INHERITANCE OF BLAST-RESISTANCE IN SOME CHINESE RICE VARIETIES AND THEIR DERIVATIVES

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Synopsis. A genetic study was conducted on Chinese varieties and their derivatives which were classified into the To-to group on the basis of their blast resistance. Results obtained by the mutant method, in which fungus mutant on pathogenicity was used to identify resistance gene, showed a complete agreement with those by the hybridization method, which was of the common for gene analysis. This indicates that the mutant method can be effectively used for identification of blast-resistance gene. To-to type varieties tested were found to have two genes for resistance, *Pi-k* and *Pi-a*. Minehikari has an additional gene, *Pi-m*.

Varieties that were used in breeding for blast-resistance at the beginning of systematic rice breeding in Japan which started in 1927 (Ito, 1965) were selections from Japanese native ones. Some field-resistant varieties including Norin 22 and Norin 23 were bred during the first 20 years.

Later, a Chinese upland rice variety, Sensho, was chosen as a source in breeding for blast-resistance, and Ginga and Homare Nishiki were bred, of which resistance should be probably classified in field resistance.

True-resistance breeding of rice in Japan started with using Chinese varieties as sources of resistance. In the Aichi Prefecture Agricultural Experiment Station, Hokushi Tami, a variety of Chinese origin, has since 1944 been used, and BR No. 1 (UJIHARA and TANABE, 1959) and Minehikari (UJIHARA, SUZUKI, TANABE and ISHIHARA, 1966) were obtained by crossing repeatedly three and four times with distinct Japanese varieties respectively. In the Central Agricultural Experiment Station, Kanto 51 and Kanto 53 were bred from Chinese varieties, To-to and Reishiko, respectively (KOYAMA, 1952).

Using Kanto 53 as an intermediate variety, several varieties were bred; these were Kusabue in the Central Agricultural Experiment Station (ITO, *et al.*, 1961), Teine and Yukara in the Hokkaido Agricultural Experiment Station, and Ōyodo in the Miyazaki Prefecture Agricultural Experiment Station (SOGA, *et al.*,

1963). In the Central Agricultural Experiment Station, To-to was used through a breeding course different from that of Kanto 51 to obtain Kagura-mochi (ITO, *et al.*, 1964a), Mangetsu-mochi (ITO, *et al.*, 1964b) and Tsukimi-mochi (ITO, *et al.*, 1965).

The Niigata Prefecture Agricultural Experiment Station obtained Senshuraku (KUNITAKE, *et al.*, 1962a) and Koshihibiki (SHIRAKURA, *et al.*, 1965) by using Kanto 51 and Kanto 53 respectively, and obtained Hatsuiwai-mochi (KUNITAKE, *et al.*, 1962b) by crossing repeatedly Reishiko with three different Japanese varieties. Sanpuku was bred by crossing repeatedly Hokushin with two different Japanese rice varieties in the Fukui Prefecture Agricultural Experiment Station (ISHIZUMI, MIZUNO, and KAWAI, 1965). The variety Hokushin was an intermediate variety obtained in breeding course to BR No. 1 mentioned above. This intermediate variety was used for breeding of Tsuyuake in the Nara Prefecture Agricultural Experiment Station (MORIMOTO, and YOSHIMOTO, 1965). Tatsumi-mochi was bred through Imochi-shirazu from Reishiko in the Fujisaka Branch of the Aomori Prefecture Agricultural Experiment Station (TSUNODA, 1965).

YAMASAKI and KIYOSAWA (1966) classified some Chinese varieties and their derivatives into 2 types (or groups) based on resistance to blast: Kanto 51 and To-to types (or groups). Kanto 51 and Reishiko belonging to the Kanto 51 group were determined to have a resistance gene *Pi-k* (YAMASAKI and KIYOSAWA, 1966;

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KIYOSAWA, 1966a).

The present study was conducted to know genic constitution of other Kanto 51 and To-to type varieties.

Materials and Methods

For gene analysis, two methods were used: hybridization of rice varieties and utilization of fungus mutant.

Hybridization method: Resistant variety to be tested was crossed with susceptible variety. Obtained hybrid and its progeny were inoculated with the spore suspension of fungus strain avirulent on the resistant variety and segregation on resistance in F_2 or F_3 generation of the hybrid was examined to determine the number of resistance genes carried in the resistant variety. Secondly, the resistant variety to be tested was crossed with a test variety which was similar to the resistant variety in reaction to blast fungus strains and of which genic constitution has been known. From the segregation in progeny of this hybrid, identity of resistance gene in the resistant variety with the one in the test variety was determined.

