# 11. GENETIC STUDIES ON HOST-PATHOGEN RELATIONSHIP IN THE RICE BLAST DISEASE

Shigehisa KIYOSAWA*

## Introduction

Genetic study on the inheritance of the resistance of rice plant to blast was begun by Sasaki[37]. In the first twenty years, many studies were accumulated as reviewed by Takahashi[41] and Yamasaki and Kiyosawa[44]. All these studies were performed under natural infection or artificial infection in which racial constitution of the blast fungus was not known.

Since then, change has taken place in the studies pertaining to the specialization of races and it has made complicated gene analysis of resistance of the rice plant to blast. Moreover, the studies on the differentiation of pathogenic races made it possible to determine the

**Table 1. Classification of rice varieties on the basis of resistance to blast.**

| Variety | Classification of rice varieties | | |
|---|---|---|---|
| | 16, 21, 22, 24) KIYOSAWA | GOTO & YAMANAKA10) | IWATA & NARITA10, 11) |
| Zenith | Zenith type | I | A |
| Te-tep, Tadukan | | II | |
| Pi No. 1 | Shimokita type | | |
| Pi No. 3 | Pi No. 4 type | | |
| Choko-to | To-to type | III | B |
| Yakei-ko, Reishiko, Kanto 51, Kanto 53 | Kanto 51 type | IV | C |
| Ishikari Shiroke, Norin 34 | Ishikari Shiroke type | V | E |
| Fujisaka 5 | | VI | |
| Homare Nishiki, Shuho, Aya-nishiki | Aichi Asahi type | | F |
| Aichi Asahi, Norin 17 | | IX | |
| Ginga | Shin 2 type | VII | G |
| Norin 22, Shin Yamabuki | | VIII | |
| Norin 20 | | X | |
| Shinsetsu* | Shinsetsu type | | D |

* This variety was not tested by GOTO and YAMANAKA10).

* Department of Physiology and Genetics, National Institute of Agricultural Sciences, Hiratsuka, Kanagawa, Japan.

**Table 2. Classification of rice varieties by KIYOSAWA and gene constitution of each type.**

| Fungus strain | | P-2b | Ken 53-33 | Ina 72 | Hoku 1 | Ken 54-20 | Ken 54-04 | Ina 168 |
|---|---|---|---|---|---|---|---|---|
| Type of variety | Genotype | *Av-k*<br>*Av-i*<br>*Av-z* | *Av-z*<br>*Av-ta2* | *Av-a*<br>*Av-i*<br>*Av-z*<br>*Av-ta*<br>*Av-ta2* | *Av-k*<br>*Av-z*<br>*Av-ta*<br>*Av-ta2* | *Av-k*<br>*Av-i*<br>*Av-z*<br>*Av-ta*<br>*Av-ta2* | *Av-k*<br>*Av-i*<br>*Av-z*<br>*Av-ta*<br>*Av-ta2* | *Av-a*<br>*Av-k*<br>*Av-i*<br>*Av-z*<br>*Av-ta2* |
| Shin 2 type | + | S | S | S | S | S | M～S | S |
| Aichi Asahi type | *Pi-a* | S | S | R | S | S | M～S | R |
| Kanto 51 type | *Pi-k* | M | S | S | $R^h$ | $R^h$ | $R^h$ | $R^h$ |
| Ishikari Shiroke type | *Pi-i* | M | S | M | S<br>M | M | M | M |
| Ta type | *Pi-ta* | S | S | M | | M | M | S |
| Pi No. 4 type | *Pi-ta2* | S | M | MR | M | MR | MR | MR |
| Fukunishiki type | *Pi-z* | M | M | M | M | M | M | M |
| To-to type | *Pi-a*<br>*Pi-k* | M | S | R | $R^h$ | $R^h$ | $R^h$ | $R^h$ |
| Shinsetsu type | *Pi-a*<br>*Pi-i* | M | S | R | S | M | M | R |
| Shimokita type | *Pi-a*<br>*Pi-ta* | S | S | R | M | M | M | R |
| Zenith type | *Pi-a*<br>*Pi-z* | M | M | R | M | M | M | R |

mutual relationship between resistance genes found in different times or by different researchers.

Recent studies on the inheritance of blast-resistance using the fungus strains with known pathogenicity were reviewed by Kiyosawa[21].

Genetic studies by using fungus strains with known pathogenicity were begun by Niizeki[33]. After that, such studies were propelled by Iwata and Narita[10,11] and Kiyosawa and his co-workers[14~28]. The progress of the researches on the differentiation of pathogenic races by Goto, *et al.*[10,11] greatly contributed to such advanced studies. Up to date, eight genes controlling true resistance to blast have been found in Japan.

## Classification of Varieties on the Basis of Resistance

In Japan, three groups of researchers attempted to classify the rice varieties which were native or bred in Japan (Table 1). Goto and Yamanaka[10] classified Japanese rice varieties including some foreign ones into ten groups, and selected the differential varieties based on the results. Iwata and Narita[10,11] divided many Japanese varieties or lines tested into seven groups. Among the varieties or lines tested by them, a large number were those which were being bred for rice cultivation in Hokkaido district and a few were the same foreign varieties as those tested by Goto, *et al.*[10,11] Based on their resistance to Japanese races, Kiyosawa and his co-workers tested native or leading varieties and newly bred varieties or lines in Japan, and divided them into eleven groups. At first Yamasaki and Kiyosawa[44] found five types, Shin 2, Aichi Asahi, Kanto 51, Ishikari Shiroke and To-to, in Japanese varieties including some Chinese varieties. Kiyosawa[16,20~22] later added six types, Shinsetsu, Ta, Pi No. 4, Fukunishiki, Shimokita and Zenith (Table 2). All of them were ones which were bred in Japan using foreign varieties as a source of resistance, except the Shinsetsu type.

