Gene Analysis of Blast Resistance in Exotic Varieties of Rice

By SHIGEHISA KIYOSAWA

1st Laboratory of Genetics, Division of Genetics, Department of Physiology and Genetics, National Institute of Agricultural Sciences (Hiratsuka)

Gene analysis of blast resistance of rice varieties was begun in using domestic blast fungus strains and domestic rice varieties including Japanese native varieties and varieties improved in Japan by using exotic varieties as a resistant parent.

And, gene analysis of Japanese rice cultivars for blast resistance was almost completed (Kiyosawa, 1967c). Therefore, exotic varieties including *indica* type varieties and Chinese *japonica* type varieties have been aimed to analyze their blast resistance.

However, there is some difficulty in gene analysis of exotic varieties with domestic fungus strains. In this paper, this difficulty and some problems encountered in gene analysis of exotic varieties will be mentioned with a brief summary of gene analysis of domestic varieties in Japan.

Gene analysis of Japanese domestic varieties

Japanese domestic varieties including native varieties and derivatives from crosses between native varieties were classified into four groups by using seven fungus strains with differential pathogenicity: Shin 2, Aichi Asahi, Ishikari Shiroke and Shinsetsu groups (Kiyosawa, 1967c). Kiyosawa studied the inheritance of some representatives of these groups for blast resistance, and found two genes, *Pi-a* and *Pi-i*.

The four groups mentioned above were explained to carry none, one or two of these two genes: Shin 2 (*Pi-a*$^+$, *Pi-i*$^+$), Aichi Asahi (*Pi-a*, *Pi-i*$^+$), Ishikari Shiroke (*Pi-a*$^+$, *Pi-i*), and Shinsetsu (*Pi-a*, *Pi-i*), where *Pi-a*$^+$ and *Pi-i*$^+$ are susceptibility alleles of dominant genes, *Pi-a* and *Pi-i*, respectively (Kiyosawa, 1967c).

Besides them, there were found some resistance genes in Ginga and Homare Nishiki, which are weakly resistant in greenhouse to the fungus strain, Ken 54-04, with weak aggressiveness (Kiyosawa 1970a).

These genes in Ginga and Homare Nishiki are weak in function and are effective only to some fungus strains under greenhouse conditions, but may be effective relatively non-specifically under field conditions. Such a resistance is very popularly called as field resistance in Japan (Kiyosawa, 1970b), although the field resistance must be more strictly definded as mentioned by Kiyosawa (1970b).

Gene analysis of Japanese varieties developed by using exotic varieties as a resistant parent

In Japan, resistance genes are lacking for controlling the blast disease with varietal resistance. Therefore, some exotic varieties were used for developing resistant variety and some resistant varieties were bred and released to farmers. These varieties were found to have the following genotypes:

Kanto 51 group
- *Pi-k* — Yamasaki and Kiyosawa, 1966

To-to group
- *Pi-k*, *Pi-a* — Kiyosawa, 1968b

Yashiro-mochi group
Pi-ta Kiyosawa, 1969c
Shimokita group
Pi-ta, Pi-a Kiyosawa, 1966a
Pi No. 4 group
Pi-ta² Kiyosawa, 1967b
Fukunishiki group
Pi-z Kiyosawa, 1967a
Zenith group
Pi-z, Pi-a Kiyosawa, 1967a

Of these genes, *Pi-k*, *Pi-ta*, *Pi-ta²* and *Pi-z* were introduced from Reishiko (Chinese), Tadukan (Philippines) and Oka-ine (Chinese), Tadukan and Zenith (American), respectively.

In addition to these, Toride 1 and Toride 2 were developed from Indian varieties, TKM.1 and CO.25 respectively, as intermediate source variety for blast resistance.

These varieties were demonstrated to have the genes, *Pi-z'*, and *Pi-z'* and *Pi-a*, respectively. (Yokoo and Kiyosawa, 1970, Kiyosawa and Yokoo, 1970). Reactions of varieties with these genes to the seven fungus strains are shown in Table 1. In spite of these breeding, resistance sources are not sufficient for controlling the blast disease because newly bred resistant varieties become susceptible one after another (Kiyosawa, 1965).

Gene analysis of exotic varieties

The author's analysis of exotic varieties was begun at the same time as the beginning of analysis of domestic varieties. However, the first publication of the data was made as late as nine years after the beginning (Kiyosawa, 1969b) except one of the U.S. variety Zenith (Kiyosawa, 1967a) and some Chinese varieties (Kiyosawa, 1968b), as compared with six years of domestic varieties (Yamasaki and Kiyosawa, 1966). This is due to a difficulty of analysis of exotic varieties.

