The Possible Application of Gene-for-Gene Concept in Blast Resistance

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Since Flor's extensive studies on the inheritance of flax and flax rust, many studies on the host-pathogen relationship were carried out. The gene-for-gene relationship was recognized by many host-pathogen systems. These were reviewed by Day (1974), Person and Sidhu (1971), and Kiyosawa (1980). This relationship was demonstrated in the rice-blast system (Yamasaki and Kiyosawa 1966, Kiyosawa 1967). This concept has been employed in breeding for blast resistance in rice plants.

The principle of gene-for-gene concept

Flor (1956) found that 25 genes controlled rust resistance in flax plants and avirulence in flax rust, respectively, and that resistant or avirulent reaction was induced in contact of a special resistance gene and the avirulence gene which corresponded specifically to the resistance gene. Such a relationship was called gene-for-gene relationship[14].

Differentiating ability of fungus strains and host varieties

Pathogenic specialization has been found in many pathogens. This corresponds to the presence of many genes for resistance in the host. Differential varieties have been used for classifying fungus strains regarding pathogenicity and differential fungus strains are required for classification of host varieties based on their blast resistance.

Let us consider differentiating ability in a system concerning two resistance genes (AB) and two avirulence genes (ab) which specifically correspond to the two resistance genes. Four genotypes are constituted from two gene-pairs: AB, A+, +B and ++ in the host varieties, and ab, a+, +b and ++ in the pathogen isolates.

The host-pathogen relationship as shown in Table 1 is obtained from all possible combinations of these genes in the host and pathogen.

Table 1. Host-pathogen relationship in two-gene-pair system

Host	Pathogen			
	ab	a+	+b	++
AB	R	R	R	S
A+	R	R	S	S
+B	R	S	R	S
++	S	S	S	S

Let us consider differentiating ability of any one fungus strain in Table 1. Any one of four fungus strains cannot classify four genotypes of the host into four groups: for example ab fungus strain divides the four genotypes into only two groups: genotypes showing resistant (R) reaction (AB, A+ and +B) and a genotype showing susceptible (S) reaction (++).

When two strains are selected from the four, ab and a+, for example, divide the four genotypes into three groups, RR (AB, A+), RS (+B) and SS (++). Of six combinations of four genotypes in the pathogen, only one combination, a+ and +b, can divide into four groups, RR, RS, SR and SS. The

use of the two strains, a+ and +b, is necessary and sufficient for classification of host varieties at least on the basis of the two resistance genes, A and B.

When three genes (A, B and C) are included in varieties, a++, +b+ and ++c are required to differentiate all possible host genotypes consisting of the three genes (Table 2). Any other combinations of three fungus genotypes cannot completely differentiate the host genotypes.

The facts mentioned above indicate that fungus strains including each one of avirulence genes are necessary to differentiate varieties on the basis of disease resistance. This indicates that fungus strains in the number which corresponds to the number of resistance genes that breeders attempt to accumulate in one variety are necessary to do so.

A similar conclusion can be drawn also on the differentiation of fungus isolates based on pathogenicity. Namely, a set of varieties having only one different gene for resistance is most effective to classify fungus isolates. This concept was established by Flor (1956) and has been used for selecting differential varieties in various crop-pathogen systems: flax-flax rust[4], oat-crown rust[3] and rice-rice blast[16].

The old Japanese set of differential varieties[8] and the international differential varieties[1,7] for rice blast are not suitable from the point that they don't have single different genes for blast resistance or are not genetically studied.

The number of genes to be found

The host-pathogen relationship in the system involving three genepairs is shown in Table 2. The left-upper quarter of this table shows the host-pathogen relation in the system including two gene-pairs (AB and ab). The left-under quarter is the case in which the third resistance gene (C) is added but not the corresponding avirulence gene (c).

On this set of varieties, the four fungus strains show the same reactions as on a set of the varieties without the resistance gene, C. When the third avirulence gene (c) is added in the differential fungus strains (right-under quarter of the table), the presence of the gene, C, is detected. This reveals that a resistance gene cannot be detected if the fungus strains used don't have the avirulence gene specifically corresponding to the resistance gene. Accordingly, the number of resistance genes found in a variety is determined not only by the number of resistance genes included in the variety but also by the number of avirulence genes in the fungus strains used.

Table 2. Host-pathogen relationship in three-gene-pair system

Host	Pathogen							
	ab+	a++	+b+	+++	abc	a+c	+bc	++c
AB+	R	R	R	S	R	R	R	S
A++	R	R	S	S	R	R	S	S
+B+	R	S	R	S	R	S	R	S
+++	S	S	S	S	S	S	S	S
ABC	R	R	R	S	R	R	R	R
A+C	R	R	S	S	R	R	R	R
+BC	R	S	R	S	R	R	R	R
++C	S	S	S	S	R	R	R	R

Similarly, the number of avirulence genes in fungus strains found, accordingly the number of races classified, is dependent not only upon the number of avirulence genes in the fungus strains, but also upon the number of resistance genes in differential varieties used.