Relatively good agreement can be found among the results of classification by the three

**Table 3. Rice varieties belonging to each type on blast resistance.**

| Japanese variety | | Foreign variety |
|---|---|---|
| Designated variety* | Others | |
| Shin 2 type<br>Shin 2, Norin 25, Norin 1, Tozan 38, Norin 22, Norin 8, Omachi, Koshiji Wase, Shin Yamabuki, Shirogane, Hatsunishiki, Tedori Wase, Koshihikari, Honen Wase, Norin 6, Norin 24, Norin 36, Norin 7, Norin 10, Norin 23, Norin 20, Norin 29, Obako Wase, Norin 12, Norin 30, Norin 31, Norin 32, Norin 35, Norin 37, Norin 38, Norin 43, Norin 44, Norin 47, Norin 48, Homasari, Futaketori, Yachi-kogane, Tone Wase, Shimotsuki, Bizennishiki, Kiyosumi, Akibae, Yaeho, Chikuma, Kokeshi Mochi$^{w}$, Yama-kogane, Shioji, Chiyohikari, Yomo-hikari, Shintsuru Mochi$^{w}$, Seto-honami, Natsuminori, Yamase-shirazu, Rikuto Norin 12. | Rikuu 132, Wase Sen-ichi, Aichi Wase Asahi, Kyushu 8, Zenkoku Wase, Kamenoo, Mokoto, St No. 1, Shinriki, Aikoku 1, Takenari, San-in 45, Toyosato, Sachi-watari, Wase Aikoku 3, Gin Bozu Chusei, Ta Sensho, Shinju, Wase Asahi 2, Togo, Kokuryo Miyako, Sen-ichi, Bozu 6. | Caloro. |
| Aichi Asahi type<br>Aichi Asahi, Senbon Asahi, Norin 17, Kinmaze, Norin 18, Norin 41, Towada, Sasashigure, Jukkoku, Eiko, Norin 27, Asakaze, Fujiminori, Zuiho, Yuki-mochi$^{w}$, Sasa-honami, Fukuminori, Norin Mochi 45$^{w}$, Hamayu, Yamabiko, Otori, Norin 21, Norin 16, Hoyoku, Norin 2, Norin 9, Norin 13, Norin 33, Nakate Shin Senbon, Mihonishiki, Gin-masari, Fukusuke, Nagiho, Akikogane, Wakasa, Toyo-chikara, Hatsukine, Asuwa, Norin 28, Asahi, Norin 14, Norin 19, Norin 42, Norin 46, Norin 49, Norin 50, Norin 51, Norin Mochi 5$^{w}$, Kotobuki Mochi$^{w}$, Hashiri Mochi$^{w}$, Hatsuminori, Kaganishiki, Hatsushimo, Azusa, Yama-tedori, Benisengoku, Akebono, Oirase, Megumi-wase, | Ayanishiki, Yutaka Senbon, Okute Eiko, Tsurugi, Shinriki 11, Koganemaru, Haruta Asahi, Chikara Senbon, Tokai Senbon, Shuho, Kyoto Asahi, Futaba, Takara, Kaori, Mikawa Nishiki, Chusei Honen, Takane Asahi, Shiro Senbon, Homare Nishiki, Kogane Mochi$^{w}$, Zensho 26, Akage, Hashiri Bozu, Hyoroku-mochi$^{w}$. | |
| Kanto 51 type<br>Kusabue, Mangetsu-mochi$^{w}$, Ugonishiki,Fukei 69, Hatsuiwai-mochi$^{w}$, Senshuraku, Tatsumi-mochi$^{w}$, Tsuyuake. | Kanto 51. Kanto 53, Kanto 59, Chugoku 31, Imochi-shirazu. | Reishiko$^{w}$, Yakei-ko. |
| Ishikari Shiroke type<br>Norin 34, Fukuyuki, Yoneshiro. | Ishikari Shiroke, Fujisaka 5, Sekiyama 2, Hokuriku 12, Fukumochi$^{w}$, Wase Bozu. | Butamachi (Doazi chall). |
| Ta type | K 1. | |
| Pi No. 4 type | Pi No. 4, Pi No. 3, Pi No. 5. | |
| Fukunishiki type<br>Fukunishiki. | 54BC–68, Ou 243, Ou 244. | |
| To-to type<br>Tei ne,Yukara, Kagura-mochi$^{w}$, Tsukimi-mochi$^{w}$,Koshihibiki, Oyodo, Sanpuku, Minehikari. | BR No. 1 (Kongo), Hokushin. | To-to, Choko-to, Hokushi Tami. |
| Shinsetsu type<br>Shinsetsu, Miyoshi, Takane Nishiki, | | |
| Shimokita type<br>Shimokita. | Pi No. 1, Pi No. 2. | |
| Zenith type | Fukei 67, Fukei 73. | Zenith. |

* : Excellent varieties which were registered in the Ministry of Agriculture and Forestry until 1967 and which are being designated in prefectures in 1967.

W: Waxy endosperm.

groups of researchers.

Matsumoto, Yamada and Kozaka[30] found high resistance to Philippine fungus strain Ken Ph-03 and found that there were at least two types of high resistance (immunity) to the strain in Japanese varieties. One of them was found in Kanto 51 and To-to type varieties (or groups) mentioned above. The second type of immunity from Ken Ph-03 was found in all of the Ishikari Shiroke type varieties tested and in some of the Shin 2 and Aichi Asahi type ones.

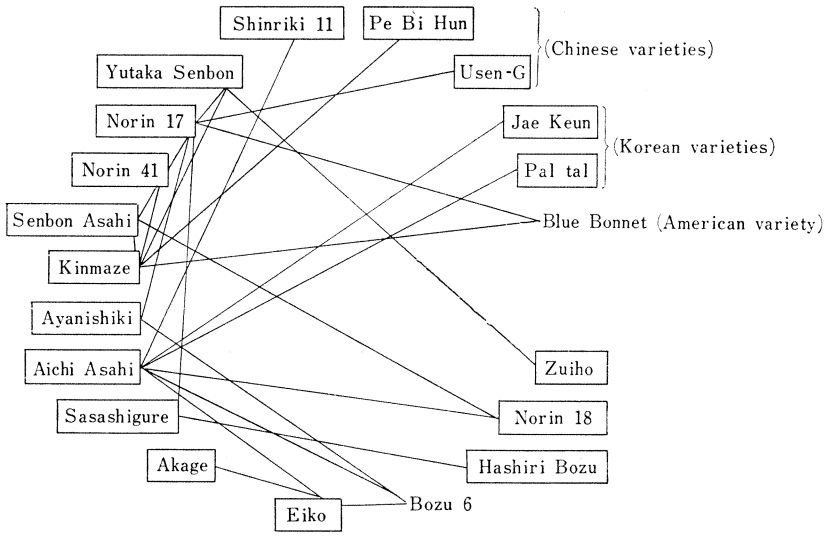
## Inheritance of True Resistance

1. Methods for Gene Analysis of Resistance

Generally, gene analysis of resistance was carried out by inoculating progenies of the hybrid between resistant and susceptible varieties with fungus strain whose pathogenicity on the differential varieties had been known. This method is called "hybridization method for gene analysis."