In general, exotic varieties have relatively many genes for resistance. For example, the West Pakistani variety Pusur and the Indian variety HR-22 were demonstrated to carry at least three genes (Kiyosawa, 1969b, Kiyosawa and Murty, 1969), respectively.

Moreover, the degree of resistance controlled by these genes differs from gene to gene. This results in a continuous distribution of intensity of resistance in F_2 or F_3 progeny of a cross which makes difficult the identification or separation of individual genes.

For this reason, various methods were devised to solve this difficulty.

Table 1. Reactions of varieties with resistance genes identified up to date to the seven fungus strains when inoculated by the injection method

(Kiyosawa, 1970c)

Vareity	Genotype	Fungus strain						
		P-2b	Ken 53-33	Ina 72	Hoku 1	Ken 54-20	Ken 54-04	Ina 168
Shin 2	*Pi-k*^s^*	S	S	S	S	S	MS	S
Kanto 51	*Pi-k*	MR	S	S	R^h	R^h	R^h	R^h
Aichi Asahi	*Pi-a*	S	S	R	S	S	S	R
Fujisaka 5	*Pi-i, Pi-k*^{s}	M	S	M	S	MS	MR	M
K 1	*Pi-ta*	S	S	M	MR	M	MR	S
Pi No. 4	*Pi-ta*^{2}	S	M	R^h	R	R	R	MR
Ou 244	*Pi-z*	M	M	M	MR	M	MR	M
Toride 1	*Pi-z*^{t}	R^h	R^h	R^h	R^h	R^h	R^h	R^h
K 2	*Pi-k*^{p}, *Pi-a*	S	S	R	R	MR	R	R
K 3	*Pi-k*^{h}	M	S	S	R	MR	R	R

*: This gene is not effective to Japanese fungus strains, but effective to the Philippine fungus strain Ken Ph-03.

1) Isolation of non-parental type. In F_2 or F_3 progeny of the hybrids of HR-22 (Kiyosawa and Murty, 1969) and Pusur (Kiyosawa, 1969b) with Japanese varieties, the author failed to estimate with certainty the number of genes in these *indica* type varieties.

Therefore, lines showing a reaction pattern different from those of its parents to the seven fungus strains were selected. These selections were divided by reaction pattern to the seven fungus strains into six and nine groups in the hybrid progenies of Pusur and HR-22, respectively.

Each one (K 2 and K 3) of lines selected from the progenies of the two hybrids was crossed again with the Shin 2 type variety in which resistance gene had not been found, and genes $Pi\text{-}k^p$ and *Pi-a*, and $Pi\text{-}k^h$ were found in the lines K 2 and K3, respectively.

Such a method was, however, successful only in a hybrid with high fertility in the F_1 with a Japanese variety.

2) The use of mutant method. Spontaneous mutants for pathogenicity of the blast fungus are often found (Kiyosawa, 1966b). By using skillfully these mutants, it becomes possible to detect easily a resistance gene. Consider a case in which a variety with a resistance gene, for example *Pi-k*, is inoculated with an avirulent fungus strain.

Susceptible type lesions are occasionally developed on this resistant variety. From these lesions, mutants in which avirulence to *Pi-k* gene of the original strain changes to virulence are isolated.

When these mutant and original strains are sprayed or injected to a variety with the gene *Pi-k*, this variety shows different reactions, susceptible and resistant, to both strains. The converse holds good. When a variety to be tested shows different reaction to both strains, this variety can be concluded to carry the gene *Pi-k*.

This method was applied to the identification of the gene $Pi\text{-}k^s$ in some exotic varieties (Kiyosawa, 1969a). The gene $Pi\text{-}k^s$ was first found in some Japanese varieties. This gene is not effective to Japanese fungus strains and is effective to the Philippine fungus strain Ken Ph-03.

Mutants attacking varieties with $Pi\text{-}k^s$ are also obtained. Using these mutant and original strains, $Pi\text{-}k^s$ was identified in some exotic varieties: To-to (long grain), Sha-tiao-tsao, Taichung 65 (Chinese), Lacrosse, and Caloro (U.S.) (Kiyosawa, 1969a).

3) An application of the mutant method to segregating population. The mutant method cannot be applied to a given variety carrying a gene epistatic or equivalent (equally effective) to the gene aimed to identify because the epistatic or equivalent gene musks the appearance of the difference in reactions to the two fungus strains, original and mutant.

