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Molecular mapping of the blast resistance gene, *Pi44(t)*, in a line derived from a durably resistant rice cultivar

Abstract A recombinant inbred line derived from a cross between CO39 and 'Moroberekan', RIL276, was found to be resistant to lineage 44 isolates of *Pyricularia grisea* in the Philippines. One hundred F_2 individuals were obtained from a backcross of RIL276 and CO39. Phenotypic analysis showed that RIL276 carries a single locus, tentatively named *Pi44(t)*, conferring complete resistance to lineage 44 isolates of *P. grisea*. RFLP probes, STS primers and AFLP markers were applied to identify DNA markers linked to *Pi44(t)*. Neither RFLP nor STS-PCR analysis gave rise to DNA markers linked to the locus. Using bulk segregant AFLP analysis, however, two dominant AFLP markers (AF_{348} and AF_{349}) linked to *Pi44(t)* were identified. AF_{349} and AF_{348} were located at 3.3 ± 1.5 cM and 11 ± 3.5 cM from *Pi44(t)*, respectively. These markers were mapped on chromosome 11 using an F_2 population derived from a cross between 'Labelle' and 'Black Gora'. The location of AF_{348} on chromosome 11 was confirmed using another F_2 mapping population derived from IR40931-26-3-3-5/ PI543851. DNA products at the loci linked to *Pi44(t)* were amplified from RIL276, 'Labelle' and PI543851 using the same primer pairs used to amplify AF_{349} and AF_{348}. Sequence analysis of these bands showed 100% identity between lines. This result indicates that these AFLP markers could be used for the comparison of maps or assignment of linkage groups to chromosomes.

Key words Disease resistance · Rice (*Oryza sativa* L.) · Rice blast (*Pyricularia oryzae* Cav.; *Pyricularia grisea* Sacc.; *Magnaporthe grisea*) · Amplified fragment length polymorphism (AFLP) · Sequence tagged site (STS) · Gene mapping

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Introduction

Rice blast, caused by *Pyricularia grisea* Sacc. (teleomorph *Magnaporthe grisea* Barr.), is one of the most widespread and destructive diseases of rice (Ou 1985). Growing blast-resistant varieties is the preferred method for protecting rice from blast and for reducing the use of fungicides. However, the sustainability of this method is dependent upon the availability and management of resistance genes.

Extensive genetic studies on blast resistance have been conducted. For instance, 13 complete resistance genes at eight loci were identified in Japan through classical genetic analysis (Kiyosawa et al. 1981). In the past few years, more than 15 major genes and ten quantitative trait loci (QTLs) associated with blast resistance have been localized through the use of molecular marker technology (Wang et al. 1994; Yu et al. 1991, 1996; McCouch et al. 1988, 1994; Naqvi et al. 1995; Naqvi and Chattoo 1996).

'Moroberekan', a traditional West African cultivar, is considered to possess durable blast resistance and has been used as resistance donor in many breeding programs. Using Philippine isolates of *P. grisea*, Wang et al. (1994) located two major blast resistance loci,

Pi5(t) and *Pi7(t)*, and nine QTLs for partial resistance in 'Moroberekan' on different chromosomal regions through restriction fragment length polymorphism (RFLP) analysis of the 'Moroberekan'/CO39 recombinant inbred (RI) population. Using this same RI population Naqvi and Chattoo (1995) and Naqvi et al. (1996) identified and mapped two more major resistance loci, *Pi10(t)* and *Pi157*, on chromosomes 5 and 12, respectively, using Indian isolates of *P. grisea*. More blast resistance loci from 'Moroberekan' might be detected if different races of *P. grisea* were to be used.

Populations of *P. grisea* in the Philippines have been characterized extensively. More than 1,500 isolates have been collected from diverse rice genotypes from two blast nurseries and analyzed by DNA fingerprinting. Ten lineages were identified from the 1,500 isolates analyzed (Chen et al. 1995). Among the ten lineages, lineage 44 was found to have unusual properties. It was not detected in the historical collection of *P. grisea* isolates gathered from around the Philippines (Nelson et al., unpublished), and its virulence spectrum was narrow and distinct from that of the other lineages (Zeigler et al. 1995; Chen et al. 1995). Because of its peculiarities, lineage 44 isolates were used to detect blast resistance loci in Moroberekan.