Mutant method: During subculturing of fungus strains, their pathogenicity often changes from avirulence to virulence. This kind of change seems to be mutation, because characters other than the concerned pathogenicity are not found to change (KIYOSAWA, 1966b). For example, a variant which is isolated from a lesion of susceptible type developed after inoculation of the variety Kanto 51 carrying the gene $Pi-k$ with an avirulent fungus strain does not show any change in resistance to other types of variety, the number of spores formed on medium, and form and colour of mycelial mat. This change is considered to be due to mutation from the avirulence allele $Av-k$ to the virulence allele $Av-k^+$. No change is recognized in other avirulence loci, $Av-a$, $Av-i$, $Av-z$, $Av-ta$ and $Av-ta2$ (KIYOSAWA, 1966b). When the mutant and its original strain were used for inoculation of a variety carrying the gene $Pi-k$, the two strains could show a difference in their reaction on the variety. By making a reverse use of this principle, one can determine presence or absence of an already known

resistance gene in a variety attempted to test. This method was first used successfully by KIYOSAWA (1967b).

In the hybridization method for gene analysis, Chinese varieties, To-to, Choko-to, Yakei-ko and Reishiko, and varieties which were bred in Japan by crossing them with Japanese varieties, Minehikari, Kanto 51 and Kusbue, were used as Kanto 51 and To-to type varieties. These varieties were crossed with each other or with some Japanese varieties, Aichi Asahi, Norin 17, Kamenoo, Norin 8, Sekiyama 2, Eiko, Kohoku-kei 11 and Shin 2. Among them, Kanto 51 (YAMASAKI and KIYOSAWA, 1966), and Reishiko (KIYOSAWA, 1966a) were known to carry the gene $Pi-k$, and Aichi Asahi (KIYOSAWA, MATSUMOTO and LEE, 1967; KIYOSAWA, 1967a), Norin 17 (YAMASAKI and KIYOSAWA, 1966), and Eiko (KIYOSAWA, 1967a) to carry the gene $Pi-a$. All the hybrids and their progenies used were obtained in the National Institute of Agricultural Sciences, Hiratsuka, except a hybrid of Minehikari $\times$ Kohoku-kei 11 and its progeny which were crossed and harvested by Messrs. TANIGUCHI and NISHIOKA in the Inahashi Branch of the Aichi Prefecture Agricultural Experiment Station.

In case of utilization of mutant method, Kanto 51 ($Pi-k$), Aichi Asahi ($Pi-a$), Zenith ($Pi-a$, $Pi-z$), Fukei 67 ($Pi-a$, $Pi-z$), 54BC-68 ($Pi-z$) (KIYOSAWA, 1967b) and Fujisaka 5 ($Pi-i$) (YAMASAKI and KIYOSAWA, 1966) were used for a confirmation of pathogenicity of mutant used.

Fungus strains used were Hoku 1- k^+ , Ken 54-20- k^+ , Ina 168- k^+ , Ina 168- a^+-k^+ and Ina 72- a^+ , in addition to the seven strains which have always been used by KIYOSAWA, P-2b, Ken 53-33, Ina 72, Hoku 1, Ken 54-20, Ken 54-04 and Ina 168. Here, for example, Hoku 1- k^+ shows a mutant that changed from Hoku 1 to attack the gene $Pi-k$. Ina 168- a^+-k^+ is a mutant of Ina 168 that changed from avirulence to virulence to the resistance genes $Pi-a$ and $Pi-k$ and that obtained by NUZEKI and MOUE (1965) in two steps of artificial and then spontaneous mutations.

For inoculation, injection and spraying methods were used. These methods were in detail described in a previous paper with the

Table 1. Comparison between reactions to mutants and their original strains of blast fungus in rice varieties including four To-to type varieties

Variety	Genotype	Fungus strain								
		Hoku 1	Hoku 1- <i>k</i> ⁺	Ken 54-20	Ken 54-20- <i>k</i> ⁺	Ina 168	Ina 168- <i>k</i> ⁺	Ina 168- <i>a</i> ⁺ - <i>k</i> ⁺	Ina 72	Ina 72- <i>a</i> ⁺
Minehikari	<i>Pi-a, Pi-k, Pi-m</i>	R ^h	MS	R ^h	MS	R ^h	R	MR	R	S
BR No. 1	<i>Pi-a, Pi-k ?</i>	R ^h	MS	R ^h	MS	R ^h	R	R	R	S
Choko-to	<i>Pi-a, Pi-k ?</i>	R ^h	M	R ^h	MS	R ^h	R	M	R	—
To-to	<i>Pi-a, Pi-k</i>	R ^h	S	R ^h	S	R ^h	R	S	R	S
Kanto 51	<i>Pi-k</i>	R ^h	S	R ^h	S	R ^h	S	S	S	S
Aichi Asahi	<i>Pi-a</i>	S	S	S	S	R	R	S	R	S
Zenith	<i>Pi-a, Pi-z</i>	M	M	MR	MR	R	R	M	R	M
Fukei 67	<i>Pi-a, Pi-z</i>	MR	MR	M	M	R	R	M	R	M
54BC-68	<i>Pi-z</i>	M	M	M	M	M	M	M	M	M
Fujisaka 5	<i>Pi-i</i>	S	S	M	M	M	M	M	M	M

A difference of reactions was detected in parts of gothic type.

method of evaluation of resistance (KIYOSAWA, 1967b).

Results

Gene analysis of four To-to type varieties by the mutant method.

Ten varieties including four To-to type varieties, Minehikari, BR No. 1, Choko-to and To-to, were inoculated with three fungus strains and their mutants (Table 1).