Accordingly, when a variety shows the same resistant reactions to the two fungus strains, it cannot be concluded that the given variety has no resistance gene aimed to identify. Only when the given variety shows different reactions to the two fungus strains, the presence of the gene is concluded in the variety. Therefore, the mutant method is not applicable to many exotic varieties.

In order to know the existence of a special gene in a given variety, the variety is generally crossed with a variety known to carry the gene, and the hybrid progeny is tested for the segregation of susceptible plants. This method is difficult to apply to the identification of the special gene in many exotic varieties which may have relatively many resistance genes because the number of genes including in the hybrid progeny increases, if the variety does not have the special gene. This makes difficult a decision of the presence or absence of the special gene in the given variety.

Therefore, the variety aimed to test, for example Dular (a West Pakistani variety), was crossed with a susceptible variety and its F_3 progeny was inoculated separately under the same conditions with two fungus strains, original (Ken 54-20) and mutant (Ken 54-20-k^+) overcoming the gene *Pi-k*.

Since F_3 lines predominated in one side of a diagonal in a correlation diagram (Fig. 1),

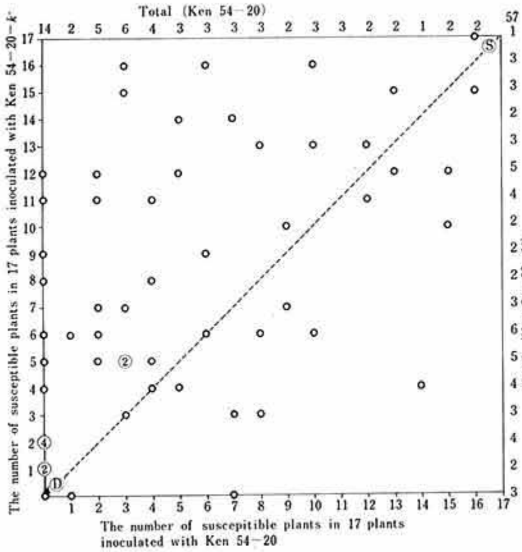

Fig. 1. Distribution of 57 lines of Dular × Shin 2.
Each of the 57 lines of Dular × Shin 2 was divided into two, and these two sets were inoculated with Ken 54-20 and Ken 54-20-k^{+} under the same conditions, respectively. The 57 lines were plotted in relation to the number of susceptible plants in 17 plants inoculated with the two fungus strains.
○, ② and ④ show the presence of 1, 2 and 4 lines at the position, respectively.
Ⓓ and Ⓢ show the position of Dular and Shin 2, respectively.

Dular was concluded to have the gene *Pi-k* or one of similar nature (Kiyosawa, unpublished). In the same way, the gene *Pi-ta* was demonstrated in the Chinese variety Pai-kan-tao (Kiyosawa, Wu and Ono, 1971).

4) A combination of isolation of non-parental line and the mutant method. An application of the mutant method to segregating population is sometimes not adequate to conclusively know the presence or absence of a special gene. In such a case, non-parental types of plants or lines were selected in F_2 or F_3 population and these progenies were tested with the original and mutant strains. Thus, the gene *Pi-k* or gene of similar nature was identified in some exotic varieties as shown in Fig. 2 and Tables 2 and 3.

