^R	23/29 ^R (P ₂)	17/17 ^R	17/17	0.063
II	s	0/17	23/29	0/30 (P ₁)	23/29	16/17 ^R	23/29 ^R (P ₂)	17/17 ^R	17/17	0.089
II	s	0/17	23/29	0/30 (P ₁)	23/29	15/17 ^R	23/29 ^R (P ₂)	17/17 ^R	17/17	0.144**
II	m+s	0/17	27/29	0/30 (P ₁)	27/29	17/17 ^R	27/29 ^R (P ₂)	17/17 ^R	17/17	0.063
III	s	0/17	4/15	0/15 (P ₁)	4/15	17/17 ^R	4/15 ^R (P ₂)	17/17 ^R	17/17	0.264**
IV	s	0/27 (P ₁)	15/17	15/17	15/17	16/17 ^R	16/17 ^R	16/17 ^R	33/33 (P ₂)	0.091
IV	s	0/27 (P ₁)	15/17	15/17	17/17	15/17 ^R	17/17 ^R	17/17 ^R	33/33 (P ₂)	0.121
IV	s	0/27 (P ₁)	14/17	14/17	17/17	14/17 ^R	17/17 ^R	17/17 ^R	33/33 (P ₂)	0.120
IV	m+s	1/27 (P ₁)	17/17	17/17	17/17	17/17 ^R	17/17 ^R	17/17 ^R	33/33 (P ₂)	0.031

Note: AABB is an abbreviation of AABB++. (AA) (A+) was employed when incomplete dominance was assumed on AA genes.

s and (m+s) are curves when class s and (m+s) are considered to be susceptible, respectively.

D and R show completely dominant and recessive, respectively.

2/17 show that 2/17 of plants with the corresponding genotype showed susceptible reactions.

** indicates significance of difference between observed and expected curves.

Fig. 2. Segregation in F₃ lines of the cross, IR 1905-81-3-1 × IR 32 when inoculated with Ph 80-50 [(m+s) curve].

to know gene constitution of such highly resistant plants, the (m+s) curve of the cross was analyzed. In this case, all plants in IR 1905-81-3-1 showed high resistance. However, there were no lines in which all plants showed high resistance in F₃ lines. This suggests that high resistance of IR 1905-81-3-1 is controlled by the action of three or more genes, because the frequency

of lines at the point 0 in Fig. 2 is much less than the frequency expected by two recessive complementary genes, $1/16=0.0625$. Therefore, three genes were considered. The two parents showed susceptible ($m+s$) reactions in 0/26 and 31/33 of plants. Therefore, two (A and C) and one (B) genes were given to P_1 and P_2 parents. In this case, the functional value of AACC is determined by the reaction of P_1 parent and that of BB by the reaction of P_2 parent. Therefore, the values 0/26 and 31/33 were given to AACC and BB, respectively. In many cases of disease resistance, the action of genes controlling more resistant reaction is epistatic over one of less resistant reaction. Accordingly, 0/26 was also given to genotype AABBC and 31/33 to AABB, BBCC and BB, and 17/17 to CC and $++$. By assuming complementary action between A and C, which are ineffective individually, the observed distribution curves were explained with a good agreement between the observed and expected distribution curves. Thus, it was thought that intermediate resistance controlled by A gene in IR 1905-81-3-1 became high resistance by complementary action with C gene.

In the second cross, IR 1905-81-3-1 $\times$ IR 2071-586-5-6-3, the reactions of two parents and of their F_3 lines to Ph 80-50 are shown in Table 1 and Figs. 3 (s curve) and 4 [($m+s$) curve]. In this case, the rates of s plants were 0/30 and 23/29 for two parents, and the frequency of lines at 0 point was zero. If major gene(s) contribute to the resistance expressed in F_3 lines, three or more genes are required to explain the segregation, since the frequency at 0 point cannot be explained by additive or complementary action of two recessive genes. Therefore, AACC genes were given to P_1 parent and BB genes to P_2 parent. To both genotypes, functional values of 0/30 and 23/29 were given, because both parents showed such reactions (Table 1).

