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Fine mapping and DNA marker-assisted pyramiding of the three major genes for blast resistance in rice

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Abstract Three major genes (*Pi*1, *Piz*-5 and *Pit*a) for blast resistance on chromosomes 11, 6 and 12, respectively, were fine-mapped and closely linked RFLP markers identified. New markers for *Pi*1 and *Pit*a were found that were flanking the genes. The three genes were pyramided using RFLP markers. A PCR-based SAP (sequence amplified polymorphism) marker was used to identify *Piz*-5 in the segregating population. The plants carrying the two- and three-gene combinations that were tested for resistance to leaf blast in the Philippines and India indicated that combinations including *Piz*-5 have enhanced resistance than when it is present alone. The genes from the pyramided lines are at present being deployed into agronomically superior rice varieties by marker-aided selection (MAS).

Key words RFLP markers · Fine-mapping · Blast resistance · Pyramiding · Marker-assisted selection

Introduction

Blast, caused by the fungus *Magnaporthae grisea* (Herbert) Borr. (anamorphe *Pyricularia oryza* Cav.) is one of the most serious diseases of rice (*Oryza sativa* L.) worldwide. The rapid changes that occur in the virulence characteristics of populations raises a continuous threat to the effectiveness of existing blast-resistant varieties. Hence, there is an urgent need for stratagies to develop varieties with durable resistance to the disease. Both major and minor genes can contribute to durable resistance (Bonman and Mackill 1988; Wang et al. 1994; Zhu et al. 1993). Several genes conferring complete and partial resistance to blast disease have been located on rice chromosome maps (Yu et al. 1991; Wang et al. 1994; Naqvi et al. 1995; Pan et al. 1996). Although major genes have often been cited as the underlying cause of resistance instability (Ahn and Ou 1982), strategies for developing durable resistance mediated by major these have been proposed (Ou 1985). These approaches depend upon careful characterization of the resistance spectrum of the genes in question and combining them such that the gene 'pyramid' is effective against the target pathogen population (Chen et al. 1995; Zeigler et al. 1994, 1995). Combining several genes and monitoring their presence is difficult by conventional methods because of their epistatic effects and the simple masking of the effect of one gene by another. Mapping blast resistance genes and locating closely linked markers has made it possible to confirm the presence of given gene in a variety with multiple genes (Paterson et al.1991; Huang et al. 1997). Thus, even when the phenotype is ambiguous, the presence of a gene can be monitored over successive segregating generations. Using near-isogenic lines (NILs) developed at IRRI, Mackill and Bonman (1992) identified four major genes and designated these as *Pi*-1(t), *Pi*-2(t), *Pi*-3(t) and *Pi*-4(t) with two alleles, *Pi*-4a(t) and *Pi*-4b(t). Allelism tests by Inukai et al. (1994) with Kiyosawa's (1984) differentials indicated that the blast resistance genes *Pi*-1(t) and *Pi*-3(t) were different from any other blast resistance genes already reported. *Pi*-2(t) was allelic to *Pi*-z and *Pi*-4a(t) was allelic to *Pit*a. Following the recommendation by the committee on rice gene symbolization (Kinoshita et al. 1994), we will use *Pi*1 for *Pi*-1, *Piz*-5 for *Pi*-2 and *Pit*a for *Pi*-4 in the paper.

In this study presented here we fine-mapped resistance alleles of three major genes for blast resistance, *Pi*1, *Piz*-5 and *Pit*a on chromosomes 11, 6, and 12, respectively. Identifying flanking DNA markers located within 5–10 cM from a gene of interest has yielded high level of selection accuracy for resistance (Hittalmani et

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Table 1 Plant materials, restriction enzymes and RFLP markers used in Southern analysis

Blast resistance gene	Chromosome	Isoline	Donor parents	Population size	Number of restriction enzymes test	RFLP markers tested	Polymorphic markers
*Pi*1	11	C101LAC	Lac23	160	30	10	5
Piz-5	6	C101A51	A5173	120	12	9	5
*Pi*ta	12	C101PKT	Pai-kan-tao	80	30	14	6

al. l995a). Further, for bacterial leaf blight resistance genes, it was possible to combine the genes using molecular markers to attain a wide spectrum of resistance (Yoshimura et al. 1995; Huang et al. 1997). Here, using closely linked restriction fragment length polymorphism (RFLP) and polymerase chain reaction (PCR)-based markers we combined blast resistance genes in a CO39 background. Using greenhouse and field challenges we were able to demonstrate that the resistance spectrum of the lines with various gene combinations match predictions based on single gene performance.

