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T. Inukai · R. S. Zeigler · S. Sarkarung · M. Bronson
L. V. Dung · T. Kinoshita · R. J. Nelson

Development of pre-isogenic lines for rice blast-resistance by marker-aided selection from a recombinant inbred population

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Abstract To increase the available set of near-isogenic lines (NILs) for blast-resistance in rice, we have developed a general method for establishing NILs from populations of fixed recombinants that have been used for gene mapping. We demonstrated the application of this method by the selection of lines carrying genes from the rice cultivar Moroberekan. Moroberekan is a West African *japonica* cultivar that is considered to have durable resistance to rice blast. Multiple genes from Moroberekan conferring complete and partial resistance to blast have previously been mapped using a recombinant inbred (RI) population derived from a cross between Moroberekan and the highly and broadly susceptible *indica* cultivar CO39. To analyze individual blast-resistance genes, it is desirable to transfer them individually into a susceptible genetic background. This RI population, and the associated data sets on blast reaction and restriction fragment length polymorphism (RFLP) genotypes, were used for selection of lines likely to carry individual blast-resistance genes and a minimum number of chromosomal segments from Moroberekan. Because skewed segregation in the RI population favored CO39 (*indica*) alleles, resistant lines carrying 8.7–17.5% of Moroberekan alleles (the proportion expected after two or three backcrosses) could be selected. We chose three RI lines carrying different complete resistance genes to blast and two RI lines carrying partial resistance genes to blast as potential parents for the development of NILs. These lines were subjected to genetic analysis, which allowed clarification of some issues that could not be resolved during the initial gene-mapping study.

Key words Disease resistance · Rice blast · RFLPs · Recombinant inbred lines · Pre-isogenic lines

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T. Inukai (✉) · R. S. Zeigler · S. Sarkarung · M. Bronson
L. V. Dung · T. Kinoshita · R. J. Nelson
Faculty of Agriculture, Hokkaido University, Sapporo 060, Japan

Introduction

Near-isogenic lines (NILs) carrying single genes for blast-resistance are useful for genetic analysis (Kiyosawa 1967; Yokoo and Kiyosawa 1970; Kiyosawa 1972; Mackill and Bonman 1992; Inukai et al. 1994a), for gene tagging (Yu et al. 1991; Miyamoto et al. 1993; Satoh et al. 1993), and for characterizing pathogen isolates (Inukai et al. 1994b; Zeigler et al. 1995). However, two problems have been encountered using the conventional approach to the development of NILs. First, while backcrossing and selection is effective for the transfer of single genes, it may not be efficient when multiple genes are present in the donor parent. If multiple genes for blast-resistance are not differentiated by the isolates used for selection, some of the loci may be "lost". In developing a set of NILs, for instance, Mackill and Bonman (1992) used four blast-resistance donors known, or suspected, to carry multiple genes for blast-resistance. To "capture" as many of the blast-resistance genes as possible in the NILs, five pathogen isolates were used to screen lines at each of several generations of backcrossing to the susceptible cultivar CO39 and at each of the subsequent generations of selfing. In spite of this, allelism tests revealed that only five blast-resistance loci were identified among the 22 NILs developed from the four blast-resistance donors (Inukai et al. 1994a). The donor cultivars are resistant to some isolates to which the NILs are susceptible, suggesting that not all of the blast-resistance genes in the donor cultivars were transferred to the NILs (T. Inukai, unpublished).

A second limitation of the conventional approach to NIL production is the difficulty of evaluating the extent of return to the recurrent parental genotype. Although the amount of genetic material from the donor is expected to be reduced by half with each generation of backcrossing to the recurrent parent, the observed level of donor DNA may be substantially higher than expected in some cases (Young and Tanksley 1989). For instance, based on RFLP data, the NILs for blast-resistance carried surprisingly high numbers of loci from the donor parents (Yu 1991). Ana-

lyzed with 41–92 probes, the lines were found to carry 10.9–27.5% of donor loci (Yu 1991).