The functional value, 0/17, was given to genotypes AACC and AABBC, and 23/29 to all genotypes including BB and not including AACC, that is AABB, BBCC and BB, and 17/17 were given to AA, CC and $++$ (Table 2). The obtained cumulative distribution curve was in an agreement with the observed distribution curve, with the largest difference, 0.063, between two curves (Fig. 3). A similar cumulative distribution curve was obtained when 16/17 was given to AA with the largest difference, 0.089, between both curves. Further decrease of functional value of AA to 15/17 leads to a significant difference. This indicates that genes, AA, BB and CC, have no or little action to induce resistance, alone, but AA and CC have complementary action to give high resistance to plants with both genes together.

The ($m+s$) curve of the second cross is described in Fig. 4. In this case, both parents showed susceptible reaction in 0 plant of 30 and 27 plants

Fig. 3. Segregation in F_3 lines of the cross, IR 1905-81-3-1 $\times$ IR 2071-586-5-6-3 when inoculated with Ph 80-50 (s curve).

of 29, respectively (Table 1). The observed cumulative distribution curve was explained by slightly changing the functional value of genotypes, AABB, BBCC, and BB, which was given to the s curve. The value is determined by reactions of P_2 parental genotype, BB, if genes, AA and CC, are not effective, alone or in interaction with BB genes. Thus, the segregation of the second cross in F_3 generation

was able to be explained by genes AACC in P_1 parent which had a complementary action to control high resistance, and BB in P_2 , which showed very variable reactions ranging from r to s class in the response to the used fungus strain and under the given experimental conditions.

The s plants in the third cross showed segregations as shown in Fig. 5 with the responses of the parents described in Table 1. Functional values were given by a way similar to one mentioned above. However, an agreement of the expected curve with the observed one was not obtained. This is mainly caused by lines located at 0 and around point 4 on the X axis in the expected cumulative distribution curve. The distribution of these lines is controlled by the functional value given to the P_2 parent genotype, BB, and genotypes including BB, except AABBCC. In order to reduce the number of lines distributing in point 0 to 5, the existence of inhibitor functional to BB genes must be assumed. It is, however, unreasonable to assume that the inhibitor is active only when this P_2 variety is used as a parent or only under the environmental conditions for this analysis. Probably, the genotype of P_2 parent in analysis may be different from that of the variety used for making cross.

Fig. 5. Segregation in F_3 lines of the cross, IR 1905-81-3-1 $\times$ IR 2071-125-1-252 when inoculated with Ph 80-50 (s curve).

Fig. 4. Segregation in F_3 lines of the cross, IR 1905-81-3-1 $\times$ IR 2071-586-5-6-3 when inoculated with Ph 80-50 [(m+s)].

The F_3 lines used in the previous analysis of the first cross were inoculated with another Philippine fungus strain, Ph 80-64. The results are shown in Table 1 on the response of the parents and in Figs. 6 and 7 on s and (m+s) curves for segregation in F_3 generation, respectively. In this case, P_2 parent showed susceptible reactions differing from the first analysis. Three

genes were given to P_1 parent, because explanation by two genes was impossible. This curve was explained by assuming the functional value of one among the three genes to be 14/17 or 15/17. Similarly, the (m+s) curve was also explained by assuming three complementary genes in P_1 parent.

The F_3 lines of these three crosses were inoculated with a Japanese fungus strain, Ken 53-33. In all crosses, the segregation of susceptible plants was not observed. This indicates that all varieties have at least one common gene controlling high resistance to Ken 53-33 and that this gene is different from at least three genes found by using the two Philippine fungus strains.

Discussion

Segregations for resistance to rice blast in F_3 lines of three crosses harvested in the International Rice Research Institute were analyzed with two Philippine and one Japanese fungus strains. These three crosses have a common parent, IR 1905-81-3-1, which always showed high resistance to the used fungus strains. Observed cumulative distribution curves were explained by assuming one, two and three genes in this variety, although any assumption failed to explain one case of four. In the first cross, the segregation for resistance to the fungus strain Ph 80-50 was explained by one major gene, of which dominance was complete or slightly incomplete, and one minor gene. The segregation to the other strain Ph 80-64 was interpreted by three complementary recessive genes, each of which had no or weak effectiveness to control resistance. It is not known whether or not the difference of segregations between experiments is due to the difference of fungus strains or environmental conditions in which the analyses were carried out.

Segregations for resistance in F_3 lines of the second cross to the fungus strain Ph 80-50 were similar to those for the first cross to the second strain Ph 80-64, although the number of genes necessary to explain both segregations is different (Figs. 3 and 6). The segregation of second cross to the fungus strain Ph 80-50 was different from that of the first cross. The first cross was explained by a major dominant gene which is effective alone and the second was by two complementary recessive genes in P_1 parent.