Materials and methods

Plant materials

The three NILs, C101LAC, C101A51 and C101PKT, each carrying the major genes *Pi*1, *Piz*-5 and *Pi*ta, respectively, in the background of the susceptible recurrent parent CO39, were used for fine-mapping and pyramiding the three genes, the details of which are given in Table 1. Each NIL was crossed separately with CO39, and the panicles of the F_1 individuals of each cross were bagged to obtain F_2 populations for further linkage analysis.

For combining the genes, we made pairwise crosses between the NILs, C101LAC(*Pi*1)/C101A51(*Piz*-5), C101LAC(*Pi*1)/C101PKT (*Pi*ta) and C101A51(*Piz*-5)/C101PKT(*Pi*ta). The DNA of the F_2 populations was used for marker analysis to identify and select plants homozygous for resistance genes based on the parental banding pattern of RFLP probes. Individual plants identified as carrying homozygous resistance genes *Pi*1+*Piz*-5 and *Pi*1+*Pi*ta were further crossed with each other to combine the three genes (Fig 1). The F_2 progenies for each of the crosses (about 150–250 plants) for the two-gene and three-gene combination used in the DNA analysis were obtained from bagged panicles of the F_1 plants. The F_3 families were progeny-tested by inoculation with putatively compatible isolates wherever possible.

DNA markers used for fine-mapping and pyramiding the genes

For the parental polymorphism survey, 30 RFLP markers each were used for *Pi*1 and *Pi*ta on chromosome 11 and 12, and another set of 12 markers were used for *Piz*-5 on chromosome 6. The polymorphic markers were subsequently identified and used to probe the segregating populations to identify any additional closely linked markers. The number of polymorphic markers used for fine mapping are presented in Table 1. The closely linked markers identified were then used to select the plants that contained the two and three genes in the homozygous state (Table 2). The RFLP markers used in this study were provided by Dr. Steve Tanksley of Cornell University and the Japanese Rice Project, Tsukuba. A PCR-based sequence-amplified polymorphic (SAP) marker for RG64 on chromosome 6 was used for identifying the *Piz*-5 gene in the segregating population (Hittalmani et al. 1995a).

Table 2 Distances of the DNA markers from the blast resistance genes on different chromosomes

Gene	Chromosome	Markers	Restriction enzymes	Distance
*Pi*1	11	Npb181	*Dra*I	3.5 cM
		RZ536	*Dra*I	7.9 cM (14 cM)[a]
Piz-5	6	RZ64	*Eco*RI	2.1 cM (2.8 cM)[a]
		RZ612	Eco*RI*	7.2 cM
		RG456	Xba*I*	(5.4 cM)[a]
		RG64-SAP	*Hae*III	(2.8 cM)[a]
*Pi*ta	12	RG869	H*Eco*RV	5.4 cM (15.3 cM)[a]
		RZ397	*Eco*RV	3.3 cM (18.1 cM)[a]
		RG241	Sca*I*	5.2 cM

[a] Numbers in the parentheses are the distances in centiMorgans (cM) from the genes that were previously mapped (Yu et al.1991)

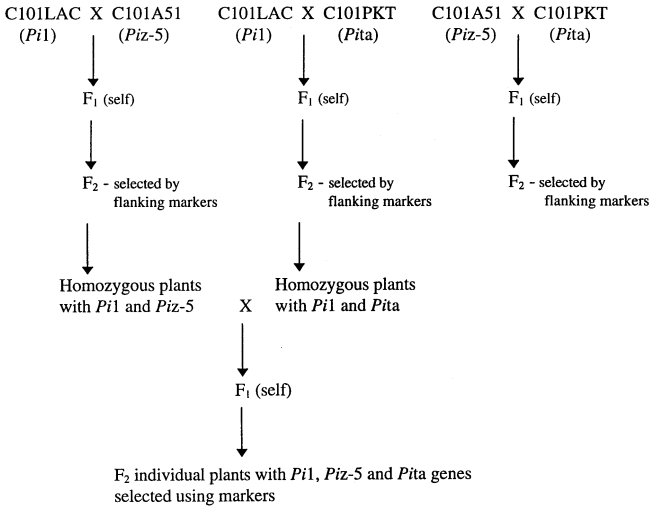

Fig. 1 Schematic diagram showing marker-assisted selection for pyramiding the three major genes for blast resistance

DNA extraction and Southern blotting

Total DNA was extracted according to the procedure of Dellaporta et al. (1983). Southern blotting, labeling and hybridization was according to standard protocols (Sambrook et al. 1989). DNA was digested with different restriction enzymes and probed with at least 9 RFLP markers for each gene for fine mapping (Table 1). For pyramiding the genes, the DNA from the F_2 progeny was digested with specific enzymes and probed with appropriate markers (Table 2). The DNA of the F_2 plants was digested with *Dra*I to identify the *Pi*1 resistance gene, with *Eco*RI for the *Piz*-5 gene and with *Eco*RV to identify the *Pi*ta genes. Markers *Npb*181 and RZ536 for *Pi*1, RG456 for *Piz*-5 and RZ397, RG241 and RZ869 for *Pi*ta were used to probe the Southern blots.

