Breeding and Some Genetical Findings for Multiracial Resistance to Blast by Using Japonica×Indica Hybrids of Rice

By KOTARO NAGAI*, HIROSHI FUJIMAKI** and MASAO YOKOO***

* Tropical Agriculture Research Center
** Crop Research Division, Central Agricultural Experiment Station
*** Division of Genetics, Department of Physiology and Genetics,
    National Institute of Agricultural Sciences

The environmental pollution by agricultural chemicals has recently created a serious problem in Japan where farmers apply a lot of chemicals to control disease and insect pests under intensive cultivation of rice. Under these circumstances, biological control of rice blast disease by the use of resistant varieties is regarded as one of the most promising approaches to overcome the blast damage.

The breeding for blast resistance has been made complicated by the physiologic specialization of the rice blast fungus (*Piricularia oryzae* CAVARA) (GOTO, 1965). Some varieties introduced from China had provided good genes for resistance and those genes had been incorporated into cultivated varieties (ITO, 1965).

But several varieties selected from different Japanese and Chinese crosses coincidentally lost their resistance due to the prevalence of new virulent races of blast fungus because an identical gene for resistance was transmitted by different Chinese varieties (KIYOSAWA, 1968). It was urgently needed to develop new varieties resistant to all fungus strains existing in the rice growing area of this country.

This report deals with the successful breeding in which two promising varieties with multiracial resistance, Toride 1 and Toride 2, were developed by the use of *indica* varieties as donor parents for blast resistance, and with genetical findings related to the resistance.

Breeding for multiracial blast resistance by using *indica* rice varieties

It is impossible in a practical screening for resistance to observe resistance reactions of a variety (or a line) to all the existing strains of blast fungus. Seven fungus strains were chosen which were typical and stable for pathogenic reactions to the differential varieties in this country. They were used throughout the present study.

According to the genetical analyses of blast resistance in rice (KIYOSAWA, 1967), cultivated varieties in Japan were classified into the seven genotypic groups or their combinations. Six genes for blast resistance were identified using the seven typical strains of blast fungus. None of them showed complete resistance to all the fungus strains (Table 1).

The Shin 2 type varieties show resistance to neither of them and no gene for resistance is supposed to be included in these varieties as far as the seven blast strains are concerned.

This type of reaction is the most frequent among the paddy rice varieties grown in Japan. The second most frequent type of reaction is the Aichi-Asahi type carrying a single gene *Pi-a* which has resistance to two of the seven strains of blast fungus.

The other type of resistance reaction, which shows moderate resistance to five of the seven blast strains is not rare among paddy rice varieties. This reaction designated as Ishikari-

Table 1. **Typical reaction types for resistance to the seven blast strains in domestic varieties of paddy rice (KIYOSAWA, 1967)**

Type of reaction	Genotype	Fungus strain (Race)							Donor variety
		Ken 53–33 (T–1)	P–2b (T–2)	Ina 72 (C–3)	Hoku 1 (N–1)	Ken 54–20 (N–2)	Ken 54–04 (N–3)	Ina 168 (N–4)	
Shin 2	$+$	S	S	S	S	S	S	S	Native or local
Aichi-Asahi	pi-a	S	S	R	S	S	S	R	Native or local
Ishikari-Shiroke	Pi-i	S	M	M	S	M	M	M	Native or local
Kanto 51	Pi-k	S	M	S	R^h	R^h	R^h	R^h	Tu-tao
Ta	Pi-ta	S	S	M	M	M	M	S	Tadukan
Pi No. 4	Pi-ta^2	M	S	MR	M	MR	MR	MR	Tadukan
Fukunishiki	Pi-z	M	M	M	M	M	M	M	Zenith

Shiroke type is induced by a single gene Pi-i. These two genes for resistance, Pi-a and Pi-i, are representatives of the genes for resistance in domestic paddy rice.

The other genes for resistance available in this country (Pi-k and its alleles, Pi-ta and its allele, and Pi-z) were introduced from exotic varieties by breeding. A characteristic found in them is resistance to most of fungus strains belonging to N-race which were prevailing on rice fields in the past but, susceptible to some strains of C- or T-race spreading rapidly in recent years.