RFLP markers have been useful for constructing the foundation of rice genetic maps (McCouch et al. 1988; Causse et al. 1994; Saito et al. 1991). Other types of DNA markers, such as random amplified polymorphic DNA markers (RAPD) (Welsh and McClelland 1990; Williams et al. 1990) and microsatellite markers (or simple sequence repeats-SSR) (Wu and Tanksley 1993) have been used to further saturate the RFLP maps. More recently, a reliable and efficient polymerase chain reaction (PCR)-based technique, amplified fragment length polymorphism (AFLP), has been developed (Zabeau and Vos 1993; Vos et al. 1995). The AFLP technique allows rapid screening of hundreds of loci, making it an attractive choice for screening DNA markers linked to certain traits and for high-resolution mapping. In this paper, we report the identification and mapping of a blast resistance locus for lineage 44 isolates of *P. grisea* using AFLP analysis.

Materials and methods

Plant materials

All seed was obtained from the International Rice Research Institute. The recombinant inbred line RIL276, derived from the CO39/'Moroberekan' cross, was selected for this study based on inoculation data and an available RFLP data set for the mapping population. RIL276 was estimated to carry 9.5% of the genome from the resistance parent, 'Moroberekan', based on the presence of 12 Moroberekan RFLP alleles out of 127 tested (Wang et al. 1994; Inukai et al. 1996). Based on RFLP and phenotypic analyses, RIL276 was inferred to carry one or more QTLs conditioning

partial resistance to the blast isolate PO6-6 but not to contain *Pi5(t)* and *Pi7(t)*, which were derived from 'Moroberekan' (Wang et al. 1994; Inukai et al. 1996). When inoculated with isolates from lineage 44 of *P. grisea*, RIL276 showed complete resistance. A set of near-isogenic lines carrying known resistance genes in the CO39 genetic background (C101LAC, C101A51, C101PKT and C104PKT; Mackill and Bonman 1992) were used for comparison of the resistance pattern of RIL276.

To identify the locus present in RIL276 that conditions resistance to lineage 44, we crossed RIL276 and CO39. One hundred F_2 progeny were obtained from the cross, and the F_2 individuals were advanced to F_3 families for phenotypic analysis. Two previously reported mapping populations and marker data sets were used for chromosomal localization of the markers linked to *Pi44(t)*. An F_2 population of a cross of 'Labelle'/'Black Gora' was utilized. The population consisted of 80 plants, and a data set was available for 182 RFLP, RAPD and AFLP markers (Redoña and Mackill 1996; Mackill et al. 1996). An F_2 population, developed from a cross of IR40931-26-3-3-5/PI543851 was also used. This population consisted of 199 individuals, and data were available for 96 RFLP and AFLP markers (Xu and Mackill 1996).

Pathogen isolates

Ten lineage 44 isolates of *P. grisea* were used to confirm the resistance in RIL276: C9240-1, C9240-2, C9240-3, C9240-4, C9240-5, C9240-6, C9240-7, C9240-8, C9240-9, C9240-10. For phenotypic analysis of the F_3 families derived from the F_2, isolate C9240-1 was used. It was collected from a near-isogenic line C101A51 [carrying *Pi2(t)*] in an upland rice breeding screening site of the International Rice Research Institute in 1992. C9240-1 was incompatible with RIL276 and compatible with CO39. It was designated lineage 44 based on DNA fingerprinting analysis using a repetitive probe MGR586 (Chen et al. 1995). To compare the reaction pattern of RIL276 with CO39 near-isogenic lines, we selected five isolates (Ca80, PO6-6, 92325-2, 92416-8, and C9240-1) representing different lineages and pathotypes for inoculation.