Identification of the Piz-5 gene by the SAP marker using PCR analysis

Confirmation of the *Piz*-5 gene was made using the co-dominant RG64 SAP marker which in our earlier work was shown to segregate similarly to the RG64 RFLP marker (Hittalmani et al. 1994, 1995a, b). The DNA of the isolines and the crosses were PCR amplified and digested with the *Hae*III enzyme to identify the bands associated with relevant phenotype. The PCR reaction consisted of 50–70 ng of template DNA, 20 ng of primers, 0.1 m*M* dNTPs, 1 × PCR buffer and 1 U of *Taq* polymerase enzyme in a 25-µl reaction. The primers synthesized for the RG64 marker to generate SAP was used (Hittalmani et al. 1995a). The PCR amplification conditions were 94°C for 5 min for the initial denaturation followed by 40 cycles of a 1-min denaturation at 94°C, 30-s primer annealing at 60°C and a 1-min primer extension at 72°C and completed with an additional primer extension for 5 min at 72°C – on a Perkin Elmer Cetus thermal cycler. The amplified product was digested with *Hae*III and run on a 1% agarose gel.

Phenotyping the populations for fine mapping

Twenty-one-day-old seedlings of F_3 families of the three populations used for fine mapping were grown in plastic trays, and the seedlings were inoculated with Philippine rice blast isolates, V89013 and IK81–25 (compatible with *Pi*1), 101–7-2 (compatible with *Piz*-5) and V86010 (compatible with *Pi*ta) in two separate trials. The inoculum was prepared following the procedure of Bonman et al. (1986). The plants in each tray were sprayed with 50 ml of inoculum of 5×10^4 conidia per milliliter and kept for 24 h at 25°C in a dew chamber and, subsequently, for 1 week at 25°C in the mist chamber before scoring.

Seven days after inoculation the plants from the F_3 families were scored as resistant (R), susceptible (S) and segregating (RS). Similarly, the RFLP banding pattern of each F_2 plant was determined and given a score of 3 when the banding pattern corresponded to a resistant genotype, 2 for segregating and 1 for the susceptible genotype. For the banding pattern where resistant dominant and heterozygous genotypes could not be distinguished, a score of 4 was given. The ratios of the segregation of the alleles was confirmed by chi-square analysis, and genetic distances between the markers and the resistance genes were estimated using the computer program MAPMAKER (Lander et al. 1987).

Phenotypic analysis of gene pyramids

The isogenic lines and the pyramids with resistance gene combination of two and three genes (*Pi*1+*Piz*-5, *Pi*1+*Pi*ta, *Piz*-5+*Pi*ta and *Pi*1+*Piz*-5+*Pi*ta) were inoculated twice with selected Philippine rice blast isolates. Twenty-one-day-old seedlings were inoculated with IK81–25 and C9232–5 compatible for *Pi*1, C9240–2 and C9240–5 for *Piz*-5 and V86010 and P06–06 for *Pi*ta and scored after 1 week for resistance (R) or susceptibility (S), respectively.

Two- and three-gene pyramid lines, the NIL parents and the recurrent parent CO39 (Table 3) were tested in the rice blast nursery at IRRI and blast hotspot at Ponnampet, India. The densely sown seedlings were surrounded by spreader rows of cvs. IR50 and IR72. Good disease development was ensured by planting infected seedlings within spreader rows. Observations on the disease reaction on the seedlings was recorded at 15, 20, 25 and 30 days after sowing. The seedlings were visually scored for percentage disease leaf area (DLA) and type of lesions (0–5 scale: resistance was scored as 0–2; 3 is moderately resistant and 4–5 is susceptible).

The two-gene combination pyramids were coded as BL12, BL14 and BL24 for *Pi*1+*Piz*-5, *Pi*1+*Pi*ta and *Piz*-5+*Pi*ta, respectively, and the three-gene pyramid *Pi*1+*Piz*-5+*Pi*ta as BL124.

