Allelism of Blast Resistance Genes in Near-Isogenic Lines of Rice

T. Inukai, R. J. Nelson, R. S. Zeigler, S. Sarkarung, D. J. Mackill, J. M. Bonman, I. Takamure, and T. Kinoshita

First, seventh, and eighth authors: Faculty of Agriculture, Hokkaido University, Sapporo 060, Japan; second and third authors: International Rice Research Institute (IRRI), P.O. Box 933, Manila 1099, Philippines; fourth author: IRRI Cooperative Project with Ministry of Agriculture and Cooperatives, P.O. Box 9-159 BKN Bangkhen, Bangkok 10900, Thailand; fifth author: USDA-ARS, Department of Agronomy and Range Science, University of California, Davis 95616; sixth author: Du Pont Agriculture Products, Stine-Haskell Building 200, P.O. Box 30, Newark, DE 19714.

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ABSTRACT

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A set of near-isogenic lines (NILs) for rice blast resistance was previously developed in the genetic background of *indica* rice cultivar CO39. The 22 CO39 NILs carry five blast resistance genes. In this report, allelism between these resistance genes in the CO39 NILs and Kiyosawa's Differential series of cultivars was analyzed. *Pi-1*(t), carried by the NILs in group I, was closely linked to *Pi-k* on chromosome 11, with a recombination value of 6.1 ± 1.7% (standard error [SE]). *Pi-2*(t), carried by the NILs in group II, was allelic or closely linked to *Pi-z* on chromosome 6. *Pi-3*(t), present in the group V NIL, was closely linked to *Pi-i*, with a recombination value of 7.0 ± 2.6% SE. *Pi-4*^{a}(t), carried by the NILs in groups III and IV, was allelic or closely linked and possibly identical to *Pi-ta* on chromosome 12. The NIL in group VI carried *Pi-4*^{a}(t) and a gene with an unknown identity, which was not *Pi-4*^{b}(t). At present, a set of NILs consisting of C101LAC (*Pi-1*(t)), C101A51 (*Pi-2*(t)), C104PKT (*Pi-3*(t)), C101PKT (*Pi-4*^{a}(t)), and C105TTP-4L23 (*Pi-4*^{a}(t)+*Pi-?*) is available for use as differentials.

Rice blast, caused by *Pyricularia grisea* (Cooke) Sacc. (16), is one of the most widespread and destructive diseases of rice (*Oryza sativa* L.). Breeding blast-resistant rice cultivars is one of the most effective means to control blast disease. Resistance breeding can be more effective if the genetics of resistance is well understood.

Extensive genetic studies on blast resistance have been conducted in Japan. Thirteen complete resistance genes have been identified, and a set of *japonica* differential cultivars and breeding lines with single resistance genes has been developed for differentiation of pathogen isolates (12). In the tropics, genetic studies have lagged behind those conducted in the temperate regions due to the presence of many resistance genes in tropical rice cultivars (27).

The production and characterization of near-isogenic lines (NILs) carrying individual resistance genes in a common genetic background allows resistance loci to be systematically analyzed. For instance, NILs can be used to assess allelic relationships between loci, the spectra of resistance in relation to pathogen subpopulations, and the virulence spectra of pathogen isolates.

A set of NILs was recently developed at the International Rice Research Institute (IRRI), Los Baños, Philippines, in the genetic background of the highly susceptible *indica* cultivar CO39 (14). The availability of these NILs makes it possible to characterize the genetic relationships among the resistance loci in the donor parents and to compare these loci with those present in the Japanese differential series (Kiyosawa's Differentials). The objectives of this study were to determine the relationships among the blast-resistance genes in the 22 CO39 NILs developed with several resistant cultivars from the tropics and to determine the relationships between the blast-resistance genes in the CO39 NILs and Kiyosawa's Differentials developed in Japan.

MATERIALS AND METHODS

Plant materials. The 22 CO39 NILs, CO39, and the 12 Kiyosawa's Differentials were used in this study (Table 1). CO39, the recurrent parent of the NILs, is a highly susceptible *indica* cultivar. The donor parents of the NILs were resistant *indica* cultivars Tetep and 5173 and resistant *japonica* cultivars Pai-kan-tao and LAC23 (14).