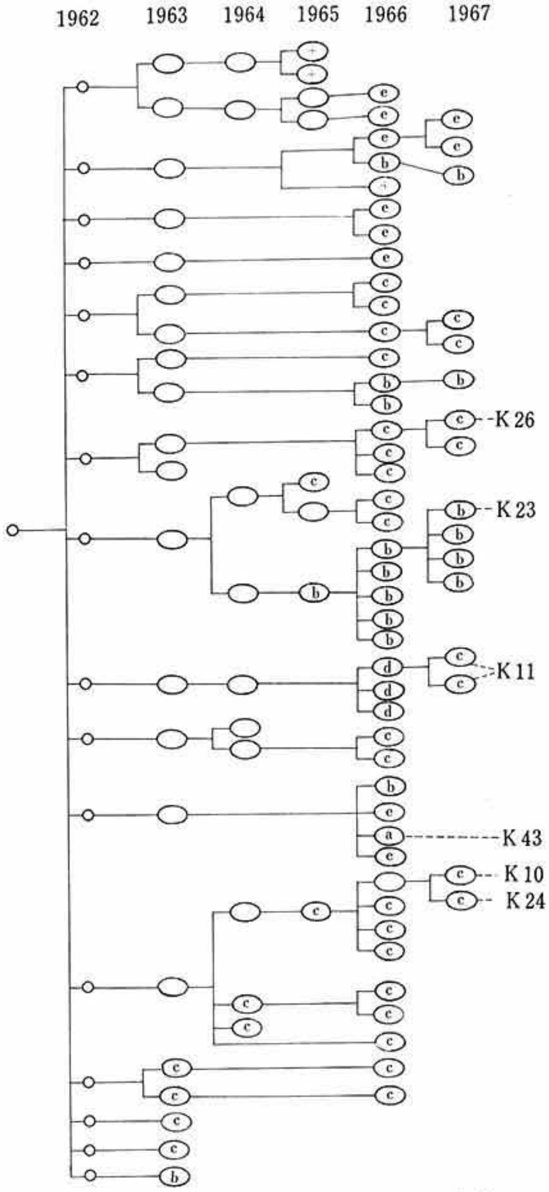

Fig. 2. The process of selections of lines showing various reaction patterns (a–e) to the 7 fungus strains. Reaction patterns of (a) to (e) types of lines are shown in Table 2.

Identification of resistance genes in exotic varieties and their distribution

By using several methods as mentioned above, resistance genes were identified in some exotic varieties as shown in Table 4. As far as

Table 2. Reactions of Norin 22 and Charnack and selections from their hybrid progenies
(Kiyosawa, unpublished)

Variety	Fungus strain						
	P-2b	Ken 53-33	Ina 72	Hoku 1	Ken 54-20	Ken 54-04	Ina 168
Norin 22	S	S	S	S	S	M	S
Charnack	R	R	MR	R	R	R	MR
a*	R^h	R^h	R^h	R^h	R^h	R^h	R^h
b	S	S	R	R^h	R^h	R^h	R^h
c	M	S	S	R	R	R	R
d	M	R	S	R	M	M	S
e	M	MS	MS	MR	MS	M	MS

Inoculated by injection method.
*: Selections from the hybrid progeny of Norin 22×Charnack were divided into five groups based on their reaction pattern to the seven fungus strains as shown in Fig. 2.

Table 3. Identification of the gene *Pi-k* or gene of similar nature in a part of selections from the hybrides of *indica* ×*japonica* by the mutant method
(Kiyosawa, unpublished)

Selection	Fungus strain		Parent (*indica*)
	Ken 54-20	Ken 54-20-*kh*+-*a*	
K 2	M	R*	Pusur
K 3	**M**	**S****	HR-22
K 4	**MR**	**S**	HR-22
K 6	**MR**	**S**	HR-22
K 9	M	R*	SM-6
K 10	**R**	**S**	Charnack
K 11	**MR**	**S**	Charnack
K 23	**R^h**	**M**	Charnack
K 24	**R**	**S**	Charnack
K 28	**MR**	**S**	Roshia No. 33
K 31	**MR**	**S**	Te-tep
K 32	**MR**	**S**	Te-tep
K 33	M	R*	SM-6
K 34	R	R	SM-6
K 40	R	R	Tadukan
K 41	R^h	MR	SM-6
K 42	M	R*	Tadukan
K 43	MR	R	Charnack
K 48	**R**	**S**	Roshia No. 33
K 49	**MR**	**S**	Tadukan

*: A higher resistance to the mutant Ken 54-20-*kh*+-*a* than to the original Ken 54-20 is due to the presence of the gene *Pi-a* in the selection. The gene *Pi-a* in selections, K 9 and K 33, is derived from *japonica* parent Norin 41 and in others from each *indica* parent.
**: Gothic letters show different reactions to both fungus strains. This difference is due to the existence of the *Pi-k* or gene of similar nature in the selection, accordingly in the *indica* type parent.

we know the gene *Pi-a* is distributed widely in the world: Japan, China, Korea, India, U.S. and Philippines.

The locus *Pi-k* has many multiple alleles, *Pi-k*, *Pi-k*^{s}, *Pi-k*^{p} and *Pi-k*^{h}. In Japanese cultivars, *Pi-k*^{s} was found, but not *Pi-k*, *Pi-k*^{p} and *Pi-k*^{h}. This indicates that the allele *Pi-k*^{s} was transported after the alleles *Pi-k*, *Pi-k*^{p} or *Pi-k*^{h} was mutated to *Pi-k*^{s} and/or that these alleles were eliminated from cultivars in Japan. On the other genes, the studies are too few to discuss the distribution in the world.