The other one is "mutant method for gene analysis." As mentioned later, pathogenicity of blast fungus strain changes sometimes spontaneously or artificially. These mutants can be efficiently used to know the presence or absence of a known gene for resistance in a given variety.

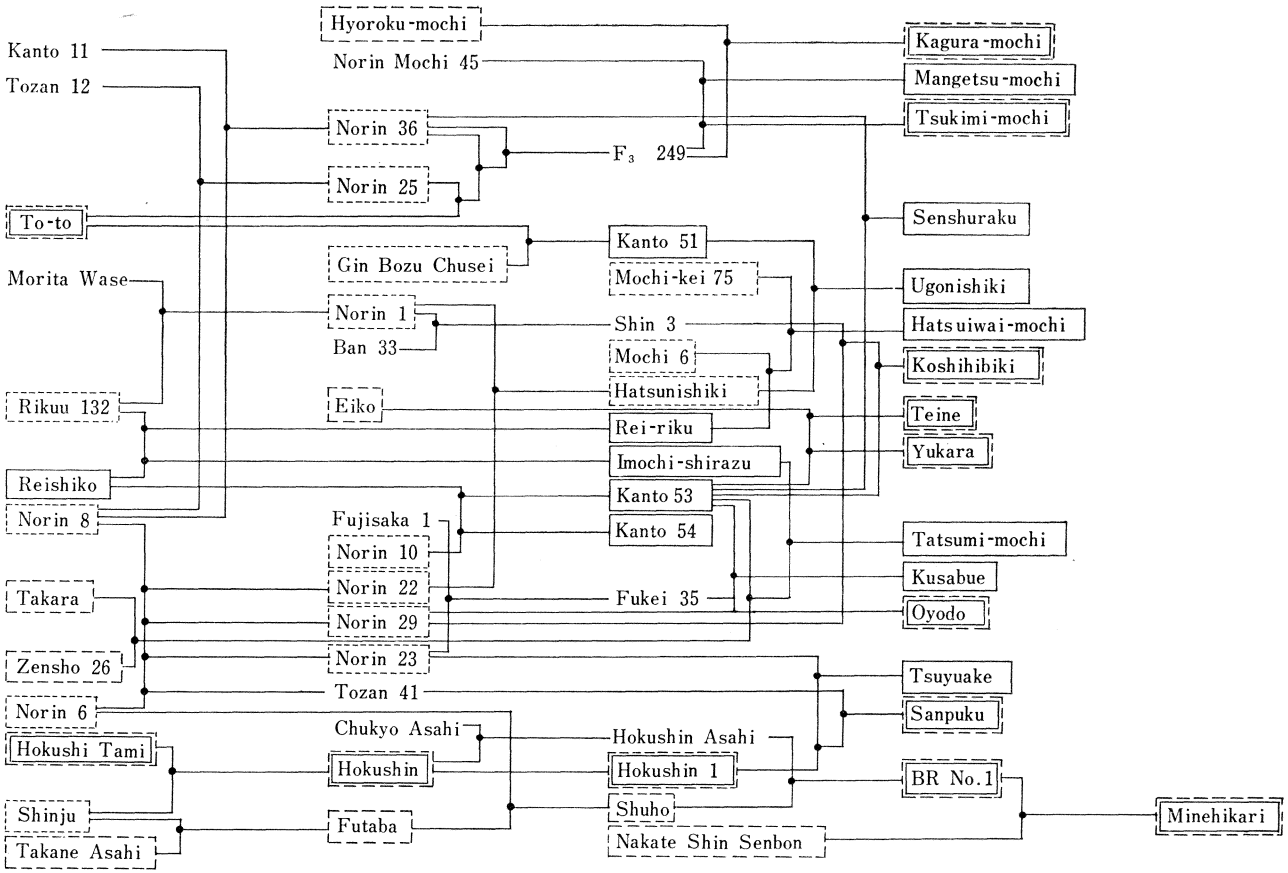
For example, when a fungus strain "a" which is avirulent to a resistant variety "A" is sprayed or injected to the variety "A", a few susceptible lesions developed occasionally on the variety. A culture which is isolated from the lesions sometimes shows susceptible reaction on the variety "A". Such a culture does not show any change of pathogenicity on other types of resistant varieties, and of characteristics on the medium. Hence, the culture is a mutant on pathogenicity. When this mutant is sprayed or injected to the varieties "A" and "B" attempted to test, many susceptible lesions should be developed on the variety "B" as well as on the variety "A", if the former has the same gene as the latter.




: Variety in which resistance to Ina 72 or Ina 168 was demonstrated to be controlled by one dominant gene.

———: No susceptible plant was segregated in $F_2$ population of hybrid between varieties at the both ends of each line.

**Fig. 1. Relationship between genes in Aichi Asahi type varieties.**




: Variety carrying *Pi-k*, : Variety carrying *Pi-a*,

: Variety having no resistance genes. Other varieties were not tested.

**Fig. 2. Breeding process of blast-resistant varieties by using the gene *Pi-k* in Japan.** (KIYOSAWA[24])

2. Aichi Asahi Type Varieties

Genetic study of resistance of Aichi Asahi type varieties was begun by Niizeki[33]. He found one dominant gene in Ayanishiki and Aichi Asahi. Iwata and Narita[10,11] reported one dominant gene in each of Norin 15, Hashiri-bozu, Hakkoda, Towada, Norin 19 and Wase Nishiki, and the gene in Hakkoda was the same as that in Wase Nishiki. Yamasaki and Kiyosawa[44] supported the theory that one dominant gene controlled resistance of Aichi Asahi type varieties including Norin 17, Kinmaze, Senbon Asahi, Norin 41, Ayanishiki and Yutaka Senbon, and concluded that genes carried by these varieties were the same. They symbolized the gene as *Pi-a*. Kiyosawa, Matsumoto and Lee[28] found one dominant gene in Aichi Asahi. Kiyosawa[20] studied, through numerous experiments, on many Aichi Asahi type varieties and obtained the same conclusion (Fig. 1). Thus, he concluded that all of the Aichi Asahi type varieties in Japan carried the same gene *Pi-a*.