During their development, each of the NILs was given a designation beginning with C (for CO39), followed by the isolate number used for selection during the development of the line (101 for IK81-3, 102 for IK81-25, 103 for PO6-6, 104 for PO3-82-51, and 105 for 43) and an abbreviation for the resistance donor (TTP for Tetep, A51 for 5173, PKT for Pai-kan-tao, and LAC for LAC23). The 22 NILs were classified into six groups based on their reaction to a set of blast isolates (14). Blast resistance was conferred by independent dominant genes in C101LAC in group I, C101A51 to C105A51 in group II, and C104PKT in group V, designated *Pi-1*(t), *Pi-2*(t), and *Pi-3*(t), respectively (14,28). Blast resistance in C101PKT, C105TTP-1 in group III, and C105TTP-4L23 in group VI was conferred by dominant alleles at an additional locus, designated *Pi-4*^{a}(t) and *Pi-4*^{b}(t), respectively (14,28).

Pathogen isolates. *P. grisea* isolates were from the collection of the Entomology and Plant Pathology Division at IRRI. Isolates IK81-3 (international race IA-125), IK81-25 (IF-3), PO6-6 (IB-47), PO3-82-51 (ID-15), 43 (IA-127), and V86010 (ID-14) were used. As described above, these isolates were used previously to develop the NILs. Isolate 43 and isolate V86010 were used for F_2 analysis of a cross between CO39 and C105TTP-4L23. The pathogenicity of isolate 86013 to the NILs in groups III and IV was reexamined to confirm the results of a previous study (14).

Inoculation methods. For F_2 analyses, a plastic tray (37 × 26 × 11 cm) was divided equally into five rows, and 30 F_2 seeds were uniformly sown in each of four rows in each tray. One row in each tray was divided into three more rows, and parental lines and a susceptible check cultivar were sown in each of three rows, with 10 seeds per row. For F_3 analyses, a plastic tray was divided equally into 12 rows, and F_3 line seeds were sown in

each of 10 rows, with a maximum of 20 seeds per F_3 line per row. Two rows were planted with parental lines and a susceptible check cultivar. In the F_2 analysis for C105TTP-4L23, parental lines and F_2 plants were sown in small cups, one seed per cup. In all experiments, nitrogen was applied at 36 g/m^2, and seedlings were grown in a greenhouse.

To produce inoculum, isolates were multiplied on oatmeal agar. About 2 ml of conidia and hyphal suspension from an agar slant was seeded to the medium in a petri plate. The inoculated plates were incubated at 25–28 C for a week until the entire agar surface was covered with mycelial growth. The growth was scraped with a sterilized glass slide or rubber spatula, and the plate was exposed to fluorescent light for 3 days to stimulate sporulation. The culture was flooded with distilled water, and the conidia was dislodged by scraping. The suspension was filtered through nylon or gauze mesh, and the concentration of conidia was estimated with a hemacytometer and standardized to 5×10^4 conidia per milliliter. Tween 20 was added to 0.02% just before inoculation.

The spraying method was described previously (1). Briefly, seedlings were inoculated 21 days after sowing (at the five to six leaf stage), using an electric sprayer to apply 50 ml of a spore suspension per tray of seedlings. Inoculated seedlings were incubated in a dew chamber at 25 C for 24 h and transferred to an air-conditioned greenhouse at 24–28 C. Disease reactions were recorded about 7 days after inoculation as either R for resistant parent type or S for susceptible parent or susceptible check cultivar type.

When individual plants were inoculated with more than one isolate, a modified needle-pricking method was used. Thirty-four days after sowing, the sixth leaf of a plant was fixed on a glass slide with cellophane tape, and droplets of an aqueous spore suspension of 1×10^5 conidia per milliliter were put into pinpoint holes. After inoculation the leaves were covered with cellophane tape to avoid evaporation of the droplets. Inoculated plants were placed in an air-conditioned greenhouse at 24–28 C. Disease reactions were scored as R or S about 7 days after inoculation.

Genetic analysis. In principle, all the NILs except C105TTP-4L23 were crossed with the differentials showing resistance to the test isolates. The NILs in group I were crossed with each other to investigate allelic relationships between resistance genes in the NILs. For the lines in groups III and IV, only C101TTP-6 was crossed with all the differentials showing resistance to the test isolates. The rest of the NILs in groups III and IV were crossed with only K1 and Pi No. 4. In crosses of C101LAC × K60, Tsuyuake × C103TTP, and Fujisaka5 × C104PKT, the F_2 plants were advanced to F_3 generation and used for progeny tests.