All the tested To-to type varieties showed different reactions to Hoku 1 and Hoku 1-*k*⁺, and Ken 54-20 and Ken 54-20-*k*⁺, as Kanto 51 did. This indicates the presence of the gene *Pi-k* in them. Three varieties, Minehikari, BR No. 1 and To-to showed different reactions to Ina 72 and Ina 72-*a*⁺, indicating the existence of the gene *Pi-a* in them. The result of inoculation with Ina 72 and Ina 72-*a*⁺ to Zenith and Fukei 67 indicates that there is *Pi-a* in these varieties. This was demonstrated in a previous paper (KIYOSAWA, 1967b).

If the To-to type varieties have only two genes, *Pi-a* and *Pi-k*, these varieties are expected to show R and S reactions to Ina 168-*k*⁺ and Ina 168-*a*⁺-*k*⁺. The results of inoculation with these mutants showed an agreement with this expectation only on To-to. In the other three varieties, moderately to highly resistant reactions remained in inoculation with Ina 168-*a*⁺-*k*⁺. This suggests the presence of gene (s) other than *Pi-a* and *Pi-k*

in these varieties.

Gene analysis of the variety To-to by the hybridization method.

To-to was crossed with the variety Kamenoo of the Shin 2 type, which was of susceptibility to six (except Ken 54-04) or sometimes all of the seven strains used by the author for resistance test of varieties (Table 14).

F₂ plants of this hybrid were first inoculated with four fungus strains, Hoku 1, Ina 72, Ken 54-04 and Ina 168-*k*⁺ as shown in Table 2. The F₂ plants showed monogenic segregation ratios for the four strains, indicating participation of one dominant gene for each of them.

In order to know a relationship of the gene controlling the resistance of To-to to Ina 72 with *Pi-a* included in the variety Norin 17, a hybrid was made between both the varieties and its F₂ plants were inoculated with Ina 72. No susceptible plants were found in 134 F₂ plants (Table 2). This suggests that To-to has *Pi-a*.

A relationship between the gene controlling the resistance of To-to to Hoku 1 and the gene *Pi-k* was examined by inoculation of F₂ plants of hybrids of Kanto 51 × To-to and Reishiko × To-to with Hoku 1. In both the hybrid progenies susceptible plants were not found (Table 2). This indicates that *Pi-k* is included in To-to, as Kanto 51 and Reishiko have been demonstrated to have *Pi-k* (YAMASAKI and

Table 2. Segregation of resistance to several strains of the blast fungus in F_2 populations of hybrids including To-to as a parent

		Fungus strain	Number of plants			P value
			r	m	s Total	
I	To-to (R^h)* Kamenoo (S) F_2	Hoku 1	17		17	(3:1)
				14	14	
			86	11	24 121	0.1~0.2
II	To-to (R) Kamenoo(S) F_2	Ina 72	17		17	(3:1)
				14	14	
			94	24	118	0.3~0.5
III	To-to (R^h) Kamenoo(S) F_2	Ken 54-04	17		17	(3:1)
				15	16	
			67	6	24 97	0.1~0.2
IV	To-to (R) Kamenoo (S) F_2	Ina 168- k^+	15		15	(3:1)
				15	15	
			98	1	25 124	0.2~0.3
V	Norin 17 (R) To-to (R) F_2	Ina 72	16		16	(1:0)
			14		14	
			134		134	
VI	Kanto 51 (R) To-to (R^h) F_2	Hoku 1	13		13	(1:0)
			11		11	
			101		101	
VII	Reishiko (R^h) To-to (R^h) F_2	Hoku 1	16		16	(1:0)
			17		17	
			127		127	

Injection method for inoculation was used in all the experiments.

*: Reaction of the parent evaluated by the method of YAMASAKI and KIYOSAWA (1966).

KIYOSAWA, 1966; KIYOSAWA, 1966 a).

F_3 lines of the hybrid between To-to and Kamenoo were inoculated with Ina 72 and Hoku 1, in order to confirm the observation obtained by the F_2 analyses and to know linkage relationship among the resistance genes in To-to and gene (s) controlling dark

purple colour in apiculus of To-to.

Colour of apiculus was observed in ears of F_2 plants. Resistance of the F_3 lines to Ina 72 was tested by the injection method at the 4.5 leaf stage. The number of plants per line was 17. Resistance to Hoku 1 was in a separate experiment tested by inoculation in the spraying method of other 17 plants of each of the same 98 lines as ones used for the test of resistance to Ina 72.

The results were shown in Table 3. Here, "r" and "s" show lines in which all or most of plants are resistant and all plants are susceptible, respectively. Table 3 shows that one dominant gene controls resistance to each of the two fungus strains and the genes controlling resistance to the two strains behaved independently of each other.

In the ears of F_2 plants of this hybrid, three classes of apiculus colour were observed, dark purple, light purple and uncolored. The segregation ratio agreed with a ratio of 36:21:7, suggesting participation of two complementary dominant genes controlling dark purple and one dominant gene controlling light purple (Table 4). A relationship between the apiculus colour and resistance to Hoku 1 suggests that there is no linkage relationship among the genes participating in both the characters (Table 5).