Table 3 List of isogenic lines and the segregating populations used in pyramiding the genes

Line\varieties	Line\cross	Resistance genes	Population size
CO39	Recurrent parent	None	
C101LAC	Isogenic line	*Pi*1	
C101A51	Isogenic line	*Piz*-5	
C101PKT	Isogenic line	P*ita*	
F_2 Populations			
BL12	*Pi*1*Piz*-5	*Pi*1 + *Piz*-5	150
BL14	*Pi*1*Pi*ta	*Pi*1 + *Pi*ta	250
BL24	*Piz*-5*Pi*ta	*Piz*-5 + *Pi*ta	150
BL124	*Pi*1*Piz*-5*Pi*ta	*Pi*1 + *Piz*-5 + *Pi*ta	180

Results

Fine mapping of the three blast resistance genes

Out of 30 RFLP markers tested for parental polymorphism for mapping *Pi*1 and *Pi*ta, 10 markers for *Pi*1 and 14 markers for *Pi*ta were found to be polymorphic. For the *Piz*-5 dominant resistant gene 12 markers were polymorphic out of 16 markers tested. The F_2 marker data for the three populations segregating for the *Pi*1, *Piz*-5 and *Pi*ta genes and F_3 phenotypic data upon analysis with MAPMAKER identified at least 3 markers on each of the chromosomes linked to the three genes (Fig 2). On chromosome 6, RG64 was at 2.1 cM from the *Piz*-5 gene, as reported earlier, while RZ612 and RG456 were located at 7.2 cM and 12.3 cM, respectively. *Npb*181 from the Japanese Rice program was located 3.5 cM from the *Pi*1 gene on chromosome 11 and a flanking marker RZ536 was located at 7.9 cM. On chromosome 12, the rice cDNA marker RZ397 was located at 3.3 cM from the *Pi*ta, while RG869 was located at 5.4 cM from the gene. A flanking marker RG241 was identified at 5.2 cM from the *Pi*ta gene. We estimated *Piz*-5 to be linked to RG64 at 2.1 cM. At least 1 marker on each chromosome was identified to be closer than distances previously mapped (Table 2).

Molecular marker analysis of plants with the two- and three-gene combinations

The F_2 plants of the cross combination C101A51/C101LAC were analyzed by PCR analysis and Southern

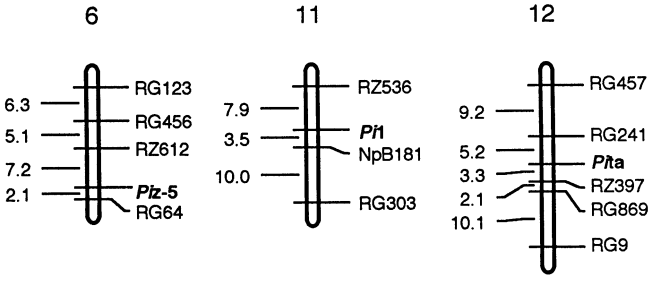

Fig. 2 The location of the three blast resistance genes, *Pi*1, *Piz*-5 and *Pi*ta, on rice chromosomes