Some problems in future

As mentioned above, several methods have been suggested for gene analysis for blast resistance of exotic varieties. Nevertheless, there are many obstacles for gene analysis of exotic varieties, especially *indica* type varieties, by crossing *japonica* type varieties. Among them, hybrid sterility and complicated segregations are included as major ones.

Table 4. Resistance genes identified in some exotic varieties

Origin	Variety	Genes identified	Literature
	Doazi chall	*Pi-i*	Kiyosawa, 1968a
Korea	Jae Keum	*Pi-a*	Kiyosawa, 1967c
	Pal tal	*Pi-a*	
	Usen	*Pi-a*, others	Kiyosawa, 1967c
	Yakei-ko	*Pi-k*	
	Reishiko	*Pi-k*	
	To-to (short grain)	*Pi-k*, *Pi-a*	Kiyosawa, 1968b
	Choko-to	*Pi-k*, *Pi-a*	
	Hokushi Tami	*Pi-k*, *Pi-a*, *Pi-m*	
China	Pe Bi Hun	*Pi-a*	Kiyosawa, 1967c
	To-to (long grain)	*Pi-k*^{s}	
	Taichung 65	*Pi-k*^{s}	Kiyosawa, 1969a
	Sha-tiao-tsao	*Pi-k*^{s}	
	Oka-ine	*Pi-ta*	Kiyosawa, 1969c
	Pai-kan-tao	*Pi-ta*, others	Kiyosawa, Wu and Ono, 1971
Philippines	Tadukan	*Pi-ta* and/or *Pi-ta*^{2}	Kiyosawa, 1966a, 1967b, 1969c
	HR-22	*Pi-k*^{h}, others	Kiyosawa and Murty, 1969
	CO. 25	*Pi-z*^{t}, *Pi-a*, others	Kiyosawa and Yokoo, 1970
India	TKM. 1	*Pi-z*^{t}, others	Yokoo and Kiyosawa, 1970
	Charnack	*Pi-k**, others	Kiyosawa, unpublished
	CO. 4	*Pi-z*^{t}	Fujimaki and Yokoo, 1971
Pakistan	Dular	*Pi-k**, others	Kiyosawa, unpublished
	Pusur	*Pi-k*^{p}, *Pi-a*, others	Kiyosawa, 1969b
Vietnam	Te-tep	*Pi-k**, others	Kiyosawa, unpublished
Malaysia	Morak Sepilai	*Pi-z*^{t}	Fujimaki and Yokoo, 1971
	Kontor	*Pi-z*^{t}	
Thailand	Leuang Tawing 77-12-5	*Pi-z*^{t}	Fujimaki and Yokoo, 1971
	Chao Leuang 11	*Pi-z*^{t}	
	Zenith	*Pi-z*, *Pi-a*	Kiyosawa, 1967a
U. S.	Caloro	*Pi-k*^{s}	Kiyosawa, 1969a
	Lacrosse	*Pi-k*^{s}	
	Blue Bonnet	*Pi-a*	Kiyosawa, 1967c
USSR	Roshia No. 33	*Pi-k**	Kiyosawa, unpublished

*: Any allele at the *Pi-k* locus.

The suggested methods are not sufficient to break through obstacles because many more years are taken than the general analysis including hybridization and the test for resistance in F_1, F_2 and F_3 generations, except a proper use of the mutant method (in section 1).

In general, gene analysis for resistance is simplest when varieties to be analyzed are crossed with other varieties native in the same country and the hybrid progeny is inoculated with a fungus strain collected in the same country.

In other words, it is generally more difficult to analyze an exotic variety by crossing with a domestic variety and by inoculating with a domestic fungus strain.

Therefore, it is more desirable to analyze a variety by crossing with some varieties indigenous in its country and by inoculating with some fungus strains collected in the country.

In this case, it is not possible to determine the identity with resistance gene found in other countries. For this purpose, we must exchange varieties each of which has been found to carry a resistance gene or fungus strain which was used for gene analysis. However, this procedure is desirable to use after resistance genes are isolated or separated into different lines in each country.

Information on the gene constitution for disease resistance in a given variety is very useful in breeding programs. Nowadays, gene constitution for blast resistance in Japanese cultivars has been mostly known. However, there is very little information on rice varieties in countries other than Japan. Accordingly, it is very desirous to advance potentially the genetics on the blast resistance of rice varieties in individual countries.