3. Kanto 51 Type Varieties

Niizeki[33] found one dominant gene in Kanto 54, using one avirulent strain of blast fungus. Yamasaki and Kiyosawa[44] studied resistance of Kanto 51 to five of seven fungus strains used by them, and concluded that the resistance to these fungus strains was controlled by one gene which was symbolized as *Pi-k* by them. When spraying method was used for inoculation the gene *Pi-k* always showed complete dominance, whereas by the injection method there was a change in dominance from complete dominance to recessiveness with variation of environmental conditions.

Kiyosawa[15] analyzed the resistance of Reishiko, and concluded that the variety carried the gene *Pi-k*.

Kiyosawa[24], furthermore, made clear the presence of the gene *Pi-k* in Kusabue and Yakei-ko.

It must be noted that all the tested Kanto 51 type varieties have been demonstrated to carry the gene *Pi-k* (Fig. 2).

4. Ishikari Shiroke Type Varieties

Yamasaki and Kiyosawa[44] studied the resistance of Ishikari Shiroke and Fujisaka 5 and found the existence of the gene *Pi-i* in these varieties. The same gene was found in Sekiyama 2[15] and in the Korean variety, Doazi chall (Butamochi)[23]. Kiyosawa[20] suggested that all the Ishikari Shiroke type varieties in Japan have the gene *Pi-i*.

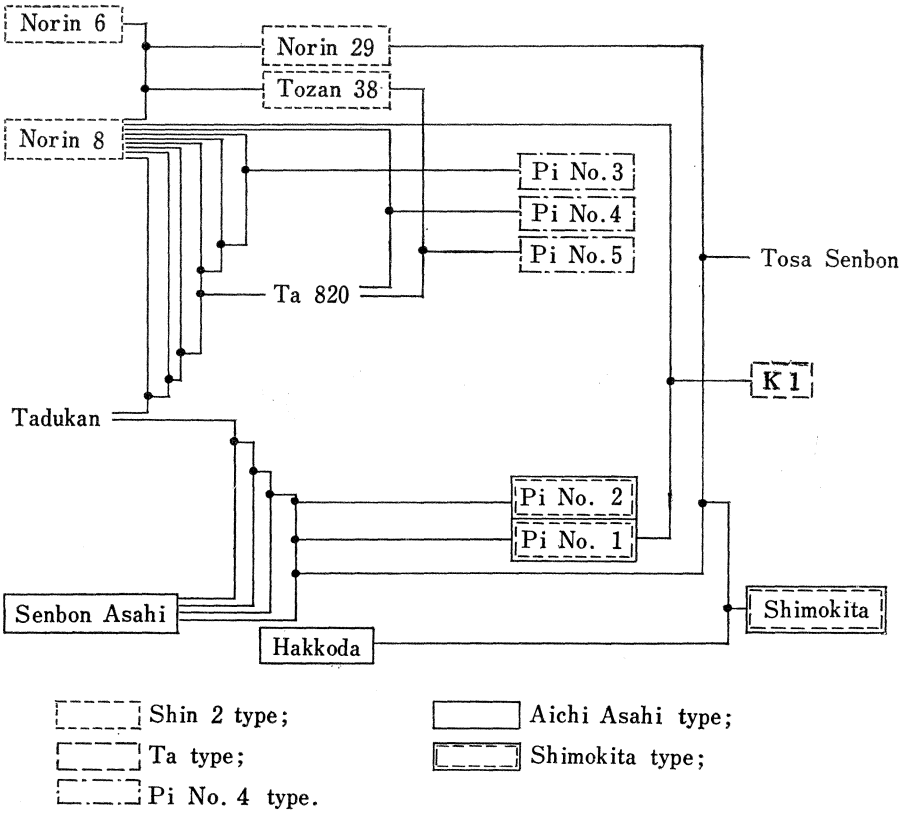
5. To-to Type Varieties

To-to type varieties were analyzed successfully with the mutant method and the results were confirmed with the hybridization method[24]. All the tested To-to type varieties, To-to, Choko-to and Minehikari, carry the genes *Pi-a* and *Pi-k*. Minehikari has the gene *Pi-m*, in addition to them[24].

All of the Kanto 51 and To-to type varieties bred in Japan were derived from hybrids of Japanese varieties with Chinese ones, Reishiko, To-to and Hokushi Tami. In addition to this, the fact that all the above mentioned tested varieties in these types have the gene *Pi-k*, indicates that all the Kanto 51 and To-to type varieties bred in Japan have the gene *Pi-k* (Fig. 2)[24].

6. Shimokita Type Varieties

The Philippine variety Tadukan which was highly resistant to Japanese races was utilized in Japan as a source of resistance for breeding of blast-resistant variety (Fig. 3). Thus, Shimokita was released in 1962 as a resistant variety. Kiyosawa[16] studied on the inheritance of resistance of Pi No. 1 which was a variety obtained in the course of breeding of Shimokita and indicated that Pi No. 1 had two dominant genes *Pi-a* and *Pi-ta*. He demonstrated this by selecting a line (K 1 in Table 3) which had only one, *Pi-ta*, of two genes contained in Pi No. 1.




Tadukan shows the M~R reactions to all the fungus strains used, except for MS or S reaction to Ken 53-33.

**Fig. 3. Genealogical relationship of derivatives from Tadukan.** (KIYOSAWA[22])

7. Pi No. 4 Type Varieties

Pi No. 4 was bred by introducing a resistance of Tadukan as well Pi No. 1. Its resistance to blast, however, differs slightly from that of Pi No. 1. Kiyosawa[22] found the gene *Pi-ta2* in this variety. He thought that this gene was an allele of the gene *Pi-ta*, a gene closely linked with *Pi-ta*, or a gene complex consisting of *Pi-ta* and another which was closely linked with *Pi-ta*.

8. Zenith Type Varieties

The U. S. variety Zenith has been utilized as a source of resistance in Japan, and Fukunishiki was bred (Fig. 4). Kiyosawa[21] studied on the inheritance of resistance of Zenith and Fukei 67; the latter was the same type as the former and was a derivative from the former. He found two genes *Pi-a* and *Pi-z* in them. Fukunishiki bred from Zenith had only the gene *Pi-z*. This *Pi-z* gene is probably the same as the gene $Pi_6$ which was found in Zenith by Atkins and Johnston[2].