Use of fungus mutants for identification of resistance gene

Fungus mutants for virulence are often obtained in inoculation experiments. They are isolated from a large lesion on resistant varieties. These mutants can be efficiently employed for identification of resistance genes, as a mutant is generally considered to differ in one gene from the original one.

In order to know the presence of a known

Table 3. Reaction pattern of Tongil and its relatives to fungus mutants

Variety		Fungus strain						
		Ken 54-20-b^+-k^+	Ina 168-b^+	Ina 168-a^+	Ken 53-33	Ina 72-b^+	Ken 54-20-k^+	Ken 54-20-z^+-k^+
Tongil		R^h	R^h	R^h	R^h	MR*	R^h	R^h
Yushin		M	R^h	R^h	R^h	R	R^h	R^h
Milyang 21		MR	R^h	R^h	R^h	MR	R^h	R^h
Milyang 23		M	R^h	R^h	R^h	R	R^h	R^h
Josaeng Tongil		R^h	R^h	R^h	R^h	MR	R^h	R^h
Differential varieties	Aichi Asahi (*Pi-a*)	S	R	S	S	R	S	S
	Kanto 51 (*Pi-k*)	S	R^h	R^h	S	S	S	S
	Fukunishiki (*Pi-z*)	R	R	R	R	M	R	S
	BL 1 (*Pi-b*)	S	S	R^h	R^h	S	R^h	R^h

*: Ina 72-b^+ was injected and others were sprayed.

resistance gene, for instance *Pi-k*, in a breeding line or a new variety, this line or variety is inoculated with a fungus strain avirulent to a variety with *Pi-k* and its mutants overcoming *Pi-k*. If the variety to be tested shows resistant and susceptible reactions to both fungus strains, respectively, the variety is concluded to have the gene, *Pi-k*.

Some Korean varieties which were derived from *indica-japonica* hybrids were tested for blast resistance with Ken 54-20-b^+-k^+*, Ina 168-a^+ and Ken 54-20-z^+-b^+, and their original fungus strains (Table 3). Among varieties tested, Yushin, Milyang 21 and Milyang 23 showed less resistant reactions to Ken 54-20-b^+-k^+ than to the other strains. This suggests that these varieties have the gene *Pi-b*. Furthermore, the highly resistant reactions of these varieties to Ina 168-b^+ and Ina 168-a^+ suggest that these varieties have genes *Pi-b* and *Pi-a*. Although gene constitution of Tongil and Josaeng Tongil was not determined in this experiment, these two varieties were suggested to have a gene or genes other than *Pi-b* and *Pi-a*.

This is a method to identify resistance genes in the host by using fungus variation and was called the mutant method for identification of resistance genes[10].

*: Ken 54-20-b^+-k^+ is a mutant which overcomes genes *Pi-b* and *Pi-k* and is originated from Ken 54-20.

Use of fungus mutants in gene analyses of disease resistance

The method mentioned above is not useful in a variety in which the expression of resistance gene to be identified is masked by an epistatic gene.

Gene analysis is generally carried out by crossing a variety to be analyzed with a susceptible variety and with varieties having known resistance genes. Thus, the number of genes included in the test variety is determined and the identity of genes found to known genes is estimated, respectively. Such procedures are, however, greatly laborious. The use of fungus mutants in gene analysis was considered as an easier method of gene identification.

An F_2 population of the hybrid of a resistant variety to be analyzed and a susceptible variety is separately inoculated with a fungus strain avirulent to the resistant variety and its mutant toward the virulence to a known resistance gene. If a significantly different segregation is obtained, it can be concluded that the resistant variety has the resistance gene which corresponds to the virulence gene differing between the mutant

and the original fungus strain.

Combined use of cumulative distribution curve method and fungus mutant

For an analysis of Tongil, fungus mutants and cumulative distribution curve method were used in combination. The cumulative distribution curve method was developed as a method to analyze in a limited space such as greenhouse and inoculation chamber. The number of plants tested is restricted in an experiment in a limited space. When the number of plants in an F_3 line is small, 3 : 1 and 15 : 1 ratios can not be differentiated from 15 : 1 and 1 : 0 ratios, respectively.

The cumulative distribution curve method was devised as a method to analyze without differentiating lines based on segregation ratios.

The principle of this method consists of the following processes. 1) Calculating the expected frequency of each genotype in F_2 generation of used F_3 lines, 2) calculating the expected frequency of susceptible plants in each F_3 line which is according to a binomial distribution, 3) adding the frequencies to obtain the frequency distribution curve, and 4) obtaining the cumulative distribution curve by adding the frequencies of the obtained curve from left to right in Fig. 1.

The expected cumulative distribution curve thus obtained is compared with the observed cumulative distribution curve obtained by testing F_3 lines for resistance. The number of resistance genes in the tested variety is estimated from an expected cumulative distribution curve most similar to the observed curve.

Fig. 2 shows the cumulative distribution curves observed in F_3 generation of the hybrid of Palkweng × Tongil inoculated separately with Ina 72 and Ina 72-a^+. The significant difference between the two observed curves suggests that Tongil has the gene *Pi-a* to which corresponding virulence gene is different between the two fungus strains used, because Palkweng (Korean *japonica* variety) is susceptible to both fungus strains.