References

1) Fujimaki, H., and Yokoo, M.: Studies on the genes for blast resistance transferred from *indica* rice varieties by backcrossing. *Japan. J. Breeding*, **21**, 9–12 (1971).

2) Kiyosawa, S.: Ecological analysis on breakdown of resistance in resistant varieties and breeding-counterplan against it. *Nōgyō Gijutsu* (Agr. Tech.) **20**, 465–470 and 510-512 (1965). [In Japanese.]

3) Kiyosawa, S.: Studies on inheritance of resistance of rice varieties to blast. 3. Inheritance of resistance of a rice variety Pi No. 1 to the blast fungus. *Japan. J. Breeding*, **16**, 243–250 (1966a).

4) Kiyosawa, S.: On spontaneous mutation of pathogenicity in *Pyricularia oryzae*. *Shokubutsu Boeki* (Plant Prot.), **20**, 159–162 (1966b). [In Japanese.]

5) Kiyosawa, S.: The Inheritance of resistance of the Zenith type varieties of rice to the blast fungus. *Japan. J. Breeding*, **17**, 99–107 (1967a).

6) Kiyosawa, S.: Inheritance of resistance of the rice variety Pi No. 4 to blast. *Japan. J. Breeding*, **17**, 165–172 (1967b).

7) Kiyosawa, S.: Genetic studies on host-pathogen relationship in the rice blast disease. Proc. Symp. Rice Diseases and Their Control by Growing Resistant Varieties and Other Measures. Tokyo 137–153 (1967c).

8) Kiyosawa, S.: Genetic relationship among blast resistance and other characters in hybrids of Korean rice variety, Doazi chall (Butamochi), with Aichi Asahi. *Japan. J. Breeding*, **18**, 88–93 (1968a).

9) Kiyoshawa, S.: Inheritance of blast resistance in some Chinese rice varieties and their derivatives. *Japan. J. Breeding*, **18**, 193–205 (1968b).

10) Kiyosawa, S.: Inheritance of resistance of rice varieties to a Philippine fungus strain of *Pyricularia oryzae*. *Japan. J. Breeding*, **19**, 61–73 (1969a).

11) Kiyosawa, S.: Inheritance of blast resistance in West Pakistani variety, Pusur. *Japan. J. Breeding*, **19**, 121–128 (1969b).

12) Kiyosawa, S.: Gene analysis of blast resistance of rice variety, Yashiro-mochi. Agr. Hort. **44**, 407–408 (1969c). [In Japanese.]

13) Kiyosawa, S.: Inheritance of blast resistance of the rice varieties, Homare Nishiki and Ginga. 1. Resistance of Homare Nishiki and Ginga to the fungus strain Ken 54–04. *Bull. Natl. Inst. Agr. Sci. D***21**, 73–105 (1970a).

14) Kiyosawa, S.: Concept of true resistance and field resistance. *Nogyo Gijutsu* (Agr. Tech.), **25**, 21–25 (1970b). [In Japanese.]

15) Kiyosawa, S.: Typical reactions of differential varieties and differential fungus strains on rice blast disease. *Nogyo Gijutsu* (Agr. Tech.), **25**, 578–580 (1970c). [In Japanese.]

16) Kiyosawa, S., and Murty, V. V. S.: The inheritance of blast resistance in Indian rice variety, HR-22. *Japan. J. Breeding*, **19**, 269–276 (1969).

17) Kiyosawa, S., Wu, Y. L. and Ono, H.: The inheritance of blast resistance in Chinese rice variety Pai-kan-tao. *Bill Natl. Inst. Agr. Sci.* D. (1971).

18) Kiyosawa, S., and Yokoo, M.: Inheritance of blast resistance of the rice variety, Toride 2, bred by transferring resistance of the Indian variety, CO. 25. *Japan. J. Breeding*, **20**, 181–186.

19) Yamasaki, Y., and Kiyosawa, S.: Studies

on inheritance of resistance of rice varieties to blast. 1. Inheritance of resistance of Japanese varieties to several strains of the fungus. *Bull. Natl. Inst. Agr. Sci.* D. **14**, 39–69 (1966). [In Japanese.]

20) Yokoo, M. and Kiyosawa, S.: Inheritance of blast resistance of the rice variety, Toride 1, selected from the cross Norin 8 × TKM. 1. *Japan. J. Breeding*, **20**, 129–132 (1971).