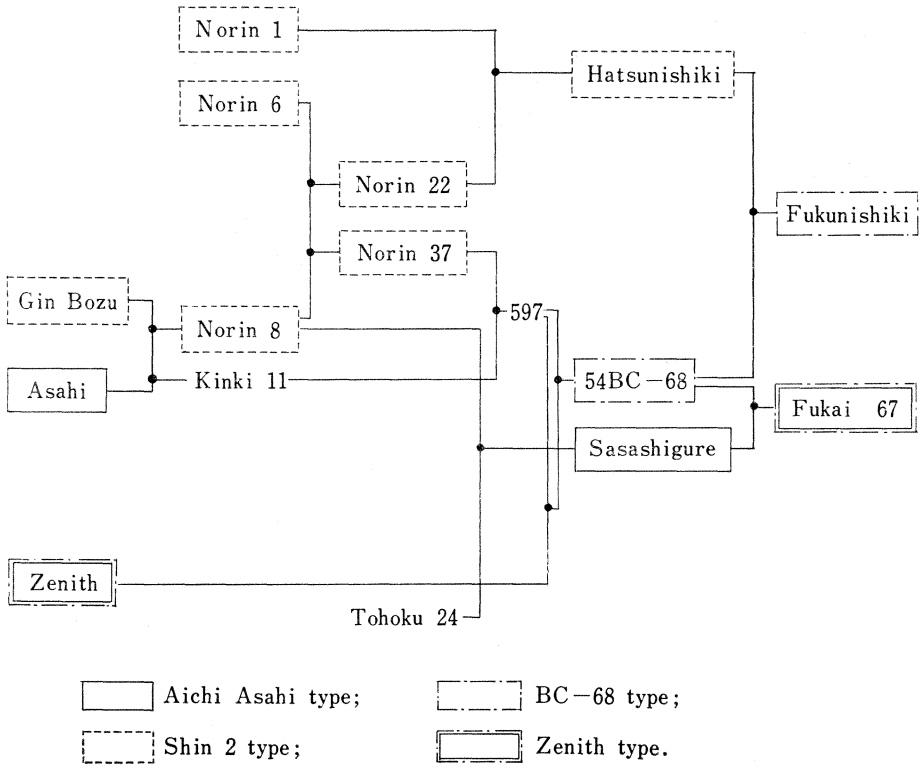



**Fig. 4. Genealogical relationship of derivatives from Zenith.** (KIYOSAWA[21])

9. Resistance of Japanese Varieties to the Philippine Fungus Strain Ken Ph-03

Matsumoto, Yamada and Kozaka[30] found that a Philippine fungus strain Ken Ph-03 showed an avirulence to some Japanese varieties in spite of susceptibility of these varieties to most of Japanese races. Kiyosawa[25] divided such an immunity from the strain of Japanese varieties into two types. Kusabue is representative of one type. Immunity from the strain Ken Ph-03 of this type is controlled by the gene *Pi-k*. Immunity of other type is represented by Shinriki. Immunity of this type is nullified by a fungus mutant which is isolated from a lesion on Shinriki. This immunity is controlled by one dominant gene, *Pi-k*$^s$, which is allelic to the gene *Pi-k*[25].

10. Linkage Relationship

Among the eight genes for resistance identified up to date, *Pi-k* and *Pi-k*$^s$ are allelic to each other, and *Pi-ta* is allelic to or closely linked with Pi-ta2, as mentioned above. In addition to these it was found that there were linkage relationships between *Pi-m* and *Pi-k* with crossing-over value of 11.3 %[24] and between *Pi-i* and *Pi-z* with that of 30.9 %[21] (Fig. 5).

Linkage relationships between genes for resistance and genes controlling characters other than resistance were studied in the following characters by Kiyosawa.

*Pi-k*: *C*, *C*$_S$ (glume colour gene in Sekiyama 2), *wx*, *s*$_Y$ (gene for yellow seedling in

Sekiyama 2) and $s_R$ (gene for yellow seedling in Reishiko).

*Pi-i*: Do. (Kiyosawa[15])

*Pi-i*: Apiculus colour (two genes) and awnedness (one) in Doazi chall.

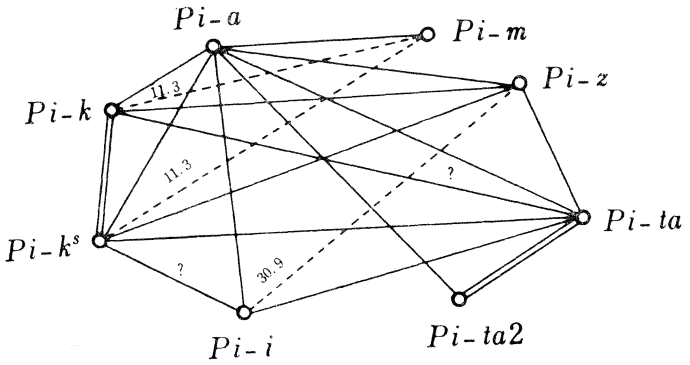
*Pi-a*: Do. (Kiyosawa[23])

*Pi-k*: Apiculus colour of To-to (three genes).

*Pi-a*: Do. (Kiyosawa[24])

He failed to find any linkage relationship among them, except the linkage (26.9 %) between *C* and *wx*.

Jodon and Atkins[13] found that the two genes $Pi_1$ in the variety Northrose and $Pi_6$ in the variety Zenith behaved independently of the genes for apiculus colour (*C*), gold hull (*gh*), gold forrow colour (*gf*), glabrous (*gl*), red bran (*Rc*) and earliness.




——: The genes at the both ends are independently behaved.

11.3: Showing linkage relationship with crossing over value of 11.3 %.

═══: Allelic or closely linked.

**Fig. 5. Linkage relationship among genes found up to date.**

11. Japanese Differential Varieties

Twelve varieties have been used for differentiation of pathogenic races in Japan[10,11]. Of these varieties, Kanto 51, Ishikari Shiroke, Aichi Asahi and Homare Nishiki were genetically analyzed and the above mentioned three genes were found in these varieties. It was already concluded that there was at least the gene *Pi-k* in Choko-to and Yakei-ko. Reactions of Yakei-ko and Choko-to to many races show that these two varieties carry gene(s) other than the gene *Pi-k*. Such a gene(s) is not yet identified.