An extensive survey of rice collection was carried out in order to search for any variety which is highly resistant to all the seven fungus strains. As the result of this survey for multiracial resistance, several *indica* varieties introduced from tropical Asia were found to be satisfactorily resistant to all the blast strains (KIYOSAWA, unpublished). The names and origins of these promising varieties are presented in Table 2 with their resistance reactions to the seven blast strains.

Backcross breeding programs were initiated in 1955 to transfer the genes for multiracial blast resistance of the two Indian varieties to a domestic variety Norin 8. TKM. 1 and

Table 2. *Indica* **varieties employed as donor parents in the backcross breeding and their resistance reactions to the seven blast strains**

Variety	Origin	Fungus strain (Race)						
		Ken 53–33 (T–1)	P–2b (T–2)	Ina 72 (C–3)	Hoku 1 (N–1)	Ken 54–20 (N–2)	Ken 54–04 (N–3)	Ina 168 (N–4)
TKM. 1	India	R^h	R^h	R^h	R^h	R^h	R^h	R^h
CO. 25	India	R^h	R	R	R^h	MR	MR	R^h
CO. 4	India	R^h	R^h	R^h	R^h	M	MR	R
Leuang Tawng 77–12–5	Thailand	R^h	R^h	MR	R	M	MR	MR
Chao Leuang 11	Thailand	R^h	R^h	MR	R^h	MR	MR	R^h
Morak Sepilai	Malaysia	R^h	R^h	R^h	R	R^h	R^h	M
Kontor	Malaysia	R^h	R^h	MR	R^h	R^h	R^h	R^h
Engkatek	Malaysia	R^h	R^h	R^h	R^h	R^h	R^h	R^h
Tjahaja	Indonesia	R^h	R^h	R^h	R	MR	MR	R
Sigadis	Indonesia	R^h	R^h	R^h	R^h	MR	R^h	R^h

Note: A part of this table was compiled from the unpublished data by S. KIYOSAWA

Inoculation was carried out by injecting the spore suspension into the inside of young folding leaves

Reactions: R^h = Highly resistant, R = Resistant MR = Moderately resistant, M = Medium
MS = Moderately susceptible, S = Susceptible

CO. 25 were used as donor parents for resistance and repeatedly backcrossed to Norin 8 (Fig. 1).

In every backcross generation, the hybrid plants were carried into the blast nursery for testing resistance to blast and resistant plants were selected to be crossed with the recurrent parent.

The morphologic characteristics of Norin 8 were reproduced by four cycles of backcrosses and selections with successful transfer of

blast resistance from *indica* donor varieties (Fig. 2).

In selfing generations following the four successive backcrosses the selection for the desirable agronomic traits and the evaluation of homozygosity were carried out.

Since the blast resistance test had been continued through backcross generations under the natural inoculation where prevailing races were supposed to have fluctuated year to year, it was not ascertained that the multi-
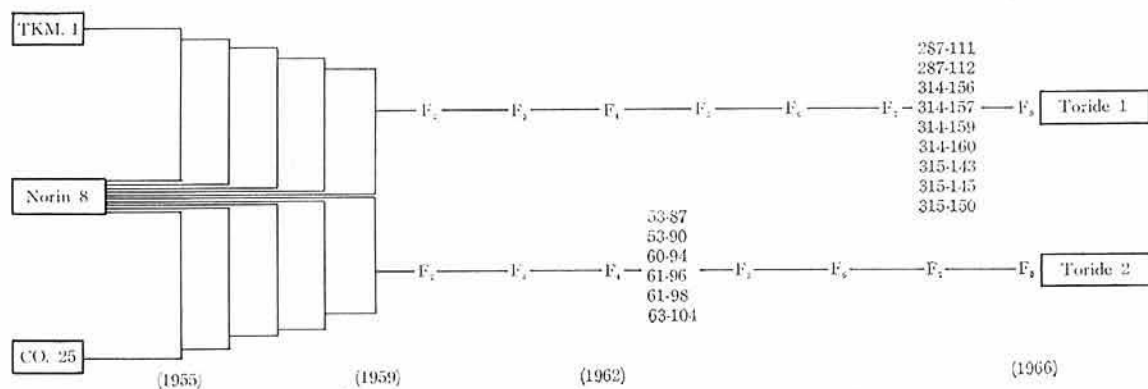