We present here an approach for developing NILs from populations of fixed segregants that have been used for molecular mapping of blast-resistance genes. All the blast-resistance genes present in a donor cultivar can be detected because every chromosome segment of the donor parent is present in at least one member of a permanent population of fixed segregants (either recombinant inbred lines or doubled-haploid lines), and the population can be analyzed with multiple pathogen isolates. Molecular marker data obtained during the gene-mapping process can be used to select lines carrying blast-resistance genes and few other loci from the donor parent. These two conditions make it possible to select lines carrying single blast-resistance genes and few other loci from the donor parent from a population of fixed segregants. We term such lines "pre-isogenic lines (PILs)" in the sense that they can be used as intermediates in the production of near-isogenic lines (NILs).

Moroberekan is an African upland *japonica* cultivar considered to have durable blast-resistance. To better understand the genetic basis of this exceptionally useful resistance, Wang et al. (1994) used RFLP markers to map major and minor genes in a cross between Moroberekan and the susceptible *indica* cultivar CO39. They developed a population of 281 recombinant inbred lines (RILs), and obtained RFLP data for each of the RILs at 127 loci selected to provide uniform genome coverage. Wang et al. (1994) identified and mapped two major genes for blast-resistance, *Pi-5*(t) and *Pi-7*(t), and ten quantitative trait loci (QTLs) conditioning partial resistance to the blast pathogen. They also found evidence for an additional blast-resistance gene in Moroberekan; some of the RILs carrying neither *Pi-5*(t) nor *Pi-7*(t) (susceptible to isolate PO6-6) showed resistance to Philippine isolate Ca65 (G. Wang, IRRI, unpublished). In the present study, we selected and further characterized lines from the Moroberekan/CO39 population, as a step towards the development of NILs carrying blast-resistance genes from Moroberekan.

Materials and methods

Plant materials and pathogen isolates

Moroberekan is an African upland *japonica* cultivar with durable blast-resistance. CO39 is an *indica* cultivar that is highly susceptible to most isolates of the rice blast pathogen, *Pyricularia grisea*. Recombinant inbred lines (RILs), previously developed and designated RIL10, RIL23, RIL29, RIL125, RIL249 and RIL276, were selected from a population of 281 F_7 RILs of a cross between Moroberekan and CO39 (Wang et al. 1994), based on available markers and phenotypic data. These were considered as putative "pre-isogenic lines (PILs)" for further genetic analysis. The CO39 near-isogenic lines (NILs) C101LAC (carrying *Pi-1*), C101A51 (carrying *Pi-z*^{5}), C104PKT (carrying *Pi-3*), C101PKT (carrying *Pi-ta*) and C105TTP-4L23 (carrying *Pi-ta* and an additional, unidentified gene) (Mackill and Bonman 1992; Inukai et al. 1994a; Kinoshita et al. 1994) were used as tester lines for genetic analysis. Isolates of the rice blast pathogen *P. grisea* were obtained from the collection maintained at the Entomology and Plant Pathology Division at the International Rice Research Institute (IRRI) in the Philippines. Isolates PO6-6, IK81-3, IK81-25, V86010 and Ca65 were used in this study. Except for Ca65, these isolates were previously used to differentiate CO39 NILs (Mackill and Bonman 1992). Isolate Ca65 was recently collected from the IRRI upland screening site at Cavinti, Laguna, The Philippines.

Data sets

The following data sets were available for a population of 281 F_7 RILs derived from a cross between Moroberekan and CO39 (Wang et al. 1994), and were used for the present study: (1) genotypes of each RIL for 127 RFLP loci distributed on the map at intervals of about 20 cM; (2) disease reaction score (0–5) of each RIL to blast isolates PO6-6 and Ca65 (data obtained using monocyclic inoculation tests); (3) diseased leaf area (DLA), susceptible lesion number and lesion size of each RIL (data obtained using polycyclic inoculation tests using blast isolate PO6-6); (4) DLA of each RIL under natural infection at the IRRI upland screening site at Cavinti, Laguna, The Philippines (data obtained from field trials conducted in 1992 and 1994, each with two replications).