Inoculation and disease evaluation

Repeated inoculations of F_3 families were conducted to determine the resistance phenotype of each of the F_2 individuals. These tests were conducted in a greenhouse at the International Rice Research Institute in 1995. Briefly, a plastic tray ($37 \times 26 \times 11$ cm) was divided equally into seven rows. About 17 seeds each of 100 F_3 families were sown in each of six rows; the seventh row was divided into two and sown with 10 seeds each of CO39 and RIL276. Plants were fertilized after sowing with ammonium sulfate at a rate of 6 g/kg of soil. Seedlings were grown in a greenhouse with temperatures ranging from $25°$ to $30°C$ for 21 days before inoculation. Inoculum was prepared as described previously (Chen et al. 1996). Before inoculation the spore suspension was standardized at a concentration of 5×10^4 conidia per milliliter in 0.02% Tween 20. Fifty milliliters of spore suspension was sprayed with an electric sprayer onto 21-day-old seedlings (five- to six-leaf stage) in each tray on a rotating platform. Inoculated seedlings were incubated in a dew chamber at $25°C$ for 24 h before being transferred to an air-conditioned greenhouse at $24°-28°C$.

Disease reactions were scored 7 days after inoculation using the following scale: 0 = no evidence of infection; 1 = brown specks (< 0.5 mm), no sporulation; 2 = brown specks (0.5–1.0 mm), no sporulation; 3 = roundish to elliptical lesions (1–3 mm) with grey center and brown margin, without or with little sporulation; 4 = typical spindle-shaped lesions with necrotic grey centers and water-soaked brown margins, capable of sporulation; 5 = lesions as in 4, but about half of one or two leaf blades killed by coalescence of

lesions. Plants with scores of 4 or higher were considered susceptible, and those with scores of 0–3 were classified as resistant.

DNA manipulations

Total rice DNA from each of the F_2 individuals, 'Moroberekan', CO39 and RIL276 were extracted following the protocol described by McCouch et al. (1988). RFLP probes, STS (sequence-tagged site) primers and AFLP markers were applied to screen DNA markers associated with resistance to lineage 44 in RIL276.

Based on the RFLP data set of RIL276 (Wang et al. 1994), five RFLP loci (RG241, RZ192, RG331, RG864 and RG64), which were linked to chromosome segments introgressed from 'Moroberekan' and two restriction enzymes (*Eco*RV and *Dra* I) were selected for this study. Two pairs of primers [designated 426 (5′CCAATCTC-TAGATCTTTGGGGATGATGCC3′) and 432 (3′GACCGGCA-TGTAACGTGACGTC5′), 431 (5′GTTGTTTGAGCTCTCCAA-TGCCTGTTC3′) and 432] were designed from the sequence of RG64 (Hittalmani et al. 1995).

Another 63 STS primers (Inoue et al. 1994) were screened to determine the level of DNA marker polymorphism between the parents. To increase the marker polymorphism for the STS-defined loci, nine restriction enzymes (*Eco*RV, *Alu*I, *Msp*I, *Hinf*I, *Mva*I, *Hha*I, *Rsa*I, *Nde*II and *Taq*I) were used to digest the PCR products. The direct PCR products and the PCR product digests were resolved in a gel composed of 0.5% agarose and 0.75% Synergel (Diversified Biotechnology).

The AFLP protocol was essentially the same as described by Zabeau and Vos (1993), with minor modifications detailed in Mackill et al. (1996). All primers and adapters for this study were synthesized by Operon Technologies. Briefly, rice genomic DNA (500 ng) was digested with *Eco*RI and *Mse*I (New England Biolabs). The restriction fragment ends were ligated with double-stranded adapters. The *Eco*RI adapter was an equal mixture of primers 92A18 (5-GACGATGAGTCCTGAG) and 92A19 (TACTCAGGACTC-AT-5). The *Mse*I adapter was an equal mixture of primers 91M35 (5-bio-CTCGTAGACTGCGTACC) and 91M36 (CTGACGCAT-GGTTAA-5). After pre-amplification with +0/+0 primers (i.e. the adapters served as the primers), selective amplification was performed with primers having three additional selective nucleotides at the 3′ end, specific for the *Eco*RI and *Mse*I adapters, and the amplified fragments were resolved in 4.5% polyacrylamide gel. Signal detection was carried out by end-labeling the primer specific for the *Eco*RI adapter with γ-[^{33}P]-ATP. The gels were dried, and autoradiography was taken with the X-ray film exposed for 3–5 days at room temperature using intensifying screens.