CO39, Aichi asahi (one of the Kiyosawa's Differentials), and C104PKT (one of the NILs) were used as susceptible parents or susceptible check cultivars for the F_2 and F_3 analyses. The number of resistance genes in the differentials to the test isolates was confirmed by F_2 of crosses with Aichi asahi or C104PKT. The test isolates for F_2 and F_3 analyses of each NIL in this study corresponded to the isolates used for the development of the NILs.

To confirm whether C105TTP-4L23 carried only *Pi-4*^{b}(t), C105TTP-4L23 was crossed with CO39, and the resulting 37 F_2 plants were inoculated with isolates 43 (used to develop C105TTP-4L23) and V86010 (which distinguished C105TTP-4L23 from the NILs in group III) by the modified needle-pricking method (described above).

Statistical analysis. F_2 and F_3 segregation ratios were analyzed

TABLE 1. Reaction pattern of rice cultivar CO39 near-isogenic lines (NILs) and Kiyosawa's Differential series of cultivars to *Pyricularia grisea* isolates

NIL group	Line or cultivar	*Pi* gene[a]	Reaction to isolates[b]					
			IK81-3	IK81-25	PO6-6	PO3-82-51	43	V86010
I	C101LAC	*Pi-1*(t)	R	S	R	R	R	R
	C103TTP		R	S	R	R	R	R
	C104LAC		R	S	R	R	R	R
II	C101A51	*Pi-2*(t)	R	R	R	R	R	R
	C102A51	*Pi-2*(t)	R	R	R	R	R	R
	C103A51	*Pi-2*(t)	R	R	R	R	R	R
	C104A51	*Pi-2*(t)	R	R	R	R	R	R
	C105A51	*Pi-2*(t)	R	R	R	R	R	R
III	C101PKT	*Pi-4*^{a}(t)	R	R	S	S	R	S
	C101TTP-1		R	R	S	S	R	S
	C101TTP-2		R	R	S	S	R	S
	C101TTP-3		R	R	S	S	R	S
	C101TTP-4		R	R	S	S	R	S
	C101TTP-6		R	R	S	S	R	S
	C102TTP		R	R	S	S	R	S
	C105TTP-1	*Pi-4*^{a}(t)	R	R	S	S	R	S
	C105TTP-2L23		R	R	S	S	R	S
	C105TTP-4L6		R	R	S	S	R	S
IV	C102PKT		R	R	S	S	R	S
	C105TTP-2L9		R	R	S	S	R	S
V	C104PKT	*Pi-3*(t)	S	S	R	R	R	S
VI	C105TTP-4L23	*Pi-4*^{b}(t)	R	R	S	S	R	R
	CO39	...	S	S	S	S	S	S
Kiyosawa's Differentials	Shin 2	*Pi-k*^{s}	I	R	R	R	R	R
	Aichi asahi	*Pi-a*	S	S	S	S	S	S
	Fujisaka 5	*Pi-i, Pi-k*^{s}	S	S	R	R	R	R
	Kusabue	*Pi-k*	R	R	R	R	R	R
	Tsuyuake	*Pi-k*^{m}	R	S	R	R	R	R
	Fukunishiki	*Pi-z*	R	R	R	R	R	R
	K 1	*Pi-ta*	R	R	S	S	R	S
	Pi No. 4	*Pi-ta*^{2}	R	R	R	R	R	R
	Toride 1	*Pi-z*^{t}	I	R	R	R	R	R
	K 60	*Pi-k*^{p}	R	R	R	R	R	R
	BL 1	*Pi-b*	I	R	R	R	R	R
	K 59	*Pi-t*	S	S	S	S	S	R

[a] Mackill and Bonman (14); Yu et al (28); Kiyosawa et al (12).
[b] R = resistant; I = intermediate; S = susceptible. Mackill and Bonman (14); T. Inukai et al (6).

by the chi-square test, and a recombination value was calculated by the maximum likelihood method. The mean of recombination values was calculated by the weighted means method.

RESULTS

NILs in group I. To test the allelism of resistance genes carried by the NILs in group I, isolate IK81-3 was used to inoculate F_2 populations derived from crosses between C101LAC and C104LAC and between C103TTP and C104LAC. No susceptible plants were observed (Table 2). These results indicated that the genes in the three NILs were allelic or closely linked.