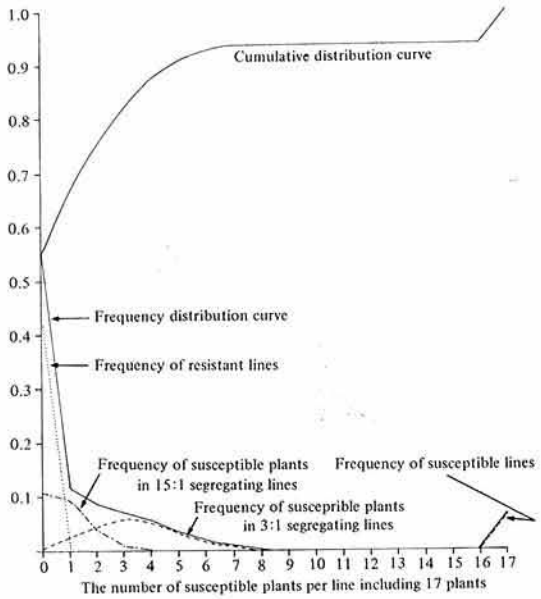

Fig. 1. Drawing of the cumulative distribution curve expected from a dihybrid relating to two dominant genes[11]

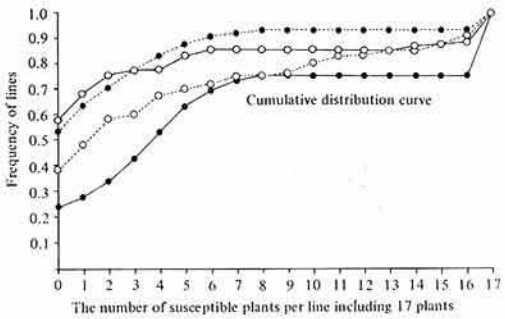

Fig. 2. Cumulative distribution curve[13] of a hybrid of Palkweng×Tongil inoculated with Ina 72 and Ina 72-a^+

—○—○— Ina 72 : The curve when inoculated with Ina 72.

···○···○··· Ina 72-a^+ : The curve when inoculated with Ina 72-a^+.

···●···●··· AB : The curve expected when two dominant genes are concerned.

—●—●— A : The curve expected when one dominant gene is concerned.

F_3 lines of the hybrid of Tongil × Koshihikari were inoculated with Ken 54-20 and

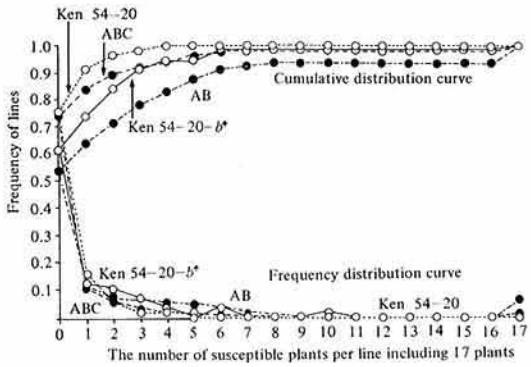

Fig. 3 Cumulative and frequency distribution curves of a hybrid of Tongil×Koshihikari inoculated with Ken 54-20 and Ken 54-20-b^+

Ken 54-20: The curves when inoculated with Ken 54-20.

Ken 54-20-b^+: The curves when inoculated with Ken 54-20-b^+.

ABC: The curves expected when three dominant genes are concerned.

AB: The curves expected when two dominant genes are concerned.

(Kiyosawa, Kikuchi and Nakane, unpublished)

Ken 54-20-b^+ (Fig. 3) (Kiyosawa, Kikuchi and Nakane, unpublished). A significant difference was observed between two cumulative distribution curves, indicating the contribution of the gene *Pi-b* in the resistance of Tongil.

These facts indicate that Tongil has at least two genes, *Pi-a* and *Pi-b*.

The results of other experiments in addition to the results in Figs. 2 and 3 indicated that Tongil had three or more genes for blast resistance. Therefore, Tongil has other gene or genes than *Pi-a* and *Pi-b*, too.

Concluding remarks

The gene-for-gene concept was established after gene analyses of disease resistance. The possibility of application of the gene-for-gene concept to the host-pathogen relationship of rice and rice blast system was confirmed through the gene analyses of Japanese rice varieties. The mutant method for identification of resistance gene revealed that many lines which were derived from many *indica-japonica* crosses have the same gene, *Pi-z*^{t} (Fujimaki and Yokoo, 1971). This is an example in which the gene-for-gene theory was efficiently applied to the breeding for blast resistance. However, utilization of the gene-for-gene theory in the breeding for blast resistance is rather a future problem in Japan. The most effective application of the concept to rice breeding for blast resistance will become possible when the number and kind of resistance genes included in the crosses are predicted from the genotype of its parents. When unknown resistance genes are introduced, gene analysis must be accompanied for an useful application of this concept to breeding or selection.

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