The results above mentioned of F_2 and F_3 analyses of hybrids of To-to show that the two genes, $Pi-a$ and $Pi-k$, control the resistance of To-to and these genes behave independently of each other. These observations agree completely with the conclusion obtained by the mutant method in the above experiment and by the hybridization method in a previous

Table 3. Relationship between resistance to Ina 72 and that to Hoku 1 observed in F_3 lines of hybrid between To-to and Kamenoo

Parent	Fungus strain	① \ ②	Number of lines			P _① value**
			r	s	Total	
To-to (R, R^h)*	① Ina 72 (Injection)	r	56	21	77	(3:1)
		s	18	3	21	0.3~0.5
Kamenoo (S, S)	② Hoku 1 (Spraying)	Total	74	24	98	(9:3:3:1)
		P _② value	(3:1)0.90~0.95			0.5~0.7

*: Reactions of the parent for the first ① and second ② fungus strains, respectively.

** : P value for the first used fungus strain ①.

Table 4. Relationship between resistance to Hoku 1 in F_3 lines and colour of apiculus on F_2 plants of hybrid between To-to and Kamenoo

Resistance to Hoku 1	Number of lines or plants				P value (Resistance)
	Dark purple	Light purple	Uncolored	Total	
r	45	23	9	77	(3 : 1)
s	8	8	3	19	0.2~0.3
Total	53	31	12	96	(36 : 21 : 7) (3 : 1)
P value (Colour)	(36 : 21 : 7) 0.8~0.9				0.7~0.8

Table 5. Relationship between resistance to Ina 72 observed in F_3 lines and colour of apiculus observed on F_2 plants of hybrid between To-to and Kamenoo

Resistance to Ina 72	Number of lines or plants				P value (Resistance)
	Dark purple	Light purple	Uncolored	Total	
r	38	23	11	72	(3 : 1)
s	15	8	1	24	1.0
Total	53	31	12	96	(36 : 21 : 7) (3 : 1)
P value (Colour)	(36 : 21 : 7) 0.8~0.9				0.7~0.8

Table 6. Segregation of resistance to several strains of the blast fungus in F_2 population of hybrid between Choko-to and Norin 8

		Fungus strain	Number of plants				P value
			r	m	s	Total	
I	Choko-to (MR) Norin 8 (M) F_2 (Corrected)*	P-2b	16			16	(3 : 1)
			8		7	15	
			108		14	122	
			92.0		30.0	122.0	0.90~0.95
II	Choko-to (R) Norin 8 (S) F_2	Ina 72	17			17	(3 : 1)
			1**		16	17	
			97		37	134	1.0
III	Chokot-o (R^h) Norin 8 (S) F_2	Hoku 1	17			17	(3 : 1)
			97		17	17	
					37	134	0.3~0.5
IV	Choko-to (MR) Norin (S) F_2	Ken 54-20	16			16	(3 : 1)
					17	17	
			96	2	27	125	0.3~0.5
V	Choko-to (R^h) Norin 8 (M) F_2	Ken 54-04	15			15	(3 : 1)
			1	8	40	15	
			182	23	41	246	0.7~0.8
VI	Choko-to (R^h) Norin 8 (S) F_2	Ina 168	15			15	(15 : 1)
					17	17	
			119	3	6	128	0.3~0.5

Injection method for inoculation was used in all the experiments.

* : As a difference of reaction between the parents was small, plants in the susceptible variety Norin 8 were divided into two groups (r and s). Accordingly, the observed segregation ratio was corrected by the following way.

$$14 \times \frac{8+7}{7} = 30.0$$

** : This seems to be due to a failure of inoculation.

Table 7. Reaction of F_2 plants of hybrid between To-to and Choko-to to Hoku 1 and Ina 72

Parent	Fungus strain	<div><div>②</div><div>①</div></div>	Number of plants						P _① value			
			P ₁			P ₂				F ₂		
			r	s	Total	r	s	Total		r	s	Total
P ₁ To-to (R ^h , R ^h)	① Hoku 1	r s	11		11	16		16	122		122	(1 : 0)
P ₂ Choko-to (R ^h , R)	② Ina 72	Total	11		11	16		16	122		122	
		P _② value							(1 : 0)			

Injection method was used for inoculation in this experiment.

paper (YAMASAKI and KIYOSAWA, 1966).

Gene analysis of the variety Choko-to by the hybridization method

Choko-to belonging to the To-to group was crossed with a variety of the Shin 2 type, Norin 8. The F_2 plants were separately inoculated with six fungus strains, P-2b, Ina 72, Hoku 1, Ken 54-20, Ken 54-04 and Ina 168, by the injection method (Table 6). Segregation ratios in inoculation with P-2b, Ina 72, Hoku 1, Ken 54-20 and Ken 54-04 agreed with a ratio of 3 : 1. Segregation for Ina 168 was in a digenic ratio of 15 : 1. These results were those expected under the existence of two genes, $Pi-a$ and $Pi-k$, in Choko-to.