Mackill and Bonman (14) suggested that the resistance gene in C101LAC was different from that in C103TTP and C104LAC because two isolates tested in France differentiated C101LAC from C104LAC and C103TTP. Because the resistance genes in both C101LAC and C104LAC were derived from the same donor parent, LAC23 (14), and both lines showed the same reaction pattern to the *P. grisea* isolates tested in this study (Table 1), it was inferred that at least C101LAC and C104LAC carried the same gene. C103TTP probably carried *Pi-1*(t). C101LAC, C104LAC, and C103TTP may carry additional resistance gene(s) that are detectable by other isolates.

In crosses between C101LAC (*Pi-1*(t)) and the differentials carrying the genes at the *Pi-k* locus (except *Pi-k*^{s}) and between C103TTP (*Pi-1*(t)) and the same differentials, only zero or one susceptible plant was observed in each F_2 population (Table 2). Because Kusabue (*Pi-k*), Tsuyuake (*Pi-k*^{m}), and K60 (*Pi-k*^{p}) carried single resistance genes for isolate IK81-3 and these genes were allelic to one another (Table 2), showing that the resistance of these cultivars or breeding lines to isolate IK81-3 was due to the genes at the *Pi-k* locus, it was inferred that *Pi-1*(t) was closely linked but nonallelic to the *Pi-k* locus. The resistance of Shin 2, carrying *Pi-k*^{s}, to isolates IK81-3 and PO6-6 appeared to be controlled by a gene with an unknown identity, which was not *Pi-k*^{s}, because both crosses of Shin 2 with C101LAC and C103TTP showed segregation ratios of 15:1 (R:S) to the test isolates (Table 2). The resistance of Shin 2 to isolate IK81-3 was

TABLE 2. Reaction of F_2 populations of crosses between rice cultivar CO39 near-isogenic lines (NILs) and Kiyosawa's Differential series of cultivars to *Pyricularia grisea* isolates

NIL group	Cross[a]	Test isolate	No. of F_2 plants observed for each class[b] R	S	Expected ratio (R:S)	Probability
I	C104LAC ×					
	C101LAC*	IK81-3	466	0	1:0	
	C103TTP*	IK81-3	430	0	1:0	
	C101LAC ×					
	Shin 2	IK81-3	141	13	15:1	0.25–0.50
	Kusabue*	IK81-3	686	0	15:1	<0.01
	Tsuyuake*	IK81-3	556	1	15:1	<0.01
	Fukunishiki	IK81-3	202	7	15:1	0.05–0.10
	K 1*	IK81-3	216	12	15:1	0.50–0.75
	Pi No. 4*	IK81-3	209	7	15:1	0.05–0.10
	Toride 1*	IK81-3	99	2	15:1	0.05–0.10
	K 60	IK81-3	284	0	15:1	<0.01
	BL 1*	IK81-3	153	16	15:1	0.05–0.10
	C103TTP ×					
	Shin 2*	PO6-6	40	2	15:1	0.50–0.75
	Fujisaka 5*	PO6-6	173	9	15:1	0.25–0.50
	Kusabue*	PO6-6	439	0	15:1	<0.01
	Tsuyuake*	PO6-6	215	0	15:1	<0.01
	Fukunishiki	PO6-6	191	7	15:1	0.10–0.25
	Pi No. 4*	PO6-6	184	18	15:1	0.10–0.25
	Toride 1*	PO6-6	65	10	15:1	0.01–0.025
	K 60	PO6-6	566	1	15:1	<0.01
	BL 1*	...	...	...		
	Aichi asahi ×					
	Kusabue	IK81-3	85	30	3:1	0.75–0.90
	Tsuyuake	IK81-3	90	30	3:1	>0.99
	K 60	IK81-3	86	31	3:1	0.75–0.90
	Tsuyuake ×					
	Kusabue*	IK81-3	356	0	1:0	
	K 60*	IK81-3	347	0	1:0	
II	C101A51 ×					
	Shin 2*	IK81-3	179	32	15:1	<0.01
	Kusabue*	IK81-3	212	9	15:1	0.10–0.25
	Tsuyuake*	IK81-3	77	3	15:1	0.25–0.50
	Fukunishiki	IK81-3	886	0	15:1	<0.01
	K 1*	IK81-3	201	8	15:1	0.10–0.25
	Pi No. 4*	IK81-3	94	3	15:1	0.10–0.25
	Toride 1*	IK81-3	341	23	15:1	0.95–0.975
	K 60	IK81-3	184	11	15:1	0.50–0.75
	BL 1*	IK81-3	184	32	15:1	<0.01
	Fukunishiki ×					
	Aichi asahi	IK81-3	83	17	3:1	0.05–0.10

(*continued on next page*)

[a] * = Differentials used as the female parent in each cross.
[b] R = resistant; S = susceptible.

intermediate (Table 1).