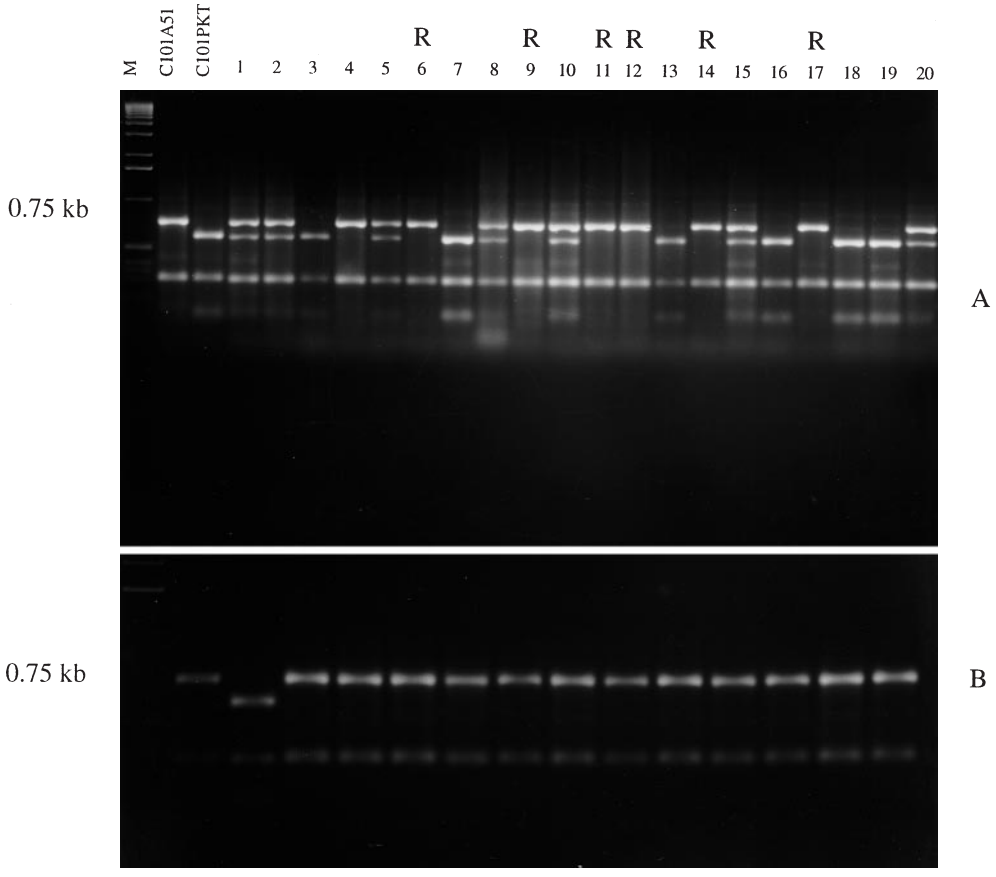

Fig. 3 A PCR banding pattern of RG64 marker linked to *Piz*-5 blast resistant gene segregating in the F_2 population of the cross C101A51 (isoline for *Piz*-5) and C101PKT (isoline for *Pi*ta). The PCR products were digested with restriction enzyme *Hae*III to obtain polymorphism. **B** The resistant plants (*R*) selected in the F_2 were confirmed in the F_3 by the RG64 PCR marker. *M* Molecular-weight standard

hybridization using RG64 (SAP) and RG456 markers for *Piz*-5 and RZ536 marker linked to *Pi*1. Three plants carrying resistant alleles for both *Pi*1 and *Piz*-5 were identified in the F_2 progeny based on the banding pattern of the parents. Similarly, 4 plants were identified with *Piz*-5 and *Pi*ta resistant alleles using the RG64 PCR marker for *Piz*-5 and RZ397 for *Pi*ta (Figs. 3 and 4). Ten plants with homozygous resistant alleles for both *Pi*1 and *Pi*ta were identified in the F_2 plants using RFLP markers RZ536 for *Pi*1 and RZ397 and RG869 for *Pi*ta. The homozygous resistant plants selected based on the banding pattern were progeny-tested in the F_3 generation and also confirmed by molecular marker analysis. Examples for *Piz*-5 and *Pi*ta gene confirmation in the F_3 by PCR and RFLP are shown in Figs. 3 and Fig. 4, respectively.

A total of 180 plants were raised to obtain the three-gene combination plants. Four plants each, carrying all three of the resistant alleles *Pi*1, *Piz*-5 and *Pi*ta were identified by markers linked to the genes. Figure 5

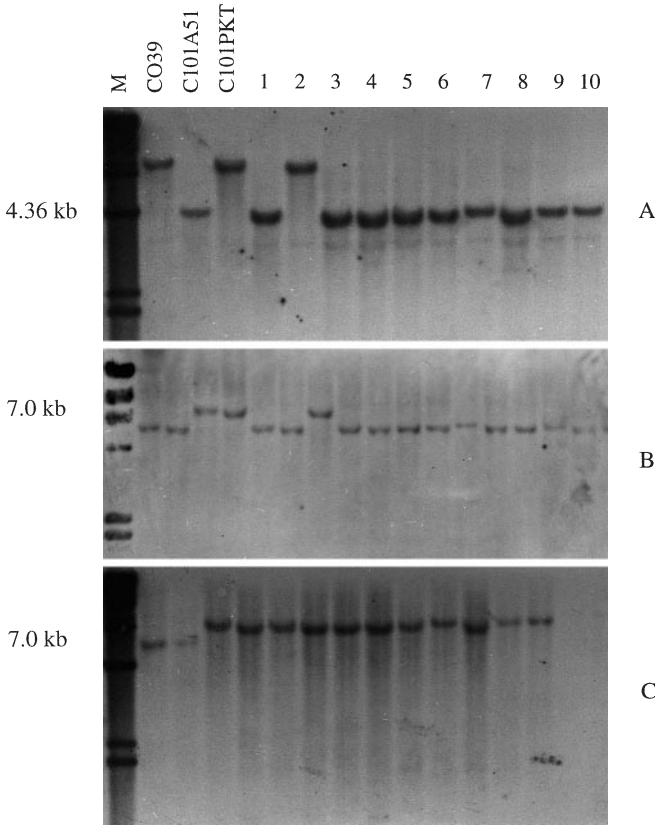

Fig. 4 A,B The identification of homozygous resistant plants with *Piz*-5 and *Pi*ta genes by RG456 (**A**) and RG869 (**B**) RFLP markers. **C** The homozygous resistant plants selected in the F_2 generation for *Pi*ta were confirmed in the F_3 by the RZ397 RFLP marker (lanes *C039, C101A51* and *C101PKT* in **Fig. 3A–C** are isogenic lines, while the resistant plants in *lanes 1–10* are those that correspond with the banding pattern of their respective isogenic lines). *M* Molecular-weight standard