Absence of susceptible segregants in F_2 population of To-to $\times$ Choko-to hybrid under inoculations with Hoku 1 and Ina 72 (Table 7) seems to indicate that Choko-to has at least two genes which are the same as those in To-to. This indication was, however, not conclusive in this experiment, because the number of plants inoculated were relatively small. A complete conformation between the results of hybridization and mutant methods for gene analysis, however, indicates conclusively inclusion of genes, $Pi-a$ and $Pi-k$, in Choko-to.

Gene analysis of the variety Minehikari by the hybridization method.

Gene analysis of Minehikari by the mutant method mentioned above showed that Minehikari might have an unknown gene (s) in addition to $Pi-a$ and $Pi-k$. F_3 lines of a hybrid of Minehikari $\times$ Kohoku-kei 11 which were given by Messrs. TANIGUCHI and NISHIOKA were used for an identification of the unknown

Table 8. Segregation for 3 fungus strains of F_3 lines of hybrid between Minehikari and Kohoku-kei 11

	Fungus strain	Number of lines			P value
		r	s	Total	
Expected value	(3 : 1)	145.5	48.5	194.0	
Observed value	Ina 72	136	58	194	0.1~0.2
	Hoku 1	138	56	194	0.2~0.3
	Ina 168- $a^+ - k^+$	136	58	194	0.1~0.2

Injection method for Ina 72 and Hoku 1 and spraying method for Ina 168- $a^+ - k^+$ were used.

gene (s). Kohoku-kei 11 used as a partner of the cross was bred in the Kohoku Branch of the Shiga Prefecture Agricultural Experiment Station. This variety at present stocked in this experiment station showed reactions of the Aichi Asahi type to the seven fungus strains. A ratio of 3 : 1 observed in F_3 analysis of the hybrid with Ina 72 (Table 8) should, however, not be expected in a cross between To-to type and Aichi Asahi type varieties, as monogenic segregation should not be obtained in the cross between resistant varieties. Parents of Minehikari are Nakate Shin Senbon and BR No. 1, both being resistant to Ina 72 and probably having $Pi-a$. Parents of Kohoku-kei 11 are Hokuchu 7 and Koganemaru; the former susceptible and the latter resistant to Ina 72. Moreover, Kohoku-kei 16 which is a sister line of Kohoku-kei 11 is susceptible to Ina 72; being the Shin 2 type. These facts suggest that a difference of genic constitution in relation to blast-resistance between the parental variety (used for making the hybrid

used here) and the variety stocked at present (used as a check for resistance test in this experiment) may be that in Kohoku-kei 11. In any event, the hybrid used in this experiment was found to be heterozygous in relation to the gene *Pi-a*. In inoculation of the same 196 lines with Hoku 1, a segregation ratio in agreement with 3:1 was obtained (Table 8). It is considered from the result obtained in the mutant method that this is due to *Pi-k*.

The same F_3 lines were tested by spraying method with Ina 168- a^+-k^+ in order to know gene(s) participating in the resistance to this strain, and a segregation ratio of 3:1 was obtained. In this case, about a half number of plants in a segregating line showed an intermediate type of resistance, a fourth highly resistant, and the other fourth susceptible, although the difference between the highly resistant and the semi-resistant reactions was not clear in some cases. This indicates that the gene controlling the resistance to Ina 168- a^+-k^+ is incompletely dominant in inoculation by the spraying method. This gene is of a new revelation. A gene symbol, *Pi-m*, is given to this gene, meaning the gene to be included in Minehikari.

In Table 9, the result of linkage analysis among *Pi-a*, *Pi-k* and *Pi-m* was shown. No

linkage relationship was found between *Pi-a* and *Pi-k* which were detected by injection of Ina 72 and Hoku 1, respectively. The observed segregation ratio for *Pi-a* and *Pi-m* detected by spraying Ina 168- a^+-k^+ was not conformed with a ratio of 9:3:3:1 expected in independence of both the genes. If there is a linkage, value of a ratio $(rs+sr)/(rr+ss)$ should be smaller than $(3+3)/(9+1)$. However, the fact was contrary to this. Accordingly, the observed segregation ratio appears to indicate that *Pi-a* behaved independently of *Pi-m*.

A relationship between *Pi-a* and *Pi-m* showed a close linkage between them. The crossing-over value was calculated to be $11.3 \pm 4.8\%$ by the IMMER's table (IMMER, 1930).

Gene analysis of the variety Yakei-ko.

Yakei-ko, a Chinese variety of the Kanto 51 type, was analyzed in F_2 population of a hybrid with Sekiyama 2 which had been known to carry *Pi-i* (KIYOSAWA, 1966 a). In the F_2 population, yellow seedlings appeared at the rate of 1/16 and they could not be inoculated because of their lethality (Table 10). In the cases of inoculation with P-2b and Ken 54-20, digenic segregations were obtained, in disregard to the existence of yellow seedlings. Segregations for Hoku 1 and Ina 168 nearly

Table 9. Linkage analysis among the three resistance genes included in Minehikari

	Fungus strain	Number of lines					P value
		rr	rs**	sr	ss	Total	
Expected value	(9:3:3:1)	109.13	36.38	36.38	12.13	194.02	
Observed value	Ina 72 : Hoku 1	94	42	44	14	194	0.1~0.2
	Ina 72 : Ina 168- a^+-k^+	90	46	46	12	194	0.02~0.05
	Hoku 1 : Ina 168- a^+-k^+	126	12	10	46	194	<0.001*

* : Crossing-over value of $11.3 \pm 4.8\%$ was obtained.

** : Line which is resistant to the left fungus strain and is susceptible to the right.

Table 10. Segregation of yellow seedlings in F_2 population of hybrid between Yakei-ko and Sekiyama 2

	Number of plants			P value
	Green	Yellow	Total	
Yakei-ko	32		32	(15:1)
Sekiyama 2	32		32	
F_2	617	35	652	0.3~0.5

agreed with monogenic and digenic ratios respectively with low P values. The experimental results indicate probably the existence in Yakei-ko of one gene controlling resistance to each of these fungus strains, as the gene *Pi-i* in Sekiyama 2 is known to show resistance to P-2b, Ken 54-20 and Ina 168. Analyses of F_2 populations of hybrids of Yakei-ko with Eiko and Shin 2 both of which are susceptible to the used fungus strains gave an evidence

Table 11. Segregation of resistance to several fungus strains in F_2 populations of hybrids including Yakei-ko as a parent

		Fungus strain	Number of plants				P value
			r	m	s	Total	
I	Yakei-ko (R^h) Sekiyama 2 (R^h) F_2	P-2b	14			14	(15 : 1)
			15			15	
			102	32	13	147	0.1~0.2
II	Yakei-ko (R^h) Sekiyama 2 (MS) F_2	Hoku 1	12			12	(3 : 1)
					13	13	
			98		49	147	0.01~0.02
III	Yakei-ko (R^h) Sekiyama 2 (MS) F_2	Ken 54-20	12			12	(15 : 1)
			15			15	
			137	3	11	151	0.5~0.7
IV	Yakei-ko (R^h) Sekiyama 2 (M) F_2	Ina 168	12			12	(15 : 1)
			15			15	
			128		15	143	0.02~0.05
V	Eiko (R) Yakei-ko (R^h) F_2	Ina 168	15			15	(15 : 1)
			16			16	
			146		9	155	0.8~0.9
VI	Eiko (S) Yakei-ko (R^h) F_2	Ken 54-04			17	17	(3 : 1)
			16			16	
			128	6	25	159	0.1~0.2
VII	Yakei-ko (R^h) Shin 2 (MS) F_2	Ken 54-20	16			16	(3 : 1)
					14	14	
			115		36	151	0.7~0.8
VIII	Yakei-ko (R^h) Shin 2 (S) F_2	Ina 168	15			15	(3 : 1)
			1*		14	15	
			122		36	158	0.5~0.7

Injection method for inoculation was used in all the experiments.

* : This seems to be due to a failure of inoculation.

to the above observations (Table 11).

In order to determine a relation of the gene (s) in Yakei-ko to $Pi-k$, Yakei-ko was crossed with Reishiko which had been known to carry $Pi-k$. F_2 populations of this hybrid were inoculated with Ina 168, P-2b, Hoku 1, Ken 54-20 and Ken 54-04. In these cases, one plant was inoculated with two strains at a week's interval (Table 12). For all the strains used, no susceptible plant was found. This indicates that the gene in Yakei-ko is $Pi-k$.

Gene analysis of the variety Kusabue.

Kusabue, a variety of the Kanto 51 type, was crossed with Kanto 51 and Aichi Asahi. F_2 population of a hybrid of Aichi Asahi $\times$ Kusabue showed a segregation ratio of 3 : 1 (Table 13), showing the presence of one dominant gene in Kusabue. Further, in F_2 -

population of a hybrid of Kusabue $\times$ Kanto 51 inoculated with Hoku 1, susceptible segregant was not found. This indicates that Kusabue has the same dominant gene, $Pi-k$, as Kanto 51 has.

Discussion

Some Chinese varieties and their derivatives are classified into two groups, the Kanto 51 and To-to types (or groups) (YAMASAKI and KIYOSAWA, 1966). The Kanto 51 group has the following varieties: Reishiko, Yakei-ko (Chinese varieties), Kanto 51, Kusabue (varieties bred in Japan by crossing Japanese variety with Chinese variety), etc. In the To-to group, To-to, Choko-to Hokushi Tami (Chinese ones), BR No. 1, Minehikari (bred in Japan by utilizing resistance gene in

Table 12. Reaction of F_2 plants of the hybrid between Yakei-ko and Reishiko against several strains of the blast fungus