Pooled DNAs, two each for homozygous resistance (RR) and homozygous susceptibility (rr), were made for bulk segregant analysis (Michelmore et al. 1991) and AFLP screening. Each bulk DNA contained equal amounts of DNA from 10 F_2 homozygous individuals. Three hundred and fourteen primer pairs were screened for AFLP markers linked to the resistance locus. Polymorphic AFLP markers associated with resistance in the bulks were confirmed in the F_2 population, from which the bulks were constructed. The segregation data of both AFLP loci and resistance phenotypes (R/S) obtained from this F_2 population were used to estimate the genetic distance between molecular marker loci and the resistance locus. To map the linked markers, parents of mapping populations ('Labelle',

'Black Gora', IR40931-26-3-3-5 and PI543851) were screened with those primer pairs producing AFLP markers linked to the resistance, and segregation data of the AFLP markers at the loci linked to the resistance in RIL276 were obtained from the F_2 populations ('Labelle'/'Black Gora', and IR40931-26-3-3-5/PI-543851). DNA bands at the loci linked to the resistance locus in RIL276 were amplified with the same primer pairs from RIL276, 'Labelle' and PI543851. These DNA bands were excised from the polyacrylamide gel, cloned using the TA cloning Kit (Invitrogen) and sequenced.

Linkage analysis

Linkage analysis was performed using MAPMARKER software (Lander et al. 1987) with a Macintosh computer. The segregation data set of the linked AFLP markers generated from the F_2 population of 'Labelle'/'Black Gora' were combined with the established RFLP, RAPD and AFLP data set for the analysis (Mackill et al. 1996; Redoña and Mackill 1996; Xu and Mackill 1996). Map distances presented in centiMorgans (cM) between markers were derived from the Kosambi function (Kosambi 1944). Standard error was calculated by the maximum likelihood method (Allard 1956). Linkage was considered significant if the logarithm of odds (LOD) score was ≥ 3.0. Mapping results from the F_2 populations of RIL276/CO39 and 'Labelle'/'Black Gora' were integrated to produce a final map of chromosome 11. The chromosomal location of the linked marker was confirmed using the F_2 population of IR40931-26-3-3-5/PI543851.

Results

Genetic and phenotypic analysis

RIL276 showed complete resistance to all ten blast isolates of lineage 44 tested, with scores of 0–2. 'Moroberekan', believed to be the resistance donor of RIL276, was incompatible to these isolates with a score of 0, and CO39, the susceptible parent, was compatible with these isolates, displaying a score of 4 in repeated inoculations (data not shown). The resistance and susceptibility genotypes of the 100 F_2 progeny developed from the cross between RIL276 and CO39 were inferred from an analysis of the corresponding F_3 families by inoculation with blast isolate C9240-1. The segregation of resistance in the F_2 population conformed to a 3:1 segregation ratio, with Chi-square values of 0.21 ($P = 0.65$) and 0.25 ($P = 0.62$) for inoculations I and II, respectively. The segregation ratio of the three genotypes in the F_3 families (RR, Rr and rr) fitted a 1:2:1 segregation ratio (Table 1). In each of the heterozygous F_3 families, resistance versus susceptibility followed a 3:1 segregation based on the Chi-square analysis

Table 1 Chi-square test for the segregation of resistance and susceptibility in F_3 families inoculated with *P. grisea* isolate C9240-1

Inoculation experiment	Homozygous resistant families (RR)	Heterozygous resistant families (Rr)	Homozygous susceptible families (rr)	χ^2 (1:2:1) in F_3 families
I	19	42	14	0.42 ($P = 0.81$)
II	23	57	20	0.27 ($P = 0.87$)

Table 2 Reaction pattern of RIL276 [*Pi44(t)*] and near-isogenic lines to representative isolates of *Pyricularia grisea* from different lineages in greenhouse inoculation (*S* susceptible, *R* resistant)

Isolate	Lineage[a]	CO39	RIL276 [*Pi44(t)*]	C101LAC [*Pi1(t)*]	C101A51 [*Pi2(t)*]	C104PKT [*Pi3(t)*]	C101PKT [*Pi4*[a]*(t)*]
Ca80	4	S	S	R	R	S	R
Po6-6	4	S	S	R	R	R	S
92325-2	7	S	S	S	R	S	S
92416-8	14	S	S	S	R	R	S
C9240-1	44	S	R	R	S	S	R

[a] Chen et al. 1995

(data not shown). These results indicated that a single locus in RIL276 confers resistance to isolate C9240-1.