To confirm the linkage relationship between the *Pi-1*(t) and *Pi-k* loci, F_3 progeny tests were conducted with the C101LAC × K60 and Tsuyuake × C103TTP crosses. For these tests, 142 resistant F_2 plants from the former cross and 100 resistant F_2 plants from the latter cross were selected randomly and advanced to the F_3 generation. The F_3 plants were inoculated with isolate IK81-3 for the C101LAC × K60 population and isolate PO6-6 for the Tsuyuake × C103TTP population. Four of 142 F_3 lines in the former and six of 100 F_3 lines in the latter showed segregation within lines (Table 3). These results confirmed that *Pi-1*(t) was closely linked but nonallelic to the *Pi-k* locus. Both the *Pi-1*(t) and *Pi-k* loci were located on chromosome 11 (22,26), and the recombination value was calculated at 6.1 ± 1.7% (standard error [SE]) from the F_3 tests.

NILs in group II. The cross between C101A51 (*Pi-2*(t)) and Fukunishiki (*Pi-z*) did not segregate for blast reaction in the F_2 (Table 2). Because Fukunishiki carried a single resistance gene to isolate IK81-3 (Table 2), it was inferred that *Pi-2*(t) in C101A51 was allelic or closely linked to *Pi-z* in Fukunishiki. Restriction fragment length polymorphism (RFLP) analysis revealed that *Pi-2*(t) in NILs belonging to group II was closely linked to a single-copy DNA marker, RG64, on chromosome 6, with a map distance of 2.8 ± 1.4 centiMorgans SE (28). This DNA marker was closely linked to the *Se-1* locus for photosensitivity as well (15). On the other hand, the *Pi-z* locus also has been mapped on chromosome 6 (3) and is closely linked to the *Se-1* locus with a recombination value of 3.5 ± 0.5% SE (25). These results, showing that the chromosomal location of *Pi-2*(t) was very close to the *Pi-z* locus, were consistent with those obtained in this study. The reaction pattern of *Pi-2*(t) and *Pi-z* was the same (both conditioned resistance to all isolates tested; Table 1), further suggesting that the two genes were the same.

The resistance of Toride 1 (known to carry *Pi-z*^{t}) to isolate IK81-3 was intermediate (Table 1). This reaction appeared to be controlled by a gene with an unknown identity, because the cross of Toride 1 with C101A51 showed a segregation ratio of 15:1 (R:S) to isolate IK81-3 (Table 2). This indicated that *Pi-z*^{t}

TABLE 2. *(continued from preceding page)*

NIL group	Cross[a]	Test isolate	No. of F_2 plants observed for each class[b] R	S	Expected ratio (R:S)	Probability
III	C101TTP-6 ×					
	Kusabue*	IK81-3	218	12	15:1	0.50–0.75
	Tsuyuake*	IK81-3	209	13	15:1	0.75–0.90
	Fukunishiki	IK81-3	218	15	15:1	0.90–0.95
	K 1*	IK81-3	441	0	15:1	<0.01
	Pi No. 4*	IK81-3	214	0	15:1	<0.01
	K 60*	IK81-3	196	8	15:1	0.10–0.25
	C104PKT ×					
	K 1*	IK81-3	133	44	3:1	0.05–0.10
	Pi No. 4*	IK81-3	104	39	3:1	0.25–0.50
	K 1 ×					
	C101PKT	IK81-3	420	0	1:0	
	C101TTP-1	IK81-3	408	0	1:0	
	C101TTP-2	IK81-3	413	0	1:0	
	C101TTP-3	IK81-3	221	0	1:0	
	C101TTP-4*	IK81-3	236	0	1:0	
	C102TTP	IK81-25	192	0	1:0	
	C105TTP-1	43	201	0	1:0	
	C105TTP-2L23*	43	119	0	1:0	
	C105TTP-4L6	43	216	0	1:0	
	Pi No. 4 ×					
	C101PKT	IK81-3	392	0	1:0	
	C101TTP-1	IK81-3	221	0	1:0	
	C101TTP-2	IK81-3	201	0	1:0	
	C101TTP-3	IK81-3	107	0	1:0	
	C101TTP-4	IK81-3	220	0	1:0	
	C102TTP	IK81-25	206	0	1:0	
	C105TTP-1	43	220	0	1:0	
	C105TTP-2L23	43	179	0	1:0	
	C105TTP-4L6	...	...	...	...	
IV	K1 ×					
	C102PKT	IK81-25	409	0	1:0	
	C105TTP-2L9	43	203	0	1:0	
	Pi No. 4 ×					
	C102PKT*	IK81-25	115	0	1:0	
	C105TTP-2L9	43	196	0	1:0	
V	C104PKT ×					
	Shin 2	PO3-82-51	173	22	15:1	<0.01
	Fujisaka 5*	PO3-82-51	155	0	15:1	<0.01
	Kusabue*	PO3-82-51	422	8	15:1	<0.01
					63:1	0.50–0.75
	Tsuyuake*	PO3-82-51	189	14	15:1	0.50–0.75
	Fukunishiki	PO3-82-51	211	12	15:1	0.90–0.95
	Pi No. 4*	PO3-82-51	208	14	15:1	>0.99
	Toride 1*	PO3-82-51	211	14	15:1	0.10–0.25
	K 60	PO3-82-51	201	9	15:1	0.10–0.25
	BL 1*	PO3-82-51	211	9	15:1	0.10–0.25
	Fujisaka 5 ×					
	Aichi asahi	PO3-82-51	124	49	3:1	0.25–0.50