Studies on gene analysis of Te-tep (Indo-Chinese variety), Tadukan (Philippine) and Usen (Chinese) by Kiyosawa[26] is in progress. $F_2$ populations of the hybrid of Te-tep × Shin 2 were inoculated with 7 fungus strains separately. Digenic segregations were observed for inoculations of P-2b, Ken 53-33 and Ina 72, and trigenic segregations for Hoku 1, Ken 54-20 and Ken 54-04. For Ina 168, susceptible plants were not found in 130 $F_2$ plants. These results suggest that at least three or more genes participate in the resistance of this variety (Table 4).

Te-tep has been used as a source of resistance in Japan. Some lines were bred. A few of them have reactions of the Kanto 51 type to seven fungus strains which have been used for gene analysis by Kiyosawa, and their mutants. This suggests that one of genes included in Te-tep is on the locus *Pi-k*.

Tadukan was crossed with Shin 2. $F_2$ populations of this hybrid werd inoculated with

**Table 4. The number of genes which were assumed to be present in some foreign varieties.**

| | P-2b | Ken 53-33 | Ina 72 | Hoku 1 | Ken 54-20 | Ken 54-04 | Ina 168 | Total |
|---|---|---|---|---|---|---|---|---|
| Tadukan (Philippines) | 2 | 2 | 3～2 | 2 | 2 | 2～3 | 2 | ≧ 3 |
| Te-tep (Indo-China) | 2 | 2 | 2～3 | 3～2 | 3～2 | 2～3 | ≧ 3 | ≧ 3 |
| Pusur (West Pakistan) | 2 | 1 | 2～3 | 2～3 | 2 | | 2～3 | ≧ 2 |
| Charnack (India) | 3～2 | >1 | 2 | 3 | | | 2～3 | ≧ 3 |
| Milok Kuning (Malaya) | | 1 | | 3 | 2 | | | ≧ 3 |

the seven fungus strains. The results suggest that two or three genes control the resistance of Tadukan. From this variety, the gene *Pi-ta* has been introduced into Japanese varieties, Pi No. 1 and Shimokita. Furthermore, the gene *Pi-ta*2 transferred into Pi No. 4 originated from this variety. Since these two genes were known to be allelic or closely linked, it is assumed that at least one or two genes are present in Tadukan besides *Pi-ta* or *Pi-ta*2.

Gene analysis of Usen was carried out by inoculating $F_2$ populations of the hybrid with Shirogane (the Shin 2 type) with the seven fungus strains. The number of genes participating in the resistance of Usen was, however, not determined, as distribution of resistance in $F_2$ population was continuous for any of these seven strains. It seems that the resistance of Usen is controlled by a relatively large number of polygenes or minor genes, or that the major gene(s) controlling the resistance of Usen is very much variable in their function.

It has been known that the variety carrying single resistance gene is most effective as differential variety, as mentioned later. From this standpoint, most of the varieties used for differential varieties in Japan are not suitable because they have two or more genes. Hence, it is necessary to exert all possible efforts to look for the varieties suitable as differential varieties.

12. Some Foreign Varieties

Studies on inheritance of blast-resistance of some foreign varieties is under observation by Kiyosawa[26]. In general, gene analysis of blast resistance is very difficult, bacause they contain many genes for resistance in one variety and weak or semi-resistance which is masked by high-resistance gene in them disturbs the grouping of $F_2$ or $F_3$ plants based on the resistance.

$F_3$ lines of the hybrid of Pusur (West Pakistani variety) × Norin 22 were inoculated with six fungus strains. Mono-or digenic segregations were observed for inoculation with P-2b, Ken 54-20 and Ken 53-33. For the first and second strains, resistance was dominant over susceptibility, whereas for the third, dominance was reverse. Di-or trigenic segregations were obtained for Hoku 1, Ina 72 and Ina 168, and in any of these cases, resistance was dominant. This indicates that there are at least two genes for resistance in Pusur. He failed to determine the relationship among the genes controlling resistance to the six fungus strains in spite of the use of $F_3$ generation for analysis. This depended upon the complexity of segregation in the hybrid progeny. Possibly, Pusur contains two or more genes for resistance.

In a similar way, Milok Kuning (Malayan variety) was genetically studied. Results were obtained that demonstrated that three or more genes were included in the variety. In this case, Kiyosawa[26] also could not determine the relationsip among the genes acting against different fungus strains, owing to the same reason as the one in the case of Pusur.

## Genetic Study of Pathogenicity

### 1. Gene Analysis of Pathogenicity

Flor[7] advocated the gene-for-gene theory on the genetic relationship between host and pathogen. Thereafter, this theory was supported by genetic studies on the host-pathogen relationship of powdery mildew of barley[31] and wheat[36], stem rust of wheat[29] and bunt of wheat[38].

Yamasaki and Kiyosawa[44] applied the theory to the rice-blast relationship, and assumed the genotype on pathogenicity of the fungus strains.

For example, fungus strains Ina 72 and Ina 168 show an avirulent reaction on Aichi Asahi which carry the gene *Pi-a*. According to the theory, a resistance gene acts only in combination with the corresponding avirulence gene. Therefore, the fungus strains carry the avirulence gene, *Av-a*, corresponding to the resistance gene *Pi-a*.

In such a way, genotypes of eight fungus strains were assumed as follows[16,21,22,24,25,44]

| | |
|---|---|
| P-2b: | *Av-a*$^+$ *Av-k Av-i Av-ta*$^+$ *Av-ta2*$^+$ *Av-z Av-ks*$^+$ |
| Ken 53-33: | *Av-a*$^+$ *Av-k*$^+$ *Av-i*$^+$ *Av-ta*$^+$ *Av-ta2 Av-z Av-ks*$^+$ |
| Ina 72: | *Av-a Av-k*$^+$ *Av-i Av-ta Av-ta2 Av-z Av-ks*$^+$ |
| Hoku 1: | *Av-a*$^+$ *Av-k Av-i*$^+$ *Av-ta Av-ta2 Av-z Av-ks*$^+$ |
| Ken 54-20: | *Av-a*$^+$ *Av-k Av-i Av-ta Av-ta2 Av-z Av-ks*$^+$ |
| Ken 54-04: | *Av-a*$^+$ *Av-k Av-i Av-ta Av-ta2 Av-z Av-ks*$^+$ |
| Ina 168: | *Av-a Av-k Av-i Av-ta*$^+$ *Av-ta2 Av-z Av-ks*$^+$ *Av-m* |
| Ken Ph-03: | *Av-a*$^+$ *Av-k Av-i*$^+$ *Av-ta*$^+$ *Av-ta2*$^+$ *Av-z*$^+$ *Av-ks* |

### 2. Mutability of Pathogenity

Pathogenicity of blast fungus is very much variable. Variability of filamentous fungi has generally been explained by recombination, heterokaryosis and mutation[1,2].