Fig. 1. Genealogical relationship of Toride 1 and Toride 2 and their sister lines
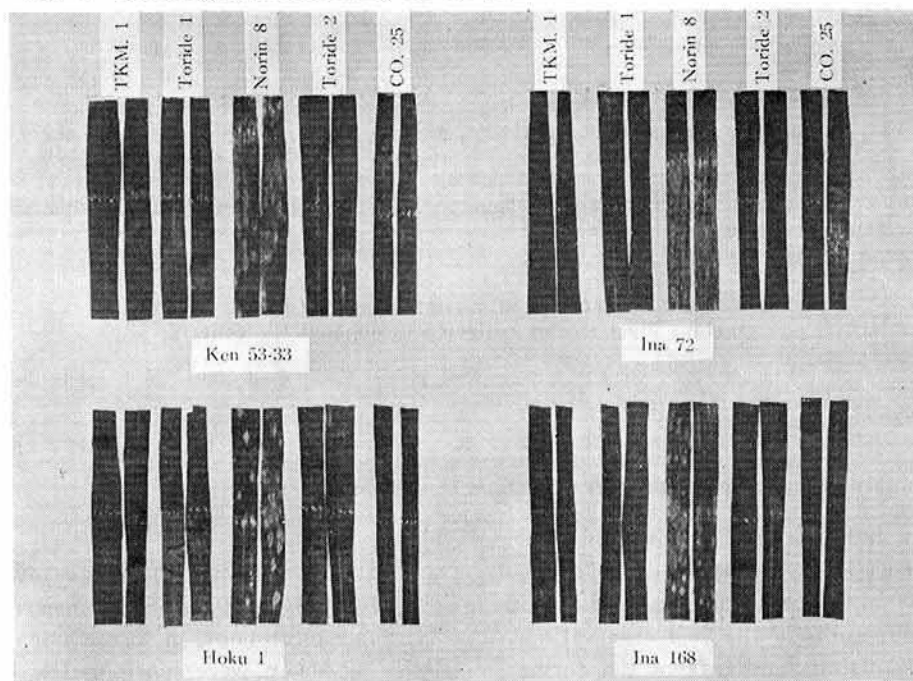

Fig. 2. Reactions of Toride 1, Toride 2 and their parents to the four of the seven strains of blast fungus

racial resistance of the donor parents had been incorporated without being lost during backcrossing.

Then the resistance reactions of self-pollinated families were observed using the seven strains of blast fungus. A part of these tests is presented in Table 3.

All the F_7 families of the cross Norin 8× TKM. 1 were true-breeding for resistance to all the seven blast strains. Among F_4 families of Norin 8×CO. 25, the only one family 61-96 was true-breeding for resistance to all the blast strains.

In order to see to what extent the multi-

racial resistance affected the other strains of blast fungus, a line of true-breeding for resistance to the seven strains was subjected to the blast test with various kinds of fungus strains collected throughout the country. The results are briefly shown in Table 4. No strain employed in this test was virulent to this line.

The true breeding families for resistance to the seven fungus strains and promising for agronomic traits were subjected to the yield trials in the randomized complete block design with three replications. These trials were done in 1966 and 1967 for the families of Norin 8×TKM. 1 and in 1963 through to

Table 3. Resistance reactions of self-pollinated families to the seven blast strains

| Cross | Line No. | Genera-tion | Fungus strain | | | | | | | Note |
			Ken 53-33	P-2b	Ina 72	Hoku 1	Ken 54-20	Ken 54-04	Ina 168	
Norin 8 ×TKM. 1	314–156	B_4F_7	S	S	S	S	S	S	S	To be Toride 1
	314–157	B_4F_7	R^h	R^h	R^h	R^h	R^h	R^h	R^h	
	314–159	B_4F_7	S	S	S	S	S	S	S	
	314–160	B_4F_7	R^h	R^h	R^h	R^h	R^h	R^h	R^h	
Norin 8 ×CO. 25	53– 87	B_4F_4	R+S	MR+S	R	R+MS	M+S	M	R	To be Toride 2
	53– 90	B_4F_4	R+S	R+S	R^h	R^h+S	R^h+S	R+S	R^h	
	60– 94	B_4F_4	S	R+S	R	R+S	MS+S	M	R	
	61– 96	B_4F_4	R	R	R	R^h	MR	R	R	
	61– 98	B_4F_4	R^h	R	R^h	R^h+M	R+MS	R	R	
	63–104	B_4F_4	M+S	M+S	R+S	M	M+S	M	R+MS	

Note: Inoculation by injecting the spore suspension into the inside of young folding leaves
Norin 8 used as a recurrent parent belongs to the Shin 2 type which is susceptible to all the seven fungus strains

Table 4. Resistance reactions of a sister line of Toride 1 (315-145) to various blast strains collected throughout the country

Race	T-1	T-2	T-3	C-1	C-3	C-4	C-6	C-7	C-8	N-1	N-2	N-3	N-4	N-5
No. of strains used	1	3	1	3	3	1	1	1	2	5	4	2	1	1
Reaction	R	R	R	R	R	R	R	R	R	R	R	R	R	R

Note: Inoculation by spraying the spore suspension of each fungus strain

1967 for the families from Norin 8×CO. 25. A part of the results is presented in Table 5.

Judging from the performances in the yield trials and the results of the field observations, the most promising family from the former cross was designated as Toride 1, and the one from the latter cross as Toride 2 (NAGAI

et al., 1970).

The two varieties almost resembled their recurrent parent Norin 8 in appearance (Fig. 3). Some physiologic or agronomic characteristics, however, were considered to be a little different from those of the recurrent parent. Toride 1 had a trend to overgrow under the

Table 5. Performances of yield trials and some agronomic characteristics of Toride 1 and Toride 2

Year	Variety	Heading date	Culm length (cm)	Panicle length (cm)	No. of panicles	1,000 grain weight (g)	Grain yield (unhusked) (kg/a)
1966	Toride 1	Sept. 6	77.1	20.4	10.7	21.5	54.60
	Toride 2	Sept. 5	81.3	19.8	11.5	21.7	48.78
	Norin 8	Sept. 5	82.3	21.4	9.1	22.1	47.45
1967	Toride 1	Sept. 5	84.0	22.0	10.7	23.1	55.46
	Toride 2	Sept. 3	88.9	20.5	11.8	21.5	51.31
	Norin 8	Sept. 3	90.4	21.5	10.2	23.4	54.40

Note: Crops in 1967 suffered from virus stripe

Fig. 3. Morphologic characteristics of Toride 1 and Toride 2

heavy fertilization of nitrogen. Response to nitrogen is considered to be one of the most important agronomic traits under the intensive cultivation.

The new varieties have not been grown on farmers' fields as practical varieties but have been employed as breeding materials for multiracial resistance in rice breeding stations in this country.

Genetical finding for multiracial resistance

The new two varieties developed in the present program are distinguished for high resistance to various races of blast fungus. The genic constitutions of the multiracial resistance of these varieties were investigated by observing segregations for resistance in hybrid populations derived from the crosses between each of these varieties and the variety carrying a single resistance gene identi-

fied so far. For instance Toride 1 was crossed with Kanto 51 carrying a gene for resistance, *Pi-k*.

In the F_2 population of this cross, a multiple digenic segregation (15:1) was observed for resistance to the five fungus strains to which both the parents were resistant, while segregations were monogenic for the other two fungus strains to which Kanto 51 was susceptible. It was concluded from these results that the resistance of Toride 1 was affected by a single dominant gene which was different from the gene of Kanto 51, *Pi-k*, and genetically independent of it.

The same procedures of genetic analysis were applied to the crosses of Toride 1 with the other varieties carrying different genes for blast resistance. These results revealed that the gene for resistance of Toride 1 is different from any other gene identified so far.

Its locus was not associated with the three loci, *Pi-i*, *Pi-k* and *Pi-ta* and weakly associated with or independent of the locus *Pi-a*. But it was considered to be allelic or very closely associated to the locus *Pi-z*.

Then the new gene of Toride 1 was designated as *Pi-z^t* (YOKOO and KIYOSAWA, 1970).


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----- : Independent
————— : Linked
═════ : Allelic
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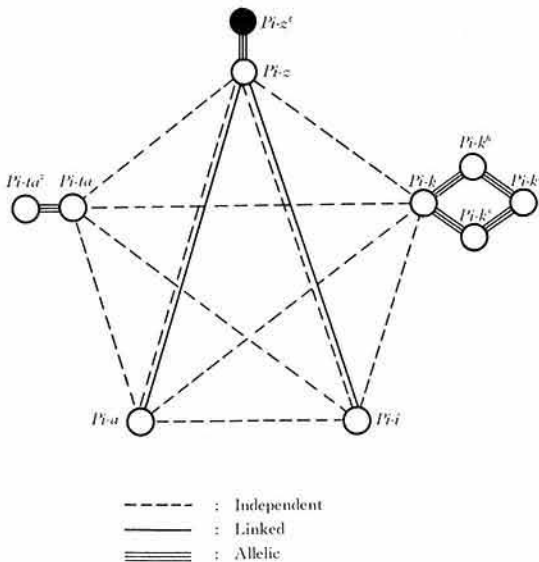
Fig. 4. Genetic relations of *Pi-z^t* with the other genes for resistance identified so far

Genetical relations of the new gene with the other genes for resistance are summarized in Fig. 4.

Another investigation was conducted for the multiracial resistance of Toride 2. It was demonstrated that the resistance of this variety was affected by the two genes *Pi-a* and *Pi-z^t* (KIYOSAWA and YOKOO, 1970).

Another experimental evidence was obtained by the use of pathogenic mutants of blast fungus to show that Toride 1 and Toride 2 carried an identical gene for high resistance to the seven blast strains. A mutant for pathogenicity Ken 53-33-*zt$^+$* was isolated from Ken 53-33 to which Toride 1 was highly resistant. This mutant strain was virulent to Toride 2 as well as Toride 1.

A pathogenic mutant of another blast strain Ina 168 was discovered, which was virulent to Toride 1 but not to Toride 2 (Table 6).

Table 6. Reactions of Toride 1 and Toride 2 to the two mutant strains for pathogenicity

Variety	Fungus strain			
	Ken 53–33		Ina 168	
	Original	Mutant	Original	Mutant
Toride 1	R^h	S	R	S
Toride 2	R^h	S	R^h	R
Norin 8	S	S	S	S

Note: Mutant strains were designated as Ken 53-33-*zt$^+$* and Ina 168–*zt$^+$* respectively
These mutant strains were isolated by H. NIIZEKI and S. KIYCSAWA

It was suggested from the reaction to these mutants for pathogenicity that the identical gene *Pi-z^t* was included in the two resistant varieties but that another gene *Pi-a* took part in expressing the resistance to Ina 168 in Toride 2. These results substantiated the conclusions obtained by the gene analyses aforementioned.

The genes for resistance of different varieties or in segregating populations are able to be identified by the use of a pathogenic mutant of blast fungus (KIYOSAWA, 1971). The genes for multiracial resistance of eight *indica* varieties listed in Table 2 were tried to be

transferred to domestic varieties by back-crossing.

On the way of the breeding, the self-pollinated populations segregating for resistance to Ken 53-33 were inoculated with the pathogenic mutant Ken 53-33-zt^+ which was previously employed to identify the genes carried by Toride 1 and Toride 2. The reactions of these hybrid populations are presented in Table 7.

Table 7. Segregations for resistance to a pathogenic mutant Ken 53-33-zt^+ in self-pollinated populations segregating for resistance to Ken 53-33

Original cross	Generation	Ken 53-33		Ken 53-55-zt^+	
		Resist.	Suscept.	Resist.	Suscept.
Norin 25 × CO. 4	B_4F_2	144	43	0	142
	B_4F_2	149	33	0	141
Fujisaka 5 × Leuang Tawng 77-12-5	B_3F_2	151	46	0	115
	B_3F_2	152	42	0	145
Chao Leuang 11 × Fujisaka 5	B_3F_2	139	47	0	116
	B_3F_2	141	51	0	130
Morak Sepilai × Fujisaka 5	B_4F_2	145	51	0	140
	B_4F_2	143	46	0	140
Fujisaka 5 × Kontor	B_4F_2	138	45	0	134
	B_4F_2	118	53	0	108
Norin 25 × Engkatek	B_4F_2	55	28	100	21
Norin 25 × Tjahaja	B_4F_4	125	55	130	56
Norin 25 × Sigadis	B_4F_3	151	41	118	35

All the populations showed monogenic segregations for resistance to the original strain Ken 53-33. To the mutant strain Ken 53-33-zt^+, however, no resistant plant appeared in the populations from the first five crosses and the populations of the other three crosses showed monogenic segregations for resistance.

These results indicated that the identical gene for resistance Pi-z^t governed blast resistance in the first five crosses but that the resistance in the other three crosses were affected by the other gene(s) (FUJIMAKI and YOKOO, 1971).

It is a very interesting fact that the identical gene was unexpectedly incorporated from different *indica* donor varieties introduced from different geographical places. Through the irony of chance the gene for resistance Pi-z^t had been already transferred to a domestic variety in the aforementioned breeding of Toride 1 and Toride 2.

Several varieties derived from various Japanese×Chinese crosses had been found to carry an identical gene Pi-k (KIYOSAWA, 1968). This may have been one of the most justifiable reasons why those commercial varieties had coincidentally lost their resistance by the rapid increase of blast races with selective virulence to Pi-k.

Judging from the results obtained in the present study and the historical evidence of breeding for blast resistance, such genes as Pi-k and Pi-z^t are supposed to distribute widely among Chinese or *indica* rice varieties.

If this deduction is correct, it will be indispensable to identify the genes affecting blast resistance in the early stage of breeding so as to avoid the repetition of incorporating the identical genes available in domestic varieties in future breeding for blast resistance.

The locus of the new gene identified in the present study was determined on chromosome by using segregating population of backcross progenies of the cross Morak Sepilai×Fujisaka 5. In this population four genes, C for apiculus color, alk for alkali digestibility of milled rice, Lm for maturity, and Pi-z^t for blast resistance were found to be segregating.

A clear linkage relation was observed among the four loci (YOKOO and FUJIMAKI,

1970). They were arranged on the linkage group I as shown in Fig. 5 from the results of the analysis of segregation for the four genes. All the four dominant alleles were transmitted by the *indica* donor Morak Sepilai. Such a large linkage block including the four alleles was held intact through four repeated backcrosses.
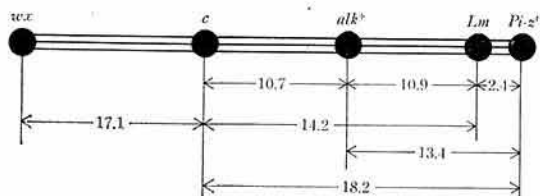

Fig. 5. A proposed arrangement of the four dominant genes incorporated from an indict donor parent Morak Sepilai

The new genes for multiracial resistance introduced in the present breeding program will be useful for efficient control of blast epidemic in the sense undermentioned. One of the utilizations of these genes is to incorporate several different genes for resistance into one commercial variety to stabilize resistance of the variety.

Another method for controlling blast epidemic is to raise a mechanical mixture of isolines carrying different genes for resistance, that is, a multiline (JENSEN, 1952). The other measure to block blast epidemic is to grow alternately several varieties with different genes for resistance.

The varieties developed in the present program would lose resistance one day when pathogenic mutation occurs to any race under the natural condition. Therefore, the genes for multiracial resistance have to be combined with the so-called field resistance in order that the dangerous epidemic may be delayed.

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