Results of inoculation experiments conducted using a set of *P. grisea* isolates representing different lineages and pathotypes indicated that the reaction pattern of RIL276 was distinct from those of the CO39 near-isogenic lines (Table 2). In greenhouse inoculations with isolate PO6-6, the lines inferred to carry *Pi5(t)* (RIL249, RIL206, RIL260) and *Pi7(t)* (RIL29 and RIL125) were highly resistant, while RIL276 displayed typical susceptible lesion types. The lesions formed on RIL276 were, however, smaller than those formed on CO39. In field trials in blast screening nurseries in both Indonesia and the Philippines, lines carrying *Pi5(t)* and *Pi7(t)* were highly resistant to field populations of *P. grisea*, while RIL276 was susceptible (Wang et al. 1994). Therefore, the locus in RIL276 was inferred to be distinct from *Pi5(t)* and *Pi7(t)* and was named tentatively as *Pi44(t)* for its resistance to lineage 44 isolates of *P. grisea*.

DNA marker polymorphism between parents

Three hundred and fourteen +3/+3 AFLP primer combinations were surveyed for AFLP marker polymorphism among bulks and parents. Bands amplified with 93 primer combinations for the parents, CO39, 'Moroberekan', and RIL276 were sampled for visual scoring. The number of bands amplified from the parental DNA varied with the primer pairs used. The visible bands ranged from 30 to 130, with an average of 87, for CO39 and 'Moroberekan', and from 21 to 120, with an average of 73, for CO39 and RIL276. Almost all the primer combinations produced polymorphism between the parents. The level of polymorphism for each of the primer combinations was variable, ranging from 12% to 54% (mean 32%) between CO39 and 'Moroberekan' and from 0% to 21% (mean 5.6%) between CO39 and RIL276.

A low level of polymorphism was detected using the 63 STS primers designed by Inoue et al. (1994). Direct PCR polymorphism was 0.63% between CO39 and 'Moroberekan' (4/63) and 0% between CO39 and RIL276. After the PCR products were digested with nine restriction enzymes, 31% polymorphism was detected between CO39 and 'Moroberekan'. Among the 63 STS markers surveyed between the parents, 18 markers (28.6%) in RIL276 were from CO39, 44 (69.8%) markers in RIL276 were monomorphic with CO39 and 'Moroberekan' and only 1 STS marker (1.6%) was inherited from 'Moroberekan'.

DNA markers linked to the resistance locus in RIL276

Among the 63 STS loci surveyed, only 1 locus gave polymorphism between CO39 and RIL276. Locus G396, amplified using primers G396F (5′ TTCGCA-TTCTTGGCTGGTGT 3′) and G396R (5′ GGTGA-TCTGGATGAATCTGA 3′) and located on chromosome 5 (Inoue et al. 1994), showed a polymorphism after the PCR products were digested with either *Rsa*I or *Alu*I. When the F_2 progeny was analyzed with either combination of G396/*Rsa*I or G396/*Alu*I, all the progeny showed the RIL276 profile. Apparently, this marker was not associated with the resistance in RIL276.