Variety	Fungus strain	② ①	Number of plants										P _① value			
			P ₁				P ₂				F ₂					
		r	m	s	—**	Total	r	m	s	—	Total	r		m	s	—
P ₁ Reishiko (R ^h , M)	① Ina 168	r m s	9			1	10	14			14	112		6	118	(1 : 0)
P ₂ Yakei-ko (R ^h , M)	② P-2b	Total	9			1	10	14			14	112		6	118	(1 : 0)
		P _② value									(1 : 0)					
P ₁ Reishiko (R ^h , R ^h)	① Hoku 1	r m s	10				10	12		4	16	143		8	151	(1 : 0)
P ₂ Yakei-ko (R ^h , R ^h)	② Ina 168	Total	10				10	12		4	16	143		8	151	(1 : 0)
		P _② value									(1 : 0)					
P ₁ Reishiko (R ^h , R ^h)	① Ken 54-20	r m s	4			4	8	11		4	15	137		16	153	(1 : 0)
P ₂ Yakei-ko (R ^h , R ^h)	② Hoku 1	Total	4			4	8	11		4	15	137		16	153	(1 : 0)
		P _② value									(1 : 0)					
P ₁ Reishiko (R ^h , R ^h)	① Ken 54-04	r m s	4			5	9	16			16	146		7	153	(1 : 0)
P ₂ Yakei-ko (R ^h , R ^h)	② Hoku 1	Total	4			5	9	16			16	146		7	153	(1 : 0)
		P _② value									(1 : 0)					

* : Reactions of the parent to two fungus strains used for inoculation.

** : Plants in failure of inoculation of the second strain.

Table 13. Segregation of resistance to Hoku 1 in F_2 populations of hybrids including Kusabue as a parent

		Number of plants				Pvalue
		r	m	s	Total	
I	Kusabue (R ^h) Kanto 51 (R ^h) F ₂	34 31 256			34 31 256	(1 : 0)
II	Aichi Asahi (S) Kusabue (R ^h) F ₂	34 206	2 3	31 58	33 267	(3 : 1) 0.3~0.5

Spraying method for inoculation was used in the experiments.

the Chinese variety Hokushi Tami), etc. are

included.

YAMASAKI and KIYOSAWA (1966) found one completely to incompletely dominant gene, $Pi-k$, in Kanto 51. KIYOSAWA (1966 a) reported the gene $Pi-k$ in Reishiko.

In the present study, To-to, Choko-to, Kusabue and Yakei-ko were found to carry the gene, $Pi-k$. Minehikari and probably BR No. 1 also contain $Pi-k$ (Table 14). This gene was introduced from Hokushi Tami into the last two varieties (UJIHARA and TANABE, 1959; UJIHARA, SUZUKI, TANABE and ISHIHARA, 1966). This indicates that Hokushi Tami has the gene $Pi-k$. Kusabue which was demonstrated to carry $Pi-k$ was bred through Kanto 53

Table 14. Synthesized results of gene analyses with the hybridization method

Variety	Fungus strain							
	P-2b	Ken 53-33	Ina 72	Hoku 1	Ken 54-20	Ken 54-04	Ina 168	Ina 168- <i>a</i> ⁺ - <i>k</i> ⁺
Norin 17	S	S	$\overset{\circ}{R}$	S	S	S	$\overset{\circ}{R}$	S
Aichi Asahi	S	S	$\overset{\circ}{R}$	S	S	S	$\overset{\circ}{R}$	S
Eiko	S	S	$\overset{\circ}{R}$	S	S	MS	$\overset{\circ}{R}$	S
Kanto 51	$\overset{\circ}{M}$	S	S	$\overset{\circ}{R^h}$	$\overset{\circ}{R^h}$	$\overset{\circ}{R^h}$	$\overset{\circ}{R^h}$	S
Kusabue	$\overset{\circ}{M}$	S	S	$\overset{\circ}{R^h}$	$\overset{\circ}{R^h}$	$\overset{\circ}{R^h}$	$\overset{\circ}{R^h}$	S
Reishiko	$\overset{\circ}{M}$	S	S	$\overset{\circ}{R^h}$	$\overset{\circ}{R^h}$	$\overset{\circ}{R^h}$	$\overset{\circ}{R^h}$	S
Yakei-ko	$\overset{\circ}{M}$	S	S	$\overset{\circ}{R^h}$	$\overset{\circ}{R^h}$	$\overset{\circ}{R^h}$	$\overset{\circ}{R^h}$	S
To-to	$\overset{\circ}{M}$	S	$\overset{\circ}{R}$	$\overset{\circ}{R^h}$	$\overset{\circ}{R^h}$	$\overset{\circ}{R^h}$	$\overset{\circ}{R^h}$	S
Minehikari	$\overset{\circ}{M}$	S	$\overset{\circ}{R}$	$\overset{\circ}{R^h}$	$\overset{\circ}{R^h}$	$\overset{\circ}{R^h}$	$\overset{\circ}{R^h}$	M $\overset{\circ}{R}$
Choko-to	$\overset{\circ}{M}$	S	$\overset{\circ}{R}$	$\overset{\circ}{R^h}$	$\overset{\circ}{R^h}$	$\overset{\circ}{R^h}$	$\overset{\circ}{R^h}$	—
Kamenoo	S	S	S	S	S	S	S	S
Norin 8	S	S	S	S	S	S	S	S
Shin 2	S	S	S	S	S	M	S	S
Kohoku-kei 11*	S	S	S	S	S	S	S	S
Sekiyama 2	$\overset{\circ}{M}$	S	$\overset{\circ}{M}$	S	$\overset{\circ}{M}$	$\overset{\circ}{M}$	$\overset{\circ}{M}$	$\overset{\circ}{M}$

— : non-segregation
 — : monogenic segregation
 : digenic segregation

when F₂ plants or F₃ lines of the cross between the varieties at both ends of these lines were inoculated with the corresponding fungus strain.

● : *Pi-k*○ : *Pi-a*▲ : *Pi-i*△ : *Pi-m*

*: Kohoku-kei 11 which has been stocked at present is the Aichi Asahi type (see the text).

from Reishiko. Accordingly, it is reasonable that Kusabue has the same gene as Reishiko. This probably indicates that Yukara and Teine belonging to the To-to group have *Pi-k* in addition to the gene *Pi-a* which has transferred from Eiko, as these two varieties have been bred from a cross of Kanto 53 and Eiko.

All To-to and Kanto 51 type varieties in Japan have been bred by using anyone of To-to, Reishiko and Hokushi Tami. In addition to this, the facts demonstrated above show that all the Kanto 51 and To-to type varieties in Japan have *Pi-k*.

Transmission course of *Pi-k* among these varieties was shown in Fig. 1.

YAMASAKI and KIYOSAWA (1966) showed no linkage between *Pi-a* and *Pi-k*. This was supported by the present study (Table 3, 6-VI, 9 and 11-V). Independence between *Pi-k* and *Pi-i* observed in hybrid progenies between Kanto 51 and Fujisaka 5 by YAMASAKI and KIYOSAWA (1966) and between Reishiko and Sekiyama 2 by KIYOSAWA (1966a) was confirmed in the present study (Table 11-I, -III).

In the present study, a new gene, *Pi-m*,

was found in Minehikari, and linkage relationship with crossing-over value of $11.3 \pm 4.8\%$ was observed between *Pi-m* and *Pi-k*. The gene *Pi-m* seemed to behave independently of *Pi-a*.

Yellow seedlings appeared in progeny of hybrid between Yakei-ko and Sekiyama 2, with digenic segregation ratio. Such an appearance of yellow seedling has been observed in progeny of hybrid of Reishiko with Sekiyama 2 by KIYOSAWA (1966a), who symbolized the genes included in the respective varieties as y_R and y_S . The gene for yellow seedling in Yakei-ko is considered to be the same as the one in Reishiko, y_R . Mutual independences among *Pi-k*, *Pi-i*, y_R and y_S were reported by KIYOSAWA (1966a). This indicates that gene analysis in disregard of the appearance of yellow seedlings in the F₂ population of the Yakei-ko × Sekiyama 2 hybrid is not unsuitable.

Summary

Blast-resistance of To-to type varieties of rice, To-to, Choko-to and Minehikari was

Fig. 1. Breeding process of blast-resistant varieties by using the gene *Pi-k* in Japan.

□ : Variety carrying *Pi-k*, □ : Variety carrying *Pi-a*,
 □ : Variety having no resistance genes. Other varieties were not tested.

genetically analyzed with hybridization method and mutant method for gene analysis. These varieties were found to carry the genes, *Pi-a* and *Pi-k*. Minehikari has an additional gene, *Pi-m*. Some Kanto 51 type varieties, Yakei-ko and Kusabue, have *Pi-k*. No linkage relationship was found between *Pi-a* and *Pi-k*, *Pi-a* and *Pi-m*, and *Pi-k* and *Pi-i*. The gene *Pi-m* linked to *Pi-k* with crossing-over value of $11.3 \pm 4.8\%$.

It was concluded from results of this and previous studies that all of To-to and Kanto 51 type varieties in Japan carry the gene *Pi-k*.

Yakei-ko has a gene for yellow seedlings, y_R , which was found in Reishiko in a previous study. This recessive gene controls chlorophyll deficiency in duplicate state with the recessive gene, y_S , in Sekiyama 2.

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イネの杜稻型品種と関東 51 号型品種のいもち病抵抗性の遺伝

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イネの杜稻型品種の杜稻・長香稻・峰光と関東 51 号型品種のクサブエ・野鷲梗の遺伝子分析を突然変異体法と交配法を用いて行なつた。抵抗性遺伝子 $Pi-k$, $Pi-a$ とその両者に対する病原性の変化した突然変異菌系と、その原菌系を用いて上記品種の抵抗性検定(突然変異体法)を行なつた結果、これらの品種は原菌系に対して抵抗性を示したが、突然変異菌系に対して罹病性を示した。

この結果から、杜稻型品種は $Pi-a$, $Pi-k$ 遺伝子をもち、関東 51 号型品種は $Pi-k$ 遺伝子をもつことが推定された。このことは、上記品種と罹病性品種との雑種後代の分析(交配法)により確認された。峰光は $Pi-a$, $Pi-k$ の他に新遺伝子 $Pi-m$ をもつ。 $Pi-m$ は、 $Pi-a$ とは独立に行動し、 $Pi-k$ とは乗換え価 $11.3 \pm 4.8\%$ で連鎖している。