was different from *Pi-2*(t).

NILs in groups III and IV. In a previous study, the NILs in group III showed complete resistance to isolate 86013, whereas the NILs in group IV showed susceptibility to the same isolate (14). When we reexamined the pathogenicity of isolate 86013 to the NILs in groups III and IV, many lesions capable of sporulation were observed on the leaves of the NILs in both groups III and IV (data not shown). Although several NILs in group III appeared to be partially resistant compared with the NILs in group IV, the reactions were not clearly differentiated. Therefore, the NILs in both groups were classified into the same group. The genetic background of those NILs may not be uniform, or the pathogenicity of isolate 86013 may be unstable.

Pi-4^a(t) in C101PKT and C105TTP-1 is located on chromosome 12 (28). The chromosomal location(s) of the genes in the other NILs in groups III and IV is unknown. C101TTP-6 in group III was crossed with the differentials showing resistance to isolate IK81-3. When the F_2 populations derived from crosses of C101TTP-6 with K1 (*Pi-ta*) or Pi No. 4 (*Pi-ta*^{2}) were inoculated with isolate IK81-3, no susceptible plants were observed (Table 2). Because the resistance of K1 and Pi No. 4 to isolate IK81-3 was controlled by a single resistance gene (Table 2), the resistance gene in C101TTP-6 was allelic or closely linked to the genes at the *Pi-ta* locus. The same results were obtained in the rest of the NILs in groups III and IV (Table 2).

Initially, the *Pi-ta* locus was located on chromosome 9 by Shinoda et al (20). However, *Pi-4*^{a}(t) in C105TTP-1 has been tagged by RFLP marker RG869 on chromosome 12 (28). According to recent reports (7,17), the *Pi-ta* locus is located on chromosome 12. In addition, Kiyosawa et al (13) reported that Pai-kan-tao (the donor parent of C101PKT) carried *Pi-ta*, and the reaction pattern of the NILs in groups III and IV to our test isolates was the same as that of K1 carrying *Pi-ta* (Table 1). Thus, we concluded that *Pi-4*^{a}(t) was closely linked or allelic and possibly identical to *Pi-ta*. All the NILs in groups III and IV carried *Pi-4*^{a}(t) (*Pi-ta*).

NIL in group V. The F_2 population derived from the cross between C104PKT and Fujisaka 5 (*Pi-i, Pi-k*^{s}) did not segregate for blast reaction (Table 2). Because Fujisaka 5 carried a single resistance gene to isolate PO3-82-51 (Table 2), this suggested that *Pi-3*(t) in C104PKT was allelic or closely linked to a resistance gene in Fujisaka 5. However, the size of the F_2 population was not large enough to determine the linkage relationship between resistance genes in C104PKT and Fujisaka 5. One hundred twenty-five F_2 plants of 155 were selected randomly and advanced to the F_3 generation and inoculated with isolate PO3-82-51. Because five of 125 lines showed segregation within lines (Table 3), we concluded that *Pi-3*(t) was nonallelic but was linked to a resistance gene in Fujisaka 5. The recombination value between *Pi-3*(t) and a resistance gene in Fujisaka 5 was calculated at 7.0 ± 2.6% SE from the F_3 test.

Fujisaka 5 carries both *Pi-i* and *Pi-k*^{s} (10). Although Shin 2, carrying *Pi-k*^{s}, showed resistance to isolate PO3-82-51 (Table 1), the result of F_2 analysis of a cross between C104PKT and Shin 2 indicated that the resistance of Shin 2 to the isolate was due to a gene that was independent or not closely linked to *Pi-3*(t) (Table 2). This meant that the resistance of Fujisaka 5 and Shin 2 to isolate PO3-82-51 was due to different resistance genes. Therefore, the resistance of Fujisaka 5 to isolate PO3-82-51 was probably due to *Pi-i*. It is possible that the Fujisaka 5 selection used in this study did not carry *Pi-k*^{s} (12). Nevertheless, the resistance of Kusabue to isolate PO3-82-51 might be controlled by two genes, one of which has an unknown identity (Table 2).

NIL group VI. The resistance gene in C105TTP-4L23 derived from Tetep has been identified as *Pi-4*^{b}(t), allelic to *Pi-4*^{a}(t) (14). Because *Pi-4*^{a}(t) also was derived from Tetep, it was suggested that Tetep might be heterogeneous at the *Pi-4* locus. On the other hand, it also was possible that C105TTP-4L23 carried an additional resistance gene, conditioning resistance to V86010. To test this hypothesis, a cross between CO39 and C105TTP-4L23 was made. The resulting 37 F_2 plants were inoculated with isolates 43 (incompatible with NILs in groups III and VI) and V86010 (compatible with the group III NILs but incompatible with group VI NIL). A modified needle-pricking method was used to allow inoculation of single plants with the two isolates. If C105TTP-4L23 carried only *Pi-4*^{b}(t), all the F_2 plants should have had the same phenotype as either C105TTP-4L23 or CO39. Nineteen and five of 37 F_2 plants showed the same phenotypes to C105TTP-4L23 and CO39, respectively. However, 13 of 37 F_2 plants showed nonparental phenotypes. Ten plants were resistant to isolate 43 but susceptible to isolate V86010, whereas three plants showed the opposite reaction to isolates 43 and V86010. These results indicated that the resistance of C105TTP-4L23 to the two isolates was controlled by two different resistance genes. Because the resistance gene to isolate 43 in C105TTP-4L23 was susceptible to isolate V86010, that gene was judged to be *Pi-4*^{a}(t). Thus, it appears that there is no *Pi-4*^{b}(t), but that C105TTP-4L23 carries *Pi-4*^{a}(t) (=*Pi-ta*) and an additional gene conditioning resistance to isolate V86010. A NIL with the additional resistance gene in C105TTP-4L23 is currently being developed.

DISCUSSION

The results of this study suggested that the donor parents of the NILs had resistance genes in common with one another and in common with other resistant cultivars. *Pi-1*(t) was present in both Tetep (a Vietnamese *indica* cultivar) and LAC23 (an African *japonica* cultivar). *Pi-4*^{a}(t), carried by both Tetep and Pai-kan-tao (a Chinese *japonica* cultivar), was the same as *Pi-ta* derived from Tadukan (a Philippine *indica* cultivar) (8). *Pi-2*(t) in 5173 (a Colombian *indica* cultivar) might be the same as *Pi-z* derived from Zenith (an American *indica* cultivar) (9). Although genetic variation for blast resistance in the tropical cultivars is considered to be large, some resistance genes appear to be widely distributed in the tropics.

Two of the five resistance genes in the CO39 NILs were closely linked but nonallelic with genes present in Kiyosawa's Differentials. Genes for resistance to other plant pathogens have often been found tightly clustered (2,4,18,19,21,24). Detailed genetic and molecular analysis of the rice chromosomal segments carrying multiple blast resistance will be of great interest for future studies.