Fig. 6. Segregation in F_3 lines of the cross, IR 1905-81-3-1 $\times$ IR 32 when inoculated with Ph 80-64 (s curve).

Fig. 7. Segregation in F_3 lines of the cross, IR 1905-81-3-1 $\times$ IR 32 when inoculated with Ph 80-64 [(m+s)].

It has been known that the segregations of same F_3 lines of a cross are different from experiment to experiment, even when same fungus strain is used for inoculation (KIYOSAWA 1970). The expression of the genes in the resistant variety, IR 1905-81-3-1, seems variable.

KIYOSAWA (1974) found 13 genes for blast resistance in Japanese and some foreign varieties. Through these analyses, such complicated segregations as the present analyses were not observed in the cases where a resistant parent showed high resistance. Therefore, the gene for resistance of IR 1905-81-3-1 is considered to be qualitatively different from the previously identified genes. The mode of inheritance for resistance in IR 1905-81-3-1 is rather similar to that of field resistance genes as found in upland rice varieties (ABE, KIYOSAWA and ONO 1974) and paddy rice varieties, Homare Nishiki and Ginga (KIYOSAWA 1970), although expression of gene group accumulated in IR 1905-81-3-1 is stronger than that in these upland and paddy rice varieties.

The above mentioned results can be summarized as shown in Fig. 8. In the first cross, P_2 parent was assumed to have BB genes, and the gene B was dominant when m

Fig. 8. Relationship between genotype and phenotype in each experiment.

plants were classified as resistant (s curve) and recessive when classified as susceptible [(m+s) curve]. This indicates that BB and BBCC genotypes belong to the m class with exception of 3 out of 33 plants in the s class, and 2 out of 33 plants in the r class.

The P_1 parent (AACC) was included in the r class with AABBC genotype, because all plants belonged to the r class. The AA genotype was located in the m class, 0/26 plants and 17/17 plants showed resistance in the s curve and (m+s) curve, respectively. The 2/17 plants of A+ genotype was, however, assumed to show a slightly different reaction in the s curve. Therefore, these were located in slightly right side as compared with AA genotype.

The AAB was different from BB in the point that there was no s class plant and

from AA in the existence of *r* plants in 2/33. Therefore, most of the plants with AABB, BB and AA genotypes showed intermediate reaction for resistance. In a similar way, other genotypes in the first and other crosses were distributed in Fig. 8.

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イネ品種 IR 1905-81-3-1 のいもち病抵抗性の遺伝

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IRRI のイネ品種 IR 1905-81-3-1 を共通の抵抗性親とする 3 つの交配組合せについて, F₃ 世代でいもち病菌の 2 つのフィリピン菌系と 1 つの日本菌系を用いて抵抗性の分離を観察した。第 1 の組合せ, IR 1905-81-3-1 × IR 32 はフィリピン菌系 Ph 80-50 に対しては IR 1905-81-3-1 の中に 1 つの主働遺伝子と 1 つの微働遺伝子を, IR 32 の中に 1 つの微働遺伝子の存在を仮定することにより説明された。

第 2 の組合せ IR 1905-81-3-1 × IR 2071-586-5-6-3 では同じく Ph 80-50 に対して IR 1905-81-3-1 中の 2 つの微働遺伝子, IR 2071-586-5-6-3 中の 1 つの微働遺伝子によって説明された。

また第 3 の組合せ IR 1905-81-3-1 × IR 36 の Ph 80-50 に対する分離については IR 36 中に IR 1905-81-3-1 中の抵抗性遺伝子を抑制する抑制遺伝子も考えない限り説明できなかった。むしろ親として用いた IR 36 が交配に用いたものと違うものと考えられた。

第 1 の組合せは第 2 のフィリピン菌系 Ph 80-64 に対して第 1 の菌系の場合とは全然異なる分離を示し, 3 つの補足遺伝子の存在を暗示した。

これらの結果から, IR 1905-81-3-1 は 3 つ以上の抵抗性遺伝子により支配され, それらの遺伝子作用の発現は主働遺伝子的な効果から補足的に働く微働遺伝子的な効果まで使用菌系, 環境条件により大きく変るものと考えられた。

何れの組合せも日本菌系研 53-33 に対しては分離を示さずこれらの品種中にこの菌系に対して働く共通の遺伝子をもつものと考えられる。