In blast fungus, the sexual stage has not been found. Accordingly, sexual recombination is not a cause of variability. Asexual recombination was studied by Goto and Yamanaka[9] and Yamasaki and Niizeki[45]. Goto and Yamanaka[6] obtained new races from mixture inoculation with two fungus strains and assumed that this new race was developed through anastomosis between the two and/or mutation. Yamasaki and Niizeki[45] observed the occurrence of anastomosis between mycelia of different fungus strains on an artificial medium and using fungus strains with many biochemical markers they demonstrated the existence of parasexual recombination in blast fungus. This suggests that asexual recombination may be a cause of variability in blast fungus.

In many fungi, it has been known that heterokaryosis is responsible for the variability[2,35]. Contribution of heterokaryosis to the variability in a given fungus is dependent upon the existence of multiple nuclei. The number of nuclei in blast fungus was studied by some researchers. Yamasaki and Niizeki[45] studied karyologically mycelia and conidia of blast fungus, and concluded that this fungus was uni-nucleate. Mizusawa[32] observed cells of blast fungus with an electron microscope, and showed the uni-nucleate character of this fungus. Chen[4,5] also indicated to be uni-nucleate. Horino and Akai[12] observed uni-nucleus in electron micrographs of conidium.

On the other hand, Suzuki[39,40] and Chu and Li[6] emphasized that mycelia and conidia of the fungus were multi-nucleate. However, staining by them is obscure and nuclei showed by them does not appear in the typical feature of nuclei as compared with the one that was observed in various filamentous fungi. Furthermore, it is hard to doubt the results shown in the electron micrographs. With the electron microscopic technique, Yamasaki and Ishii[43] succeeded in getting lengthwise section of mycelial cells and observed only one nucleus in

them. Such considerations lead the present author to the conclution that the number of nuclei in the blast fungus is one in conidia as well as in mycelia.

Accordingly, it is likely that heterokaryosis is not responsible for the variability in this fungus.

Kiyosawa[17] studied mutability of the pathogenicity in blast fungus. He calculated the percentage of mutant spores using the following formula.

$$\frac{Ra}{Sa} \times \frac{Sv}{Rv}$$

Ra: The number of susceptible lesions (due to mutant spores) formed when resistant variety "R" is inoculated with avirulent strain "a".

Sa: The number of susceptible lesions formed when susceptible variety "S" is inoculated with the same strain "a".

Sv: The number of susceptible lesions formed when the variety "S" is inoculated with virulent strain "v".

Rv: The number of susceptible lesions formed when the variety "R" is inoculated with the strain "v".

Here, Sv/Rv is a factor to correct the difference of genetic background between the varieties "R" and "S" other than the gene in question which controls the major difference in resistance of both varieties.

He found differences of percentage of mutant spores between strains and between concerned resistance genes. Niizeki[34] obtained similar results in the same way. Namely, the percentage of mutant spores from *Av-k* to *Av-k*$^+$ was larger in fungus strains Ken 54-20 and Ina 168 than in Hoku 1 and Ken 54-04, and the percentage of mutant spores in relation to the gene *Av-k* was larger than that of the gene *Av-a* (Table 5).

**Table 5. Frequency of mutant spores, which changed from avirulence to virulence to various resistance genes, in various fungus strains.**

| Variety (Resistance gene) / Fungus strain | KIYOSAWA[17] | | NIIZEKI[34] | | | |
|---|---|---|---|---|---|---|
| | *Pi-k* variety* | *Pi-a* variety* | Kanto 51 (*Pi-k*) | Fukunishiki (*Pi-z*) | Pi No. 1 (*Pi-ta*)** | Pi No. 4 (*Pi-ta2*) |
| P-2b | | | 49.0**** | 4.1 | | |
| Ken 53-33 | | | | 2.4 | | < 0.1 |
| Ina 72 | | 20.0 | | | | |
| Ina 72-*a*$^+$ *** | | | | 1.8 | 19.0 | < 0.7 |
| Hoku 1 | 660.0 | | < 22.0 | < 8.4 | 220.0 | <24.0 |
| Ken 54-20 | 2290.0 | | 130.0 | 12.0 | 160.0 | <13.0 |
| Ken 54-04 | 90.0 | | 96.0 | 2.7 | 1.0 | 2.2 |
| Ina 168 | 2630.0 | 20.0 | 420.0 | 59.0 | | <10.0 |

* Average obtained on varieties carrying the gene *Pi-k* or *Pi-a*.

** Pi No. 1 has two genes, *Pi-a* and *Pi-ta*. However, only the frequency of mutant spores acquiring the virulence to the gene *Pi-ta* was related in this case.

*** A mutant which originated from Ina 72 and acquired the virulence to the *Pi-a* gene.

**** $\times 10^4$.

Data of KIYOSAWA[17] were accumulated by a large number of tests for resistance for the past 5 years. In a few cases of these tests, all the inoculum used consisted of mutants which changed in pathogenicity during subcultures. This is responsible for a relatively large difference of data of KIYOSAWA[17] from ones of NIIZEKI[34].

Such an inter-genic difference of mutation frequency on pathogenicity should be noted in selecting the resistance gene for breeding of resistant variety. That is, resistance gene, to which the corresponding avirulence gene has low mutation frequency, must be chosen as a source of resistance.

## Inheritance of Field Resistance

Norin 22 is evaluated as semi-resistance and susceptibility based on the type of predominated lesions and of the largest lesion, respectively, when sprayed or injected with the fungus strain Ken 54-04. This variety shows relatively high resistance in the field.

Kiyosawa, Matsumoto and Lee[28] studied the inheritance of resistance of this variety. When $F_3$ lines of the hybrid of Norin 22 × Aichi Asahi were inoculated with Ken 54-04 and Ken Ph-03, monogenic segregation was observed in each of the two fungus strains. In this case, the difference of degrees of resistance among $F_3$ plants was not clear-cut, and it was suggested that two or more and one or more minor genes complicated the segregation for Ken 54-04 and Ken Ph-03 respectively. Ken 54-04 used in this experiment showed weaker pathogenicity to many varieties as compared with Ken 54-20; both strains showing similar specificity. Possibly, this weak pathogenicity is nonspecific. Field resistance is considered to be weak resistance that is able to appear in a field but not in a greenhouse. Therefore, it is reasonable to attempt to test this resistance with the weakly (nonspecificially) pathogenic strain.