Inoculation methods

For pathogenicity tests, seeds of PILs and CO39 NILs were sown in plastic trays (11×23×11 cm). Ten seeds were planted in each of eight rows in each tray with three replications. For F_2 analysis, plastic trays (37×26×11 cm) were 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 two or three more rows, and parental lines and a susceptible check cultivar were sown in each row, with ten seeds per row. For F_3 analysis, plastic trays were divided equally into ten rows, and F_3 line seeds were sown in each of nine rows, with 20 seeds per F_3 line per row. One row in each tray was divided into two more rows, and parental lines were sown in each row, with ten seeds per row. In all experiments, nitrogen fertilizer was applied at 36 g/m^2 as ammonium sulphate, and seedlings were grown in a green house. Seedlings were inoculated 21 days after sowing (at the fifth-to-sixth leaf stage) by the spraying method previously described (Inukai et al. 1994a). The disease reactions were scored about 7 days after inoculation.

Selection of PILs

To allow selection of lines carrying genes derived from Moroberekan that condition complete resistance to isolate PO6-6 or isolate Ca65, RILs showing complete resistance to isolate PO6-6 or isolate Ca65 (those lines with disease scores of 0–2 to one or both of these isolates) were identified. Among each group with a particular reaction pattern to the two isolates, those lines carrying the fewest RFLP alleles from Moroberekan were selected. The reaction patterns of the selected lines were tested with the five isolates described above, and the lines showing different reaction patterns were selected as PILs and used for the following genetic analysis.

To allow selection of PILs carrying Moroberekan genes conditioning partial resistance to blast, RILs not carrying any of the resistance genes to isolates PO6-6 and Ca65, but showing partial resistance to blast in polycyclic tests and field experiments, were identified. From among the lines with less than half the number of susceptible lesions relative to CO39 in polycyclic tests and showing low levels of disease in field experiments, those carrying the fewest Moroberekan alleles for the 127 RFLP loci were selected as PILs. RFLP marker data for loci linked to putative QTLs conditioning partial resistance to blast were used to estimate which QTLs were present in the selected lines.

Genetic analysis

CO39 was used as a susceptible parent or as a susceptible check cultivar for the F_2 and F_3 analyses. F_2 and F_3 segregation ratios were an-

alyzed by the chi-square test, and a recombination value was calculated by the maximum-likelihood method. Distances between markers are presented in centimorgans derived using the Kosambi function.

RFLP analysis

DNA was extracted from the leaves of CO39, RIL29, RIL249 and the F_2 plants using the procedure described by Dellaporta et al. (1984). DNA extracts were digested with the restriction enzymes *Dra*I, *Eco*RI, *Eco*RV, *Hin*dIII and *Sca*I. The digested DNAs were subjected to electrophoresis on 1% agarose gels and transferred to BIODYNE B membranes (Pall Corp.) according to the manufacturer's instructions. The DNA clones that were reported to be linked to *Pi-5*(t) or *Pi-7*(t) (Tanksley et al. 1992; Wang et al. 1994) were labeled by ECL direct nucleic-acid labelling and detection systems (Amersham Corp.) and used as probes for F_2 segregation analysis. Probe labelling, hybridization and signal detection conditions were done according to the instructions accompanying the ECL direct nucleic-acid labelling and detection systems (Amersham Corp.).

Results

PILs with genes conditioning complete resistance to *P. grisea* isolate PO6-6

As reported by Wang et al. (1994), Moroberekan has at least two genes for complete resistance to *P. grisea* isolate PO6-6. These loci, designated *Pi-5*(t) and *Pi-7*(t), were located on chromosomes 4 and 11, respectively (Wang et al. 1994). As a first step towards selecting PILs for these blast-resistance genes, the RILs that were resistant to isolate PO6-6 and carrying less than 20% of Moroberekan alleles at RFLP loci were selected from a population of 281 F_7 RILs of a cross between Moroberekan and CO39 (Table 1). Three RILs (RIL29, RIL125 and RIL249) satisfying the above criteria were selected and the reaction patterns of those RILs to the five test isolates were determined. While RIL29 and RIL125 showed the same reaction patterns to the test isolates, RIL249 showed a different reaction pattern from the other two RILs. The reaction patterns of these RILs were similar to those of the CO39 NILs carrying *Pi-1* and *Pi-3*, respectively (Table 2). The morphology of RIL29 was more similar to CO39 than was that of RIL125, so the former was selected for further analysis, along with RIL249. RIL29 and RIL249 had Moroberekan alleles at 17.5 and 10.3% of the RFLP loci tested, respectively (Table 1). These proportions corresponded to the amounts of introgressed donor genetic material expected after two or three backcrosses.

To determine the number of genes conditioning resistance to isolate PO6-6 in each of these lines, RIL29 and RIL249 were each crossed to CO39, and F_2 populations derived from each cross were inoculated with PO6-6. For both crosses, the results fit the expected 3:1 ratio for resistant:susceptible progeny, confirming that each line carried a single gene. To determine whether the two lines carried the same or different genes, a cross was made between RIL29 and RIL249, and again the F_2 plants were inoculated with isolate PO6-6. The ratio of resistant to susceptible plants fit the 15:1 ratio expected for two independent loci (Table 3). Thus, it was confirmed that RIL29 and RIL249 each carried single resistance genes to isolate PO6-6, and that those blast-resistance genes were independent.

When the reaction profiles of the RILs were compared with those of CO39 NILs, the reaction pattern of RIL29 was found to be similar to that of C101LAC carrying *Pi-1*, while the reaction pattern of RIL249 was similar to that of C104PKT carrying *Pi-3* (Table 2). To test the hypothesis that the gene in RIL29 was allelic to *Pi-1*, and that the gene in RIL249 was allelic to *Pi-3*, crosses were made between the two RILs and the selected CO39 NILs. The cross between RIL29 and C101LAC yielded no progeny showing susceptibility to isolate PO6-6, indicating that the blast-resistance gene in RIL29 was either allelic with or else closely linked to *Pi-1*. In the F_2 population derived from the cross between RIL249 and C101LAC, a 15:1 ratio of resistant and susceptible plants was seen for isolate PO6-6, confirming that the gene in RIL249 is not allelic with or linked to *Pi-1* (Table 3).

To test allelism between the gene in RIL249 and *Pi-3* in C104PKT, isolate PO3-82–51 was used, because this isolate is incompatible to both C104PKT and RIL249, but compatible to CO39. When F_2 plants from the cross between RIL249 and CO39 were inoculated with this isolate, a 3:1 segregation ratio was seen for blast-resistance, indicating that a single gene for resistance to isolate PO3-82–51 was present in RIL249. Among the 466 F_2 progeny of the cross between C104PKT and RIL249, no progeny susceptible to isolate PO3-82–51 were found, indicating that the gene in RIL249 conditioning resistance to this isolate is allelic, or else closely linked, to *Pi-3* (Table 3).

Table 1 Pre-isogenic lines (PILs) for complete or partial resistance genes to blast, selected from recombinant inbred lines (RILs) of a cross between Moroberekan and CO39

Line no.	Generation	R gene or QTL	No. of RFLP markers from Moroberekan	%
RIL29	F_7	R gene to PO6-6	22/127	17.5
RIL125	F_7	R gene to PO6-6	11/127	8.7
RIL249	F_7	R gene to PO6-6	13/127	10.3
RIL10	F_7	R gene to Ca65	15.5/127	12.3
RIL23	F_7	QTL linked to RZ398, RG64 (chr. 6) and RG612 (chr. 1)	12/127	9.5
RIL276	F_7	QTL linked to RG64 (chr. 6) and CDO 920 (chr. 1)	12/127	9.5

Table 2 Reaction pattern of pre-isogenic lines (PILs), selected from recombinant inbred lines of a cross between CO39 and Moroberekan, and CO39 near-isogenic lines (NILs) to *P. grisea* isolates

Line	*Pi* gene[a]	Reaction to isolates[b]				
		PO6-6	IK81-3	IK81-25	V86010	Ca65
RIL29	*Pi-7*(t)	R	R	S	R	S
RIL125	*Pi-7*(t)	R	R	S	R	–
RIL249	*Pi-5*(t)?	R	S	S	I	S
RIL10	*Pi-12*(t)	S	S	S	R	R
CO39	–	S	S	S	S	S
C101LAC	*Pi-1*	R	R	S	R	–
C101A51	*Pi-z*^{5}	R	R	R	R	–
C104PKT	*Pi-3*	R	S	S	S	–
C101PKT	*Pi-ta*	S	R	R	S	–
C105TTP-4L23	*Pi-ta*, *Pi-?*[c]	S	R	R	R	–

[a] Wang et al. (1994); Inukai et al. (1994a); Kinoshita et al. (1994)
[b] R=resistant; I=intermediate; S=susceptible
[c] An additional, unidentified gene in C105TTP-4L23 (Inukai et al. 1994a)

Table 3 Reaction of F_2 populations of crosses between pre-isogenic lines (PILs) and CO39 near-isogenic lines (NILs) to *P. grisea* isolates

Cross	Test isolate	No. of F_2 plants observed for each class		Expected ratio (R : S)	Probability
		R	S		
CO39/RIL29	PO6-6	88	32	3 : 1	0.50–0.75
CO39/RIL249	PO6-6	61	19	3 : 1	0.75–0.90
RIL29/RIL249	PO6-6	227	11	15 : 1	0.25–0.50
RIL29/C101LAC	PO6-6	234	0	1 : 0	
RIL249/CO101LAC	PO6-6	225	13	15 : 1	0.50–0.75
CO39/RIL249	PO3-82-51	84	32	3 : 1	0.25–0.50
C104PKT/RIL249	PO3-82-51	466	0	1 : 0	
CO39/RIL10	V86010	66	33	3 : 1	0.05–0.10

Table 4 Observed frequencies of genotypes on R genes to isolate PO6-6 and RFLP markers in crosses CO39/RIL29 and CO39/RIL249

Cross	Gene pair	Genotype[a]									Total	Probability
		AABB	AABb	AAbb	AaBB	AaBb	Aabb	aaBB	aaBb	aabb		
CO39 (aabb)/ RIL29 (AABB)	R gene-RZ537	6 (3.4)[b]	5 (6.9)	1 (3.4)	5 (6.9)	20 (13.8)	6 (6.9)	1 (3.4)	7 (6.9)	4 (3.4)	55	0.05–0.10
CO39 (aabb)/ RIL249 (AABB)	R gene-RG788	2 (2.7)[c]	10 (12.9)	3 (9.3)	5 (4.7)	26 (22.2)	12 (16.1)	3 (2.5)	11 (11.9)	9 (8.6)	91	0.10–0.25

[a] A and a represent the genotypes of each complete resistance gene. B and b represent the genotypes of each RFLP marker
[b] Expected value estimated by theoretical ratio 1:2:1:2:4:2:1:2:1
[c] Expected value estimated by observed value. Because the segregation at RG788 locus was significantly skewed (BB:Bb:bb=10:47:34, χ^2=12.76**), a test for independence was done

Wang et al. (1994) estimated that *Pi-7*(t) was flanked by the RFLP markers RG16 and RG103A on chromosome 11. Because the recombinant inbred population used in this estimation showed skewed segregation (Wang et al. 1994), the chromosomal location of *Pi-7*(t) could not be determined precisely. Yu et al. (1991) reported that *Pi-1* was linked to RZ536 on chromosome 11, at a distance of 14.0 cM. Mew et al. (1994) reported that *Pi-1* was linked to RZ536 and G181 [the previous registration was Npb181 (Inoue et al. 1994)] with distances of 7.9 and 3.5 cM, respectively. Although RIL29 did not carry Moroberekan alleles at the RG16, RG103A and RZ797 loci, this line had the Moroberekan allele at RZ537, which is located near RG103A (Fig. 1). To test the hypothesis that the blast-resistance gene in RIL29 is located near RZ537 on chromosome 11, the segregation of blast-resistance genes and RZ537 was analyzed for 55 F_3 lines derived from the cross between CO39 and RIL29. The results showed that RZ537 was loosely linked to the blast-resistance gene in RIL29 with distance of 27.0±5.5 (SE) cM (Table 4). Since the map distance between RZ537 and G181 was estimated to be about 30 cM by previous data (Tanksley et al. 1992; Mew

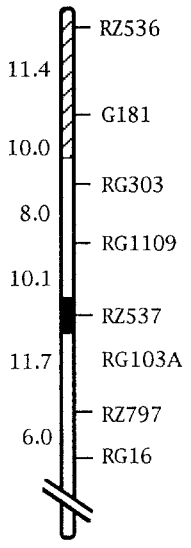

Fig. 1 Graphical genotype of a part of chromosome 11 in RIL29. The *black region* indicates the segment derived from Moroberekan; *white regions* indicate segments derived from CO39; the *shaded region* has not yet been checked. *Designations* to the right indicate marker names; *numbers* to the left indicate the map distance s(cM). Order of markers and the map distances were according to Tanksley et al. (1992) and Mew et al. (1994)

et al. 1994) (Fig. 1), it appeared that the blast-resistance gene in RIL29 was located in the same region of chromosome 11 as the *Pi-1* locus. This conclusion was consistent with the result of the allelism test with C101LAC. *Pi-7*(t) in RIL29 appeared to be allelic, or else closely linked, to *Pi-1*.

If *Pi-5*(t) and *Pi-7*(t) were the only genes in Moroberekan conditioning resistance to isolate PO6-6, then RIL249 would be expected to carry *Pi-5*(t), because the blast-resistance gene in RIL249 was independent of *Pi-7*(t) in RIL29 (Table 3). Since RIL249 had a Moroberekan allele at the RG788 locus, which was found to be linked to *Pi-5*(t) (Wang et al. 1994), linkage between the RG788 locus and the blast-resistance gene in RIL249 was analyzed. Ninety one F_3 lines derived from the cross between CO39 and RIL249 were inoculated and scored for reaction to isolate PO6-6. DNA was extracted from the lines, and membrane-bound digested DNAs were probed with RG788. Although the segregation at the RG788 locus in the F_2 population was significantly skewed to the CO39 allele, it was shown that the blast-resistance gene in RIL249 was independent of, or else loosely linked to, the RG788 locus (Table 4). This result suggested that *Pi-5*(t) was actually not linked to the RG788 locus, or else that Moroberekan carried an additional resistance gene to isolate PO6-6.

PIL with a gene conditioning complete resistance to *P. grisea* isolate Ca65

Twelve of the one-hundred and fifty RILs that were susceptible to isolate PO6-6 were resistant to isolate Ca65, suggesting that Moroberekan carried at least one additional blast-resistance gene (G. Wang, IRRI, unpublished). Among the RILs showing resistance to isolate Ca65, but not to isolate PO6-6, RIL10 carried the lowest number of Moroberekan alleles (Table 1), and was selected as a PIL.

Table 5 Partial blast-resistance of pre-isogenic lines (PILs) RIL23 and RIL276

Line no.	Polycyclic test			Field test[a]
	Lesion no.	Lesion size	DLA[b]	DLA[b]
RIL23	47[c]	100[c]	30[c]	31[c]
RIL276	41	45	18	43
CO39	100	100	100	100
Moroberekan	–	–	–	1

[a] In Caliraya experimental station, Laguna, Philippines
[b] Diseased leaf area
[c] Relative ratio to CO39 (100)

Since the reaction pattern of RIL10 to the five test isolates was clearly different from that of the existing CO39 NILs, or indeed any of the identified PILs carrying resistance genes to isolate PO6-6 (Table 2), this putative blast-resistance gene was temporarily designated *Pi-12*(t).

PILs with QTLs conditioning partial resistance to blast

Of the original 281 Moroberekan/CO39 RILs, 138 were susceptible to both isolates PO6-6 and Ca65. Among these, 43 RILs showed fewer than half the number of susceptible lesions than did CO39 in polycyclic inoculation tests (Wang 1992). The DLA of most of these RILs was also less than half that of CO39 in polycyclic tests and field experiments (Wang 1992; Bronson 1994). These lines were considered likely to carry quantitative trait loci (QTLs) conditioning partial resistance to blast. Among these lines, RIL23 and RIL276 carried the lowest number of Moroberekan alleles at the RFLP loci analyzed, and were thus selected as PILs (Table 5).

While the lesions formed on RIL276 were less than half the size of those formed on CO39 in polycyclic tests, the lesions formed on RIL23 were similar to those on CO39 (Table 5). Both RIL23 and RIL276 carried 9.5% Moroberekan alleles (Table 1). RIL23 carried Moroberekan alleles at RFLP loci linked to three different QTLs affecting lesion number and/or DLA: RG612 on chromosome 1, and RZ398 and RG64 on chromosome 6 (Fig. 2). RIL276 carried Moroberekan alleles at RFLP loci linked to two QTLs affecting lesion number, DLA and/or lesion size: CDO920 on chromosome 1, and RG64 on chromosome 6 (Fig. 2).

Discussion

Near-isogenic lines are useful for studies aimed at characterizing blast-resistance genes, and for studies aimed at characterizing pathogen virulence. NILs for blast-resistance have been developed by conventional backcrossing and selection (Mackill and Bonman 1992; Sasaki et al. 1994). We propose that NILs could be more easily and efficiently produced by marker-aided selection of lines from

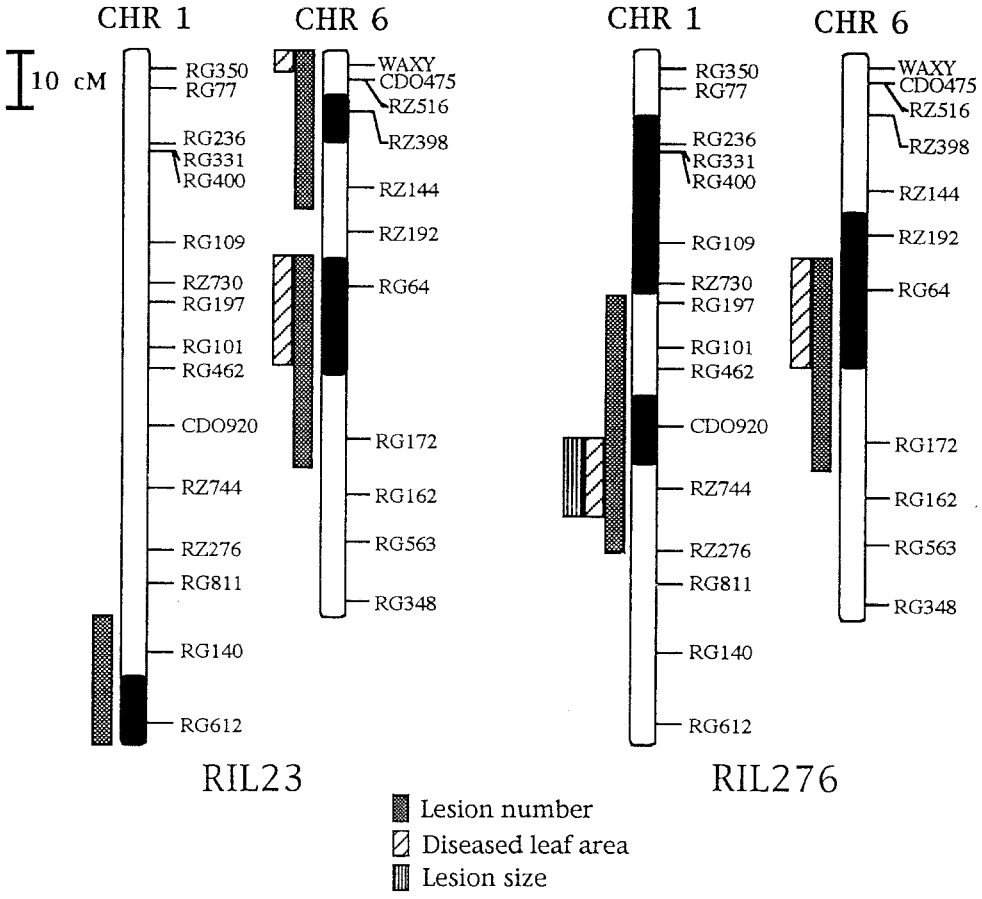

Fig. 2 Graphical genotype of chromosomes 1 and 6 in RILs23 and 276. *Black regions* indicate segment derived from Moroberekan; *white regions* indicate segments derived from CO39. *Designations* to the right indicate marker names; *stippled bars* to the left represent supporting intervals around the chromosomal regions associated with partial resistance to blast (Wang et al. 1994)

populations of fixed segregants that have been used for RFLP mapping. In the present study we demonstrated that a set of PILs carrying individual blast-resistance genes could be selected from a population of recombinant inbreds by marker-aided selection. Three lines carrying distinct major genes were selected based on marker and phenotypic data, and subjected to further genetic analysis. Based on the data available for 127 RFLP loci, each of the selected PILs carried less than 20%, and in some cases nearly 10%, of the genome of the resistant parent, Moroberekan. The extent to which the genetic background of the susceptible parent genome was recovered corresponded to that expected for two or three backcrosses. Based on available RFLP data, the PILs selected in this study do not possess more genetic material from the donor parent than do other near-isogenic lines carrying blast-resistance genes (Yu 1991).