Table 4 Evaluation of the susceptibility of isolines and the gene pyramids to selected blast isolates

Lines	IK81-25	C9232-5	C9240-2	C9240-5	V86010	P06-06
C101LAC	S	S	MR	R	R	R
C101A51	R	R	S	S	R	R
C101PKT	R	R	MR	R	S	S
BL12	R	R	S	R	R	R
BL14	R	R	S	R	R	R
BL24	R	R	S	R	R	R
BL124	R	R	S	R	R	R
CO39	S	S	S	S	S	S

R, Resistant; S, susceptible; MR, moderately resistant

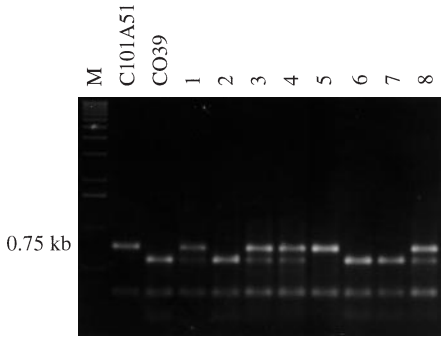

Fig. 5 Identification of the *Piz*-5 resistant gene in the F_2 generation segregating for the three genes. The 0.75-kb band represents the *Piz*-5 resistant gene. *M* Molecular-weight standard

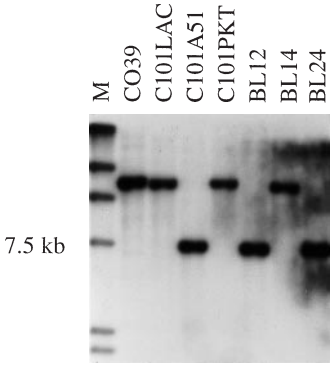

Fig. 6 Confirmation of the two gene pyramids carrying the *Piz*-5 gene by the RG456 RFLP marker. *M* Molecular-weight standard

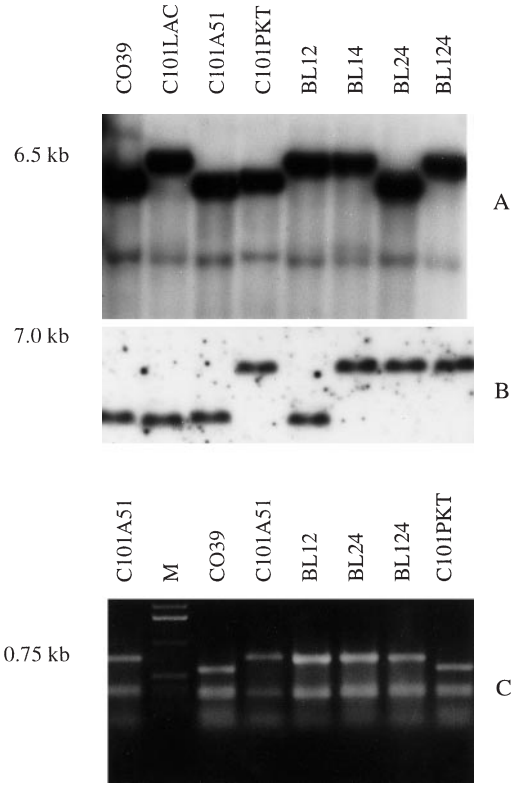

Fig. 7 A–C Two- and the three-gene pyramids as identified by RZ536 for *Pi*1 (**A**), RZ397 for *Pi*ta (**B**) and the RG64 PCR marker for the *Piz*-5 gene(**C**) *M* Molecular-weight marker used as the standard

shows the segregation of the *Piz*-5 gene and identification of the resistant genes based on the banding pattern of the marker. The plants that were homozygous for each gene were all confirmed in the F_3 generation by RFLP and PCR markers (Figs. 6, 7).

Phenotypic analysis of the two-gene and three-gene pyramids

The four sets of pyramid lines and the parents were inoculated twice with six rice blast isolates compatible with at least one of the resistance genes. The pyramid lines with two- and three-gene combinations were found to be resistant to the compatible isolates tested for all the three genes with the exception of C9240–2 (Table 4). The pyramids with two-gene combinations *Pi*1+*Piz*-5, *Pi*1+*Pi*ta and *Piz*-5+*Pi*ta and the three-gene pyramid with *Pi*1+*Piz*-5+*Pi*ta were tested in the blast nursery and scored for disease reaction for leaf blast at different intervals starting from 15th day after sowing (Table 5). On the 30th day after sowing, the susceptible check (CO39) had 95–100% infection and the isolines with *Pi*1 showed 15–20% DLA in both locations tested. *Piz*-5 remained resistant and *Pi*ta was susceptible in both the locations tested. Among the two-gene combinations *Pi*1+*Piz*5 was resistant and *Piz*-5+*Pit*a had lower levels of blast infection. *Pi*1+*Pi*ta was moderately resistant with a percentage DLA equal to one of the parents, while the pyramids with the three genes were resistant in both the locations.