Using varieties with the different degrees of resistance in the field, Kiyosawa obtained a parallel relationship between resistance in the field and the greenhouse[18], and the same was carried out on sister varieties of Norin 22 and their parents[19]. These support the theory that the resistance gene caught in the greenhouse by using Ken 54-04 is the same as that acting under field conditions. If it is true, field resistance of Norin 22 is controlled by one major gene and two or more minor genes.

Goto[8] reported that the resistance of Sensho was controlled by a few additive genes, one of which was linked with *lazy* gene.

## What does the gene-for-gene theory teach us?

1. Differentiating Ability of Varieties or Fungus Strains

Now let us consider two gene pairs controlling host-pathogen relationship ($A$ $+^A$, $B$ $+^B$, $a$ $+^a$, $b$ $+^b$; $+^A$ and $+^B$ are susceptibility alleles of $A$ and $B$ resistance genes in the host plants, respectively, and $+^a$ and $+^b$ are virulence alleles of $a$ and $b$ avirulence genes in the fungus strains, respectively. These are in many cases simplified as $+$). Two pairs of avirulence genes also make four genotypes. When the hosts of the four resistance genotypes are separately infected with the fungus strains of the four avirulence genotypes, the reactions as shown in Table 6 are expected according to the gene-for-gene theory. Namely, the resistance gene $A$ is effective only against the fungus strain having the corresponding or complemetary avirulence gene $a$. The correspondence or complementation between a resistance gene and an avirulence gene is highly specific.

Let us choose two genotypes out of the four genotypes in order to differentiate the four geneotypes (races) of fungus strains. The two genotypes, $AB$ and $A+$ or $AB$ and $+B$, divide the four fungus genotypes only into three groups [$ab$, $a+$ (RR against the two host genotypes) : $+b$ (RS) : $++$ (SS)]. The other two host genotypes, $AB$ and $++$, divide the four genotypes only into two groups [$ab$, $a+$, $+b$ (RS) : $++$ (SS)]. In the same way, the two genotypes, $A+$ and $++$ or $+B$ and $++$, divide into two groups, RS and SS. As compared with those, the two genotypes, $A+$ and $+B$, can classify into four groups, RR, RS, SR and SS. Therefore, the two genotypes, $A+$ and $+B$, are most effective to classify

the fungus genotypes, *ab*, *a*+, +*b* and ++. In other words, the choice of hosts, each of which carrys different single resistance gene, promise the most effective differentiation of fungus genotypes (races). The same principle can be applied to the choice of fungus genotypes for classification of host varieties based on their resistance,

The differential varieties must be chosen on the basis of such a principle[14].

2. Limitation of the Number of Resistance Genes Found by the Fungus Strains Used

Supposing the third gene pair, *C*+, in addition to the gene pairs shown in Table 6, the host-pathogen relationship as shown in Table 7 is expected. Out of them, ++*C* plant does not show resistant reaction to the four fungus races in the table, *ab*+, *a*++, +*b*+ and +++, in spite of the existence of *C* gene in the host plant. This host plant shows resistance to the four fungus races, *abc*, *a*+*c*, +*bc* and ++*c*, for they have the avirulence gene *c*. The resistance gene *C* can be found only when the fungus strain carrying the avirulence gene *c* is used for gene analysis. In other words, only the resistance genes corresponding to the avirulence gene in the fungus strains used can be found. That is, the number of genes which are included in the fungus strains used shonld determine the maximum numbcr of resistance genes detected. Even if it was experimentally determined using a limited number of fungus strains that a given variety has only one gene, it is not logically correct to lead to the conclusion that all the fungus strains showing an avirulent reaction to them have the avirulence gene corresponding to the resistance gene which has already been found in the variety, although the conclusion may be practically correct in many cases.

3. The Minimum Number of Fungus Strains Which are Required for Selection of Resistant Plant Carrying More than Two Genes

Let us consider on the selection of the *AB* plants from the $F_2$ or $F_3$ population of *A*+ × +*B* hybrid (Table 6). When we use only one fungus strain out of the four strains shown in Table 6, we can not differentiate the four host genotypes. As mentioned above,

**Table 6. Host-pathogen relationship in participation of two gene pairs.**

| Host \ Pathogen | *ab* | *a*+ | +*b* | ++ |
|---|---|---|---|---|
| *AB* | R | R | R | S |
| *A*+ | R | R | S | S |
| +*B* | R | S | R | S |
| ++ | S | S | S | S |

**Table 7. Host-pathogen relationship in participation of three gene pairs.**

| Host \ Parthogen | *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 |

the choice of the two fungus strains, $a+$ and $+b$, is most effective for the differentiation of the four genotypes of hosts. That is, at least two fungus strains are required for selection of the plants which carry two resistance genes. If we can not use the suitable fungus strains, three or more fungus strains are necessary for the selection. For identifying the plants or lines which possess a given number of resistance genes, the use of fungus strains of the number equivalent to the number of the resistance genes is necessary and sufficient, if the suitable genotypes of fungus strains are available.

If there is no interaction between two fungus strains, mixture inoculation of more than two fungus strains may be enable to select the plants or lines carrying more than two reristance genes in one test. Kiyosawa and Fujimaki[27] found an interaction between two fungus strains injected, and called this interaction as mixture inoculation effect. When a mixture of virulent and avirulent fungus strains were injected to the variety carrying the *Pi-a* gene, pathogenicity of the former was inhibited by the presence of the latter. Such an inhibitive action by avirulent fungus strain was not found on the variety carrying the *Pi-k* gene. This difference between resistance genes in mixture inoculation effect must be noted in the use of mixture inoculation.

## Discussion

**H. I. Oka,** Japan: May I ask you the number of races the resistances to which are controlled by a resistant gene? It might differ according to resistant genes. How many for *Pi-k*?

**Answer:** The number of fungus strains used by me is very small as compared with the numerous number of strains collected in Japan. So, I can not answer to your question without further experiments. But, I think the gene *Pi-k* may be effective to all the races belonging to a predominant race group, N, in Japan.

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