It was possible to recover a set of resistant lines with relatively little of the Moroberekan genome because the recombinant inbred population utilized in this study showed skewed segregation, favoring alleles from the susceptible parent (Wang et al. 1994). The RI population represented a set of F_7 lines derived from a cross between an *indica* and a *japonica* cultivar. Although 1:1 segregation would be expected with an F_7 population, skewed segregation is often observed with *indica/japonica* crosses (Oka 1955; McCouch et al. 1988). Averaged across RFLP loci, the frequency of *indica* (CO39) alleles was 75% in this population; averaged across lines, the frequency of *indica* alleles was 80% (Wang et al. 1994). Thus, segregation distortion in *indica/japonica* crosses may be advantageous for the selection of PILs with an *indica* genetic background. If a recombinant inbred or doubled haploid population showing normal segregation was used, the probability of recovering PILs from the population would be lower, in comparison to a population showing segregation distortion. In this case, the use of a population generated from BC_1F_1 plants without selection would be an alternative way to reduce the proportion of alleles coming from the donor parent.

Although segregation distortion may be advantageous for the selection of PILs, it may reduce the accuracy of gene mapping. The accuracy of gene mapping in the study of Wang et al. (1994), in which the Moroberekan/CO39 RI population was used to identify and locate genes for blast-resistance, was affected by this. The results of the initial mapping work should be viewed as establishing an hypothesis about the number of genes effective for the isolates tested, and their approximate locations. For instance, *Pi-7*(t) was estimated by Wang et al. (1994) to reside between RG16 and RG103A on chromosome 11. The selection of a line carrying only this gene effective against isolate PO6-

6 allowed more precise genetic analysis, involving allelism tests and segregation analysis, which led to the conclusion that *Pi-7*(t) is allelic, or closely linked, to *Pi-1*. This gene is also present on chromosome 11, but is likely to reside at a more distal position, more closely linked to G181.

Based on the analysis of the RI population, Wang et al. (1994) identified two major genes effective for isolate PO6-6, and designated these as *Pi-5*(t) and *Pi-7*(t). In the present study, two genes effective for this isolate were also identified. It is clear that the gene in RIL29 corresponds to *Pi-7*(t), and that this gene is apparently allelic, or closely linked, to *Pi-1* in the existing NILs. Further work is, however, needed to identify the chromosomal location of the gene in RIL249, and to determine whether or not this gene is the same as *Pi-5*(t). The gene in RIL249 was apparently unlinked to RG788, which was found to be linked to *Pi-5*(t) by Wang et al. (1994). Lines carrying the chromosomal segment to which *Pi-5*(t) was mapped are currently under analysis.

The RI population utilized in the present study was previously used to map both major genes and QTLs in Moroberekan. Twenty RFLP loci defining ten chromosomal segments of Moroberekan have been found to be associated with quantitative effects on blast resistance, and the individual contribution of QTLs to partial resistance to blast has been estimated (Wang et al. 1994). If a set of near-isogenic lines carrying individual QTLs can be obtained, it will be possible to systematically evaluate the magnitude of the effects of individual QTLs relative to a wide range of pathogen isolates, and to evaluate the associations of other traits with these chromosomal segments. Such a set of NILs would also be useful for investigating the mechanism of partial resistance to blast. The PILs selected here should be useful as donor parents for developing NILs carrying individual QTLs.

Although the PILs selected in this study carry relatively few Moroberekan loci, the lines each contain several chromosome segments from the blast-resistance donor. These introgressed segments may also carry unrecognized blast-resistance genes. The PILs should be back-crossed to CO39 to remove introgressed segments from Moroberekan that are not associated with blast-resistance genes of interest with the aid of RFLP analysis. Only a few backcrosses will be needed, because the RFLP genotype of each PIL is known, and the chromosomal segments from the resistant parent can be efficiently removed by RFLP-based selection (Tanksley et al. 1989). The purification of the PILs obtained here is now under way.

We used the approach presented in this paper to analyze only blast-resistance genes. However, this approach should also be applicable to the analysis of major or minor genes associated with other traits. The RI population and the method employed here for the development of NILs from the RI population will be a powerful tool to understand the genetic basis of agriculturally important traits in rice.

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