Tale 5 Evaluation of the isogenic lines and gene pyramids with respect to percentage disease leaf area

Lines	15 DAS[a]		20 DAS		25 DAS		30 DAS	
	DLA%	(LT)[b]	DLA%	(LT)	DLA%	(LT)	DLA%	(LT)
	I[c]	II[d]	I	II	I	II	I	II
C101LAC	1 (3)	3 (4)	5 (4)	10 (4–5)	8 (4–5)	13 (4–5)	15 (5)	20 (4–5)
C101A51	0	0	0 (p)	(2)*	0 (p)	(2)*	0 (p)	(2)*
C101PKT	0.5 (4)	1 (4)	10 (5)	20 (5)	20 (5)	60 (4–5)	80 (5)	95 (5)
BL12	0	0	0	0	0 (p)	0	0 (p)	0
BL14	0	0.25 (3)	0 (p)	8 (4–5)	4 (2)	13 (4–5)	15 (5)	20 (4–5)
BL24	0	0	0	0.25 (3)	0.25 (2)	1.5 (4)	3 (4)	4 (4–5)
BL124	0	0	0 (p)	0 (p)	0 (p)	0 (p)	0 (p)	0 (p)
CO39	20 (4)	25 (4–5)	50 (4–5)	60 (5)	85 (5)	90 (5)	95 (5)	100 (5)
IR50	5 (4)	3 (2–3)	10 (5)	8 (5)	60 (5)	15 (4–5)	90 (5)	25 (4–5)
IR72	4 (4)	2 (3)	10 (4–5)	5 (4)	50 (5)	10 (4–5)	85 (5)	20 (4–5)

[a] DAS, Days after sowing
[b] DLA%, Percentage disease leaf area (LT), Type of lesions, presented in parentheses on a scale of 0–5: 0–3 = resistant; 4–5 = susceptible; p = pinhead-type lesions
[c] I, IRRI, Philippines
[d] II, Ponnampet, India
* Negligible infection with a few spores of Type 1–2

Discussion

Most efforts in blast resistance breeding have been directed towards incorporating single genes. Rice varieties carrying only one or few major resistance genes have a tendency to break down as unpredictable changes occur in the race composition of the pathogen populations (Ahn and Ou 1982; Kiyasawa 1982). Efforts have been made to develop partial resistance via the accumulation of putative quantitative trait loci (QTLs) (Bonman et al. 1992). Combining major genes that have wider spectrum of resistance would nonetheless still be useful (Chen et al. 1995; Yoshimura et al. 1995; Zeigler et al. 1994, 1995; Huang et al. 1997). In addition to overlapping resistance, it could decrease the selection pressure on the pathogen and provide cross protection. A workable technique for manipulating multiple resistance genes ultimately will be required to combine major genes and QTL for resistance to reproduce the apparent genotypes of durably blast resistant rice cultivars (Wang et al. 1994).

To improve the efficiency of marker-assisted selection (MAS), it is important that the recombination frequency between the target gene and the marker be as low as possible, and this could be achieved by identifying markers as close as possible to the gene. With the saturation of the molecular marker maps (Tanksley et al. 1992), it is now possible to identify new markers closely linked to the gene of interest and also the flanking markers. In our study on fine-mapping the major genes for blast resistance, four markers linked to the three genes were identified. We confirmed previous results that RG64 is linked to *Piz*-5 on chromosome 6 and RG869, RG241 and RZ397 are linked to *Pit*a on chromosome 12. However, we obtained closer distances between the target resistance genes and the markers, RG64 (2.1 cM), RG869 (5. 4 cM), RZ397 (3.3 cM) and RZ536 (7.9 cM) than reported earlier. A new cDNA marker *Npb*181 was identified at 3.5 cM from the *Pi*1 gene. The closer distances and new markers linked to the genes obtained in this study could be due to the larger size of the mapping populations and the saturation of the RFLP map. With the saturation of the rice molecular maps in future, new markers even closer to the genes would further increase the accuracy of identifying the right genotype and successfully avoid recombinants.

It is difficult to select plants with multiple resistance genes based on phenotype alone as the action of one gene may mask the action of another. In such situations, the DNA markers help in identifying single genes in pyramided plants without inoculation and/or progeny testing. In this study both RFLP and the PCR markers were used to select lines carrying the pairs of resistance genes. The availability of the NILs carrying the major genes *Pi*1, *Piz*-5, and *Pit*a in the common CO39 genetic background allowed us to combine the resistant genes easily and quickly. The flanking markers identified by fine mapping made it possible to select the genes in the segregating populations and then pyramid them. Since each NIL (resistant parents in this study) has the resistant allele linked to the RFLP markers it was easy to select those plants putatively carrying resistant alleles based on the polymorphic RFLP banding pattern. Unless there is recombination between the gene and the marker this linkage relationship remains the same from population to population and, hence, any selection made based on this marker would be reliable (Abenes et al. 1993). Plants with different combinations of genes were identified unambiguously as markers do not exhibit epistatic interaction.

The two pyramids *Pi*1+*Piz*-5 (BL12) and *Pi*1+*Piz*-5+*Pit*a (BL124) were equally resistant in both locations tested compared to the isoline with the *Piz*-5 gene. This was expected as *Piz*-5 has a wide resistance spectrum for most of the isolates studied in the Philippines (Chen et al. 1995). The two-gene pyramids in general had a lower level of infection than one of the parents, and their DLA was equal to the best parent. The compatible isolates for each of the genes that could infect single genes failed to attack the pyramided lines, indicating that the two non-allelic genes have a complementary effect when present together. Even though the performance of these pyramids is on par with the *Piz*-5 gene alone, the pyramids could be useful for managing the blast disease by reducing the selection pressure of a single pathogenic race and impart longer life for the varieties (Chen et al. 1995) since it is known that selection solely based on

this gene is likely to be unstable. The only isolates in the Philippines known to be compatible with *Pi*z-5 belong to the genetic lineage 44 (Chen et al. 1995, 1996). All isolates fitted to this lineage are incompatible with *Pi*1. Therefore, a combination of *Pi*1 and *Pi*z-5 should confer resistance to a broader population. *Pi*z-5 is also known to have a broad spectrum of resistance for most South Indian isolates (S Hittalmani, unpublished).

The performance of the lines challenged with isolates C9240–2 and C9240–5 (lineage 44) is intriguing. The latter isolate yielded a virulence pattern fully consistent and compatible with the individual genes of the parental NILs. However, C9245–5, fully compatible with *Pi*z-5 (as are most isolates in lineage 44), was only intermediately incompatible with *Pi*1 and *Pi*z-5. The two- and the three-gene pyramids rather than showing incomplete incompatibility with this isolate were generally fully compatible. It appears that some isolates may carry factors that modify the interactions of their avirulence genes with major resistance genes. Other studies have shown that NILs carrying *Pi*ta can interact with lineage 44 in an analogous manner (Zeigler et al. 1995; DH Chen et al. unpublished). Regardless, the apparent absence of isolates compatible with the *Pi*1+*Pi*z-5 and *Pi*1+*Pi*z-5+*Pi*ta pyramid lines in the field may indicate that while they may occur under laboratory conditions, such strains may not survive in nature. Studies are underway on the relative and absolute fitnesses of strains carrying all compatibility combinations (Silue et al. 1992, Tohme et al. 1993).

Indiscriminate combinations of major genes may be ineffective against some populations, as indicated by the performance of the *Pi*1+*Pi*ta combination in the field (Sivaraj et al. 1996). Thus, knowledge of the resistance spectrum of genes with respect to the target pathogen population will remain important for the success of a MAS gene pyramiding program. The combination of major genes if properly deployed after studying the population structure of the pathogen and its resistance spectrum should be of immense importance.

The pyramids that we developed provide excellent tools for plant pathologists studying the reactions of the different isolates across lineages to different combinations of genes. Studies for testing the durability of the resistance and for their wide applicability is in progress at IRRI (SW Ahn, personal communication). The pyramids could also be useful parental material for integrating the major genes into varieties having partial blast resistance to further enhance host plant resistance. The genes from the pyramids with *Pi*1+*Pi*z-5 and *Pi*1+*Pi*z-5+*Pi*ta have been transferred to popular rice varieties with good partial resistance to combine QTLs and the major genes into varieties more acceptable than CO39 (K Girishkumar and S Hittalmani, University of Agricultural Sciences, Bangalore, India, personal communication).

The use of DNA markers permits the selection of plants with more than one gene without phenotyping. Rice plants now available with two and three known resistance genes selected on the basis of closely linked DNA markers exemplify the power of marker-aided selection. With the availability of PCR-based primers for all the RFLP markers-linked genes, simple and rapid marker-assisted selection could be adopted in rice breeding programs.

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