We hypothesized that *Pi44(t)* in RIL276 could be an allele of the *Pi2(t)* locus for two reasons. First, RIL276 had been previously shown to carry a segment of 'Moroberekan' DNA on chromosome 6 that includes the marker RG64, which is linked to *Pi2(t)* (Yu et al. 1991; Wang et al. 1994). Second, the resistance spectrum of RIL276 is the inverse of that of *Pi2(t)* (Table 2). That is, while *Pi2(t)* conditions resistance to the isolates tested, *Pi44(t)* is compatible with these isolates; *Pi2(t)* is compatible with lineage 44 isolates, while *Pi44(t)* is incompatible with these isolates. To test this hypothesis we applied 2 STS primer pairs (431/432, 426/432) designed from the sequence of RG64 (Hittalmani et al. 1995) to analyze the DNA marker segregation among the F_2 progeny. Pair 431/432 amplified polymorphic bands between CO39 and RIL276; these displayed a 1:2:1 segregation ratio in the F_2 population, as expected for a codominant marker. Pair 426/432 amplified one band for CO39 but no band for RIL276, and displayed a 3:1 segregation ratio in the F_2 progeny. When the segregation of the DNA markers with the resistance was analyzed, none of the RG64-STS markers were associated with the resistance in RIL276 specific for lineage 44. This indicated that *Pi44(t)* in RIL276 is not an allele of the *Pi2(t)* locus.

In addition to the introgression on chromosome 6, available RFLP data indicated that RIL276 carries
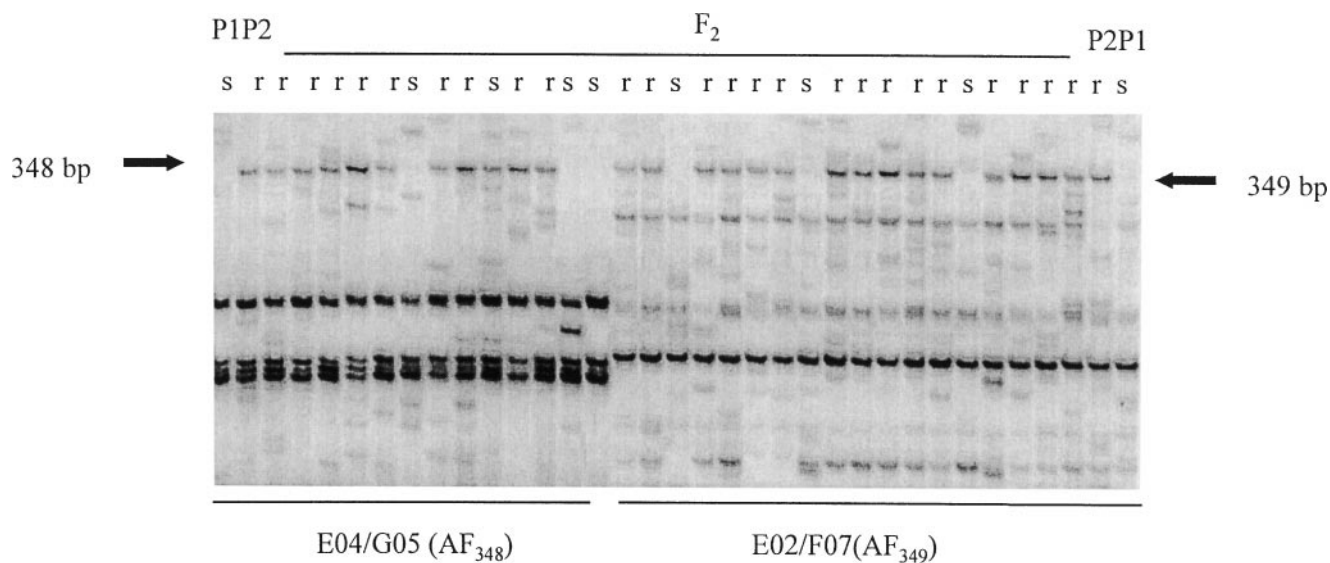
Fig. 1 AFLP profile showing cosegregation of AFLP markers AF_{348} and AF_{349} and resistance phenotypes in the F_2 population of the CO39/RIL276 cross. *Arrows* indicate the loci linked to *Pi44(t)*. AF_{348} and AF_{349} were amplified by primers E04/G05 and E02/F07, respectively. *P1* CO39, *P2* RIL276, *r* is for resistance phenotype, *s* is for susceptible phenotype. CO39 was susceptible to the blast isolate C9240-1, and 'Moroberekan' and RIL276 were resistant to this isolate

'Moroberekan' chromosomal segments from chromosomes 1, 4 and 10 (Wang et al. 1994; Inukai et al. 1996). To determine whether one of these segments is associated with resistance to lineage 44, we selected probes RG331 (chr. 1) RG864 (chr. 4) and RG241 (chr. 10) for analyzing of the F_2 progeny of RIL276. RG331/*Eco*RV, RG864/*Dra*I and RG241/*Eco*RV gave rise to polymorphism between CO39 and RIL276. While these markers showed a 1:2:1 ratio in the F_2 progeny of CO39/RIL276, none of them were associated with the resistance in RIL276 to lineage 44 isolates.

Because linked markers were not found using RFLP and STS, bulk segregant analysis using the AFLP technique was employed to identify the DNA markers linked to the resistance locus in RIL276. Out of 314 primer combinations screened, primer pairs E04/G05 and E02/F07 were found to amplify the dominant markers AF_{348} and AF_{349}, respectively, that were associated with the resistance phenotype in the F_2 population of CO39/RIL276 cross (Fig. 1). AF_{349} was present in RIL276 and 'Moroberekan' and absent in CO39, while AF_{348} was present in RIL276 but absent in 'Moroberekan' and CO39. To determine if the DNA sequence of AF_{348} was present in 'Moroberekan' CO39, we digested genomic DNA of 'Moroberekan', RIL276 and CO39 with *Eco*RI, *Eco*RV, *Mse*I, *Pst*I, *Hin*dIII and double-digested it with *Eco*RI and *Mse*I. Southern analysis using AF_{348} as a probe showed that a single band was detected from RIL276, 'Moroberekan' and CO39, indicating the presence of the
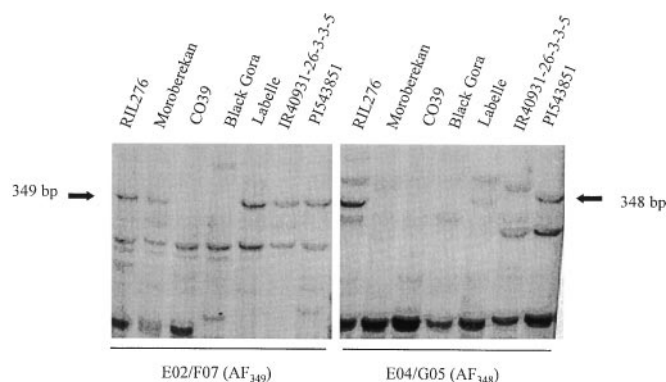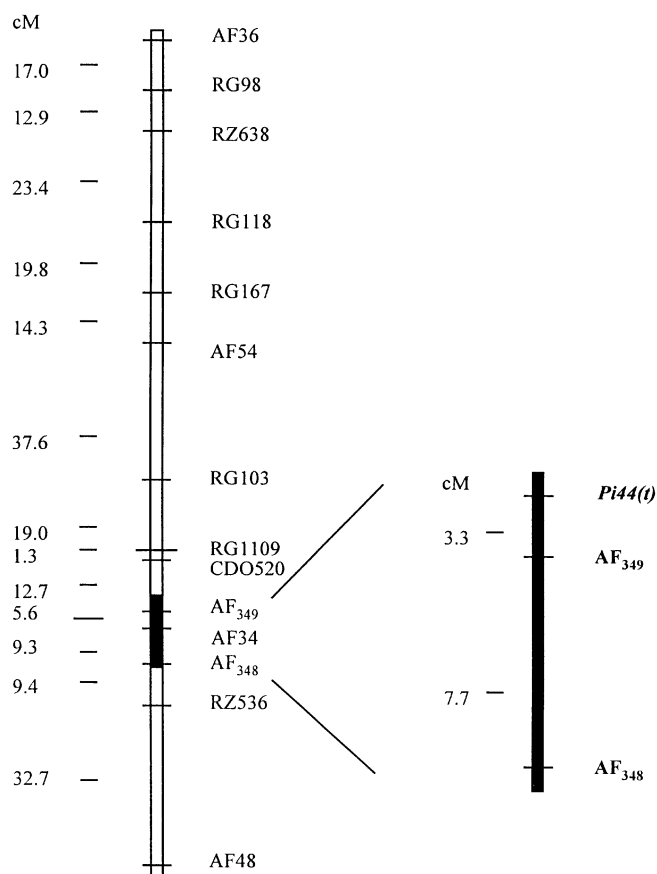

Fig. 2 AFLP survey of markers AF_{348} and AF_{349} between parents of mapping populations. *Arrows* indicate the polymorphic loci linked to *Pi44(t)* in RIL276. 'Black Gora' and 'Labelle' were the parents of one F_2 mapping population, and IR40931-26-3-3-5 and PI543851 were the parents of another F_2 mapping population

AF_{348} DNA sequence in 'Moroberekan' and CO39. Among the six enzyme digestions, the *Eco*RI digestion gave rise to polymorphism between 'Moroberekan' and CO39. RIL276 was found to inherit the 'Moroberekan band'. The absence of AF_{348} in 'Moroberekan' from the AFLP analysis was thus tentatively attributed to a mutation at an *Eco*RI or *Mse*I restriction site during the generations of developing RIL276.

AF_{348} and AF_{349} were found to be polymorphic between 'Labelle' and 'Black Gora', parents of an F_2 mapping population (Fig. 2) (Redoña and Mackill 1996), and these bands segregated in the F_2 population. AF_{348} was found to be polymorphic between IR40931-26-3-3-5 and PI543851 (Fig. 2), parents of another F_2 mapping population (Xu and Mackill 1996), and it segregated in this F_2 population. The segregation ratios of these markers in both F_2 populations were as expected for a 3:1 ratio by Chi-square analysis at the $P = 0.05$ level.

To determine if the co-migrating AFLP products amplified by the same primer combination present in different mapping populations represented a similar DNA sequence, we cloned and sequenced the amplified products. The DNA segment (AF$_{349}$) amplified by E02/F07 and associated with the resistance in RIL276 was 349 bp, and the DNA segment (AF$_{348}$) amplified by E04/G05 and associated with the resistance in RIL276 was 348 bp. The DNA segment amplified from 'Labelle' by primers E02/F07 at the AF$_{349}$ locus was 100% identical with AF$_{349}$ from RIL276; and the DNA segment at the AF$_{348}$ locus amplified from 'Labelle' and PI543851 by E02/G05 was identical with AF$_{348}$ from RIL276 (sequence not shown). These results indicated that the DNA segments of AF$_{348}$ and AF$_{349}$ were conserved among these rice genotypes and that the resistance locus Pi44(t) in RIL276 can be mapped using these available mapping populations.

Linkage analysis revealed that AF$_{349}$ was 3.3 ± 1.5 cM and AF$_{348}$ was 11 ± 3.5 cM away from Pi44(t), respectively. The linked markers AF$_{349}$ and AF$_{348}$ were then mapped in the F$_2$ population of 'Labelle'/'Black Gora' and found to be located on chromosome 11 between RFLP markers CDO520 and RZ536 (Mackill et al. 1996) (Fig. 3). The location of AF$_{348}$ linked to Pi44(t) on chromosome 11 was confirmed in the F$_2$ population of IR40931-26-3-3-5/ PI543851 (data not shown).

To further assess the origins of the chromosome segment associated with resistance to lineage 44 isolates, we tested two microsatellite markers located between RG1109 (a marker tightly linked to CDO520) and RZ536 for RIL276, 'Moroberekan' and CO39 (Chen et al. 1997). RM254 was found to be monomorphic for 'Moroberekan' and CO39. RM224 gave non-parental bands in RIL276.

Fig. 3 AFLP map of Pi44(t) on rice chromosome 11. This map was generated from the 'Black Gora'/'Labelle' F$_2$ mapping population. The segregation data of AF$_{348}$ and AF$_{349}$ in this population were integrated with the existing marker segregation data set for linkage analysis (Redoña and Mackill 1996). The linkage of Pi44(t) with AF$_{348}$ and AF$_{349}$ were analyzed using the F$_2$ population of CO39/RIL276. The location of AF$_{348}$ on chromosome 11 was confirmed using the F$_2$ population of IR40931-26-3-3-5 and PI543851

Discussion

The West African upland rice variety 'Moroