Inheritance of Blast Resistance in Near-Isogenic Lines of Rice

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ABSTRACT

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Resistance to blast disease is an important objective of most rice breeding programs. Genetic studies of resistance have been complicated by variability of the pathogen and lack of rice genotypes with single resistance genes. Near-isogenic lines (NILs) with single blast resistance genes were developed by backcrossing four donor cultivars to the recurrent parent CO39. Five pathogen isolates were used to screen the populations during backcrossing. The 22 NILs were classified into six groups by their reaction to a diverse set of blast isolates. Blast resistance was conferred by independent dominant genes in the NILs C101LAC, C101A51, and C104PKT, designated *Pi-1(t), Pi-2(t),* and *Pi-3(t),* respectively. Blast resistance in C101PKT and C105TTP-4 was conferred by dominant alleles at an additional locus, designated *Pi-4^a(t)* and *Pi-4^b(t),* respectively.

Blast is one of the most destructive diseases of rice (*Oryza sativa* L.), causing crop loss in both temperate and tropical rice-growing regions. In the tropics, blast is particularly severe in upland and rainfed lowland environments that are prone to drought. However, susceptible cultivars can be severely damaged by blast even under irrigated conditions (1). Genetic variability exists for both complete and partial resistance in rice, the former being conditioned by a few genes of major effect (7,8).

Genetic studies on blast resistance have been conducted in Japan, where 13 dominant resistance genes at eight loci were identified, and cultivars or breeding lines with single resistance genes were developed (7). In the tropics, genetic studies have been hindered by a lack of continuity in the studies conducted, driven by the extremely changeable virulence of the pathogen *Pyricularia grisea* Sacc. (= *P. oryzae* Cav.) (9) and by the presence of many resistance genes in rice cultivars (8,10).

A program to develop near-isogenic lines (NILs) of rice with single resistance genes was begun at the International Rice Research Institute (IRRI) in the Philippines. Such a set of NILs would offer several advantages to researchers studying rice blast in the tropics. Allelism tests would be much easier in NILs than in donor cultivars where resistance is often conferred by two or more major genes, thus allowing more accurate determination of the number and distribution of the resistance genes. Additionally, NILs could be used as an improved differential set for describing pathogen isolates. A set of NILs in an indica background would be more appropriate for experimental use in tropical countries than the japonica differential set being used in Japan. This is because indica NILs would be easier to cross with local indica cultivars for gene identification, and the temperate japonica differentials do not grow well in the tropics. Some japonica differentials have more than one resistance gene against Philippine isolates (J. M. Bonman, *unpublished*). We report here results of initial studies on the set of NILs developed.

MATERIALS AND METHODS

A set of near-isogenic lines was developed by backcrossing using the indica rice cultivar CO39 as the recurrent parent. This cultivar is highly susceptible to almost all tropical blast isolates and has a short growth duration (about 95 days to maturity). It also has little partial resistance to matching pathogen races, showing many large lesions in standard inoculation tests conducted in the greenhouse.

Four resistance donors were selected: the indica rice cultivars 5173 and Tetep, the upland cultivar LAC23, and the temperate japonica cultivar Pai-Kan-Tao. According to the subspecific classification scheme for *O. sativa* proposed by Glaszmann (4), LAC23 and Pai-Kan-Tao are both in isozyme group VI and are therefore more closely related to one another than to indica rices. CO39 was used as the female parent in all crosses so that plants resulting from accidental selfing could be identified by a susceptible F_1 reaction and then removed. Five isolates of the pathogen (designated 101 for IK81-3, 102 for IK81-25, 103 for PO6-6, 104 for PO3-82-51, and 105 for 43) were used. Isolates 102, 103, and 105 were used in a previous study (11). All donor parents were resistant to the five isolates used with the exception that Pai-Kan-Tao was susceptible to 103. Backcross F_1 plants were inoculated with the same isolate of *P. grisea* in each generation. As the total number of resistance genes in the donors was not known, all isolates were used within each cross. The resulting populations were designated C (for CO39), followed by the isolate number, and an abbreviation for the resistant donor (A51 for 5173, TTP for Tetep, LAC for LAC23, and PKT for Pai-Kan-Tao). Thus, C101LAC is the NIL developed from inoculating the LAC23 cross with the isolate 101.

Previous information (8) indicated that Tetep had two dominant genes for resistance to isolates 101 and 105. For these combinations, several lines were backcrossed separately to try to isolate both genes. These lines were designated with a number (e.g., C101TTP-1, C101TTP-2, etc.). At an early stage of backcrossing, several of the donor-isolate combinations (LAC 23 with 102, 103, and 105; Pai-Kan-Tao with 105; Tetep with 104) could not be maintained because of the difficulty in completing all the backcrosses. They were dropped from the backcrossing program.

The F_1 plants of the last backcross (BC_6F_1) were inoculated, and the number of resistant and susceptible plants was counted to determine if a single gene was responsible for resistance. BC_6F_2 plants harvested from resistant BC_6F_1 plants were inoculated, and resistant plants were selfed. Resistant BC_6F_3 lines showing no segregation for blast resistance were selfed and bulked to form the NILs. These lines were morphologically similar to the recurrent parent CO39.

The NILs were tested against 73 Philippine isolates of *P. grisea* from the collection of the Plant Pathology Division at IRRI. The lines were classified into groups based on the reaction pattern to these isolates. NILs of the same group were resistant to the same isolates. One member of each group was selected to make crosses for allelism tests. The F_1 plants of the crosses between NILs were inoculated with isolates virulent on the female parent and avirulent on the male parent. Only resistant plants were selfed to obtain F_2 seed. The resulting F_2 populations were inoculated with isolates avirulent on both NILs, and the goodness of fit to the expected ratio for two independent, duplicate-dominant genes (15:1) was measured with the chi-square test.

Inoculation methods were as described previously (3). Briefly, seedlings were grown in the greenhouse in plastic trays, about 100 seedlings per tray, and inoculated at the fifth- to sixth-leaf stage by spraying 50 ml of an aqueous spore suspension of 5×10^4 conidia per milliliter to each tray of seedlings. Inoculated seedlings were placed in a dew chamber for 24 h at 26 C, and transferred to an air-conditioned greenhouse room at 24–28 C. Disease reactions were scored 7 days after inoculation on a scale from 0 to 5 in which 0 = no evidence of infection; 1 = brown specks smaller than 0.5 mm in diameter; 2 = brown specks about 0.5–1 mm in diameter; 3 = roundish to elliptical lesions about 1–3 mm in diameter with gray centers and brown margins; 4 = typical spindle-shaped blast lesion, 3 mm or longer with little or no coalescence of lesions; 5 = same as 4 but half of one or more leaves killed by coalescence of lesions. Plants rated 0–3 were considered resistant, and those rated 4–5 were considered susceptible.

TABLE 1. Segregation for blast resistance of BC_6F_1 and BC_6F_2 plants from seed harvested from resistant BC_6F_1 plants[a]

Near-isogenic line	BC_6F_1 plants (no.)			BC_6F_2 plants (no.)		
	Resistant	Susceptible	χ^2 (1:1)	Resistant	Susceptible	χ^2 (3:1)
C101A51	18	38	3.571	71	29	0.540
C102A51	34	47	1.043	82	30	0.101
C103A51	64	74	0.362	240	88	0.392
C104A51	11	11	0.000	101	36	0.060
C105A51	28	62	6.422*[b]	104	35	0.002
C101LAC	34	122	24.821**	169	44	1.494
C104LAC	42	55	0.871	231	80	0.048
C101PKT	74	99	1.806	192	69	0.180
C102PKT	29	59	5.114*	100	29	0.266
C104PKT	24	59	7.380**	112	120	65.566**
C101TTP-1	15	31	2.783	91	24	0.680
C101TTP-2	19	27	0.696	63	22	0.11
C101TTP-3	9	11	0.100	51	13	0.448
C101TTP-4	12	8	0.400	71	18	0.692
C101TTP-6	12	11	0.022	62	24	0.221
C102TTP	3	4	0.071	63	12	2.199
C103TTP	33	23	0.893	64	54	19.799**
C105TTP-1	7	6	0.083	78	6	10.246**
C1-5TTP-2	7	20	3.130	57	20	0.012
C105TTP-4	8	10	0.111	59	21	0.025

[a]The same isolate used in selection during backcrossing was used for inoculation.

[b]* and ** = Significantly different from the expected ratio at the 5 and 1% level of probability, respectively.

RESULTS AND DISCUSSION

Most lines showed a good fit to the expected ratio for a single resistance gene in the BC_6F_1 and BC_6F_2 generations (Table 1). Three of the BC_6F_1 populations that did not show a good fit were later confirmed by the BC_6F_2 populations that were harvested from the resistant BC_6F_1 plants. Two populations (C103TTP and

TABLE 2. Reaction of near-isogenic lines to differential isolates of *Pyricularia grisea*

Group	Near-isogenic line	Reaction to isolate number[a]				
		1	2	3	8	9
I	C101LAC	0	4	0	0	0
	C103TTP	0	4	0	3	3
	C104LAC	0	4	0	0	3
II	C101A51	0	3	0	3	3
	C102A51	3	3	0	0	0
	C103A51	0	0	0	0	0
	C104A51	0	0	0	3	0
	C105A51	3	3	0	0	0
III	C101PKT	0	0	4	0	4
	C101TTP-1	0	0	4	0	4
	C101TTP-2	0	0	4	0	4
	C101TTP-3	0	0	4	0	4
	C101TTP-4	0	0	4	0	4
	C101TTP-6	0	0	4	0	4
	C102TTP	0	0	4	0	4
	C105TTP-1	0	0	4	0	4
	C105TTP-2L23	0	0	4	0	4
	C105TTP-4L6	0	0	4	0	4
IV	C102PKT	0	0	4	4	4
	C105TTP-2L9	0	0	4	4	4
V	C104PKT	4	0	0	3	4
VI	C105TTP-4L23	0	0	4	0	0
Recurrent Parent CO39		5	5	5	4	5

[a]Isolates are: 1 = 101, 2 = 102, 3 = 103, 8 = 86013, 9 = V86010. Disease reaction scores are: 0 = no evidence of infection; 1 = brown specks smaller than 0.5 mm in diameter; 2 = brown specks about 0.5–1 mm in diameter; 3 = roundish to elliptical lesions about 1–3 mm in diameter with gray center and brown margins; 4 = typical spindle-shaped blast lesion, 3 mm or longer with little or no coalescence of lesions; 5 = same as 4 but half of one or more leaves killed by coalescence of lesions.

TABLE 3. Segregation patterns and chi-square analysis for allelism studies of blast resistance genes in near-isogenic lines

Cross	Groups represented	No. of plants with score[a]						χ^2 (15:1)
		0	1	2	3	4	5	
C101LAC/C101A51	I/II	136	8	3	19	7	7	0.480
C101LAC/C101PKT	I/III	169	3		6		11	0.009
C101LAC/C104PKT	I/V	155	15		7	5	13	2.467
C101LAC/C105TTP-4L23	I/VI	155		2	4	6		1.792
C101PKT/C101A51	II/III	158	8	1	7	9	3	0.001
C104PKT/C101A51	II/V	88	60	12	15	7	9	1.134
C105TTP-4L23/C101A51	II/VI	274	5	2	11	40	10	39.474**[b]
C104PKT/C101PKT	III/V	146	28	2	9	4	8	0.003
C101PKT/C105TTP-4L23	III/VI	382			8			
C104PKT/C105TTP-4L23	V/VI	122	47		7	6	11	1.744

[a]Scores of 0–3 were considered resistant and 4–5, susceptible.
[b]** = Significantly different from the expected ratio at the 1% level of probability.

TABLE 4. Resistance genes identified in blast near-isogenic lines

Designation	Group	Representative NIL	Source	Reaction pattern to isolates[a]				
				1	2	3	8	9
Pi-1(t)	I	C101LAC	LAC23	R	S	R	R	R
Pi-2(t)	II	C101A51	5173	R	R	R	R	R
Pi-3(t)	V	C104PKT	Pai-kan-tao	S	S	R	R	S
Pi-4^a(t)	III	C101PKT	Pai-kan-tao	R	R	S	R	S
Pi-4^b(t)	VI	C105TTP-4	Tetep	R	R	S	R	R

[a]R = resistant, S = susceptible.

C105TTP-1) showed a good fit to a 1:1 ratio (resistant:susceptible) in the BC_6F_1 but did not show a good fit to a 3:1 ratio in the BC_6F_2. Only C104PKT did not fit the expected ratios in both generations. However, C104PKT was used in subsequent allelism tests, because it was the only representative of its reaction group (Table 2), and its progeny showed the ratios expected if a single dominant resistance gene were present.

All the BC_6F_4 homozygous isogenic lines were screened against a large number of isolates in the collection at IRRI. Six groups of NILs were identified based on reaction pattern, and the differential reactions of the NIL groups to five isolates are given in Table 2. Two isolates tested in cooperation with J. L. Notteghem in France were able to differentiate C101LAC from C104LAC and C103TTP in group I (unpublished data). Allelism tests with NIL C104LAC are now under way. Inoculation tests are being continued in an effort to identify isolates that can further differentiate NILs within the groups. In two cases (C105TTP-2 and C105TTP-4), F_4 lines within the same NIL showed a different reaction pattern. These NILs were subsequently identified with a line number (e.g., C105TTP-2L23).

Initially, one member of each group was selected for crossing to determine if the genes were independently inherited. Results with the line C102PKT are not reported here because some segregation for resistance in this line was encountered. Most of the crosses showed the expected ratio (15:1) for two independently inherited, duplicate dominant resistant genes (Table 3). With the exception of C104PKT × C101A51, the proportion of plants with intermediate reactions (scores 1–3) was relatively low. The cross C101PKT × C105TTP-4L23 gave no susceptible recombinants, indicating that the genes are either allelic or tightly linked. The number of susceptible recombinants in C105TTP-4L23 × C101A51 was higher than that expected for a 15:1 ratio. This cannot be explained by linkage because the F_1 would be in repulsion phase, and few susceptible plants would be expected.

Four independent loci were identified for blast resistance in these NILs. Previously, resistance genes have been designated as *Pi-*, followed by a letter that usually referred to the resistant donor. As these were identified in Japan, with Japanese blast isolates, we cannot presently determine the relationship between the genes in these NILs and those in the Japanese differentials. In conformity with rules for gene designation in rice, we have designated these genes as *Pi-1* through *Pi-4*, with the allelic genes distinguished with a superscript letter (Table 4). The tentative nature of this designation is indicated by the letter *(t)*.

Studies on the genetics of resistance to blast in the tropics have been consistently plagued by the difficulties in working with *P. grisea*. The description by early workers of the seemingly unlimited variability of the pathogen (9) suggested that traditional genetic analysis would have limited usefulness. More recently, with improved experimental techniques (2), simple inheritance of resistance has been confirmed (8,10). Aberrant ratios are observed in some crosses; however, expected ratios for individual dominant genes can be observed with follow-up studies on the same crosses. If some BC_5F_1 plants used in backcrossing were actually escapes and not truly resistant, then an excess of susceptible plants would be observed in the BC_6F_1 plants (Table 1).

Another possibility is that some NILs contained a few susceptible plants due to residual segregation or outcrossing. Outcrossing should have been minimized by the placing of bags over all panicles. Instability of the fungus in culture may also occasionally influence results. For the most part, however, the integrity of the race reaction patterns of the near-isogenic lines has been maintained.

Intermediate reactions may complicate the classification of resistance in *P. grisea*. In our work, type 3 and type 4 lesions are clearly distinguished and appear to represent the boundary between resistance and susceptibility. Breeding lines with type 3 lesions do not develop much disease and do not exhibit type 4 reactions in subsequent tests. Blast resistance, however, is known to be influenced by environmental conditions. For example, Kiyosawa (5,6) observed differences in segregation ratios under different inoculation conditions. Some genes conferred high resistance under spray inoculation but not with injection.

Crosses with C101A51, which showed a category 3 reaction to several isolates, seemed to give more intermediate progeny in crosses and was a parent in the only nonallelic cross that did not fit the expected 15:1 F_2 ratio (Table 3). Although not considered important when the parents were chosen, it may have been preferable to choose a group II NIL with more category 0 reactions, such as C103A51. It is probable that the intermediate reaction is conditioned by a gene or genes in C101A51 independent of the resistance gene *Pi-2(t)*.

Because previous genetic studies indicated that Tetep had two resistance genes for isolates 101 and 105 (8), several lines from these crosses were maintained throughout the backcrossing. All of the C101TTP lines fell into group III (Table 2), suggesting that they probably all have the same gene. The lines from C105TTP, however, would not be expected to fall into three reaction groups (III, IV, and VI). Furthermore, the genetic studies indicate that the gene in C105TTP-4L23 (*Pi-4^b*) is allelic to that in C101PKT (*Pi-4^a*), which shows the same reaction pattern as other TTP lines. There are two possible explanations. The gene in C101PKT may indeed not be allelic to the TTP lines in group

III, even though they show the same race reaction pattern. Alternatively, the original seed source used for Tetep may have been polymorphic at the *Pi-4* locus, as was observed also for the two *Pi-ta* alleles from the cultivar Tadukan (7).

The existence of NILs with individual blast resistance genes provides a powerful tool for future studies on the rice blast disease. The virulence patterns of tropical blast races will be easier to study with lines possessing known resistance genes (2). Moreover, it should be easier to identify additional resistance genes. Additional near-isogenic lines are now being tested, and the genes from parents with novel reaction patterns are being backcrossed into the CO39 background. NILs have been shown to be very effective in mapping resistance genes with molecular markers (10). These blast resistant NILs are currently being used to tag the resistance genes using restriction fragment length polymorphism markers (12). This information may be useful in practical breeding programs as well as in the eventual cloning of the resistance genes.

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