We were able to estimate the minimum number of resistance genes in the donor parents of the NILs. Tetep carried at least three resistance genes: *Pi-1*(t), *Pi-4*^{a}(t), and an additional gene in C105TTP-4L23. Kiyosawa (11) previously reported that Tetep carried *Pi-k*^{h} that might be allelic to *Pi-k*. It is necessary to confirm the allelism relationships between *Pi-k*^{h} and the resistance gene(s) in Tetep for which chromosomal locations are undetermined. Pai-

TABLE 3. Reaction of F_3 lines of rice cultivar crosses of C101LAC × K 60, Tsuyuake × C103TTP, and Fujisaka 5 × C104PKT to *Pyricularia grisea* isolates

NIL group[a]	Cross	Test isolate	No. of F_3 lines observed[b] R	H	Expected ratio (R:H)	Probability
I	C101LAC × K 60	IK81-3	138	4	7:8	<0.01
	Tsuyuake × C103TTP	PO6-6	94	6	7:8	<0.01
V	Fujisaka 5 × C104PKT	PO3-82-51	120	5	7:8	<0.01

[a] Near-isogenic line.
[b] R = resistant; H = segregating.

TABLE 4. Complete set of rice cultivar CO39 near-isogenic lines (NILs) in minimum

NIL group	NIL	Donor	Resistance gene
I	C101LAC	LAC23	*Pi-1*(t)
II	C101A51	5173	*Pi-2*(t)
III	C101PKT	Pai-kan-tao	*Pi-4*^{a}(t)
V	C104PKT	Pai-kan-tao	*Pi-3*(t)
VI	C105TTP-4L23	Tetep	*Pi-4*^{a}(t), *Pi-?*

kan-tao carried at least two resistance genes, *Pi-3*(t) and *Pi-4*^{a}(t). LAC23 carried at least one resistance gene, *Pi-1*(t). Cultivar 5173 also carried at least one resistance gene, *Pi-2*(t). These numbers are likely to be underestimated. Individual donor parents showed resistance to isolates that were compatible to all the NILs (data not shown), indicating that the donor parents are likely to carry more resistance genes than those "captured" in this set of NILs.

Based on the results of this study, we concluded that the 22 CO39 NILs carried five blast resistance genes, only four of which were carried separately in the lines and were subjected to systematic analysis. At present, a set of NILs consisting of C101LAC (*Pi-1*(t)), C101A51 (*Pi-2*(t)), C104PKT (*Pi-3*(t)), C101PKT (*Pi-4*^{a}(t)), and C105TTP-4L23 (*Pi-4*^{a}(t)+*Pi-?*) are available for use as differentials (Table 4).

On the other hand, it was suggested that Kiyosawa's Differentials was not a suitable differential set for the tropics, because some of the differentials showed intermediate reactions to Philippine isolates (6) and some carried more than one resistance gene effective against the Philippine isolates. At least the intermediate reaction of Shin 2 and Toride 1 to isolate IK81-3 appeared to be controlled by a gene with an unknown identity. Imbe and Matsumoto (5) have already reported that Shin 2, Kusabue, Fukunishiki, Toride 1, and BL1 carried *Pi-sh*, which was identified with local races on the island of Kyushu in Japan. The reaction of *Pi-sh* to isolates fluctuated with varying temperature after infection. Assuming that *Pi-sh* was effective against Philippine isolates, it may be that the intermediate reaction of the cultivars to Philippine isolates is due to *Pi-sh*.

Based on this study, it appears that the production of NILs by backcrossing and selection was inefficient. A larger number of resistance genes could probably have been captured if more diverse isolates were used for selecting lines. Based on DNA fingerprinting, the isolates used for selection of the NILs represented only two genetic lineages (D. Chen, IRRI, *personal communication*). DNA fingerprinting data could allow genetically diverse pathogen isolates to be selected. The extraction and purification of NILs from populations of fixed recombinants (doubled haploid lines or recombinant inbred lines) used for gene-mapping studies also could increase the efficiency with which resistance genes are transferred to NILs (T. Inukai, *unpublished data*). This CO39 NIL set is currently being extended using these approaches. Recently, Wang et al (23) identified two additional resistance genes in the durably resistant, west African cultivar Moroberekan. We are now developing additional NILs with the Moroberekan resistance genes, using marker-aided selection of lines from the mapping population developed and analyzed by Wang et al (23).

The set of NILs will not only be a powerful tool for analyzing resistance genes and pathogen isolates, but will also serve as donors for blast-resistance breeding. The genes in the NILs have been tagged with molecular markers and could be transferred from the NILs by marker-aided selection ([28]; N. Huang, IRRI, *personal communication*; T. Inukai, *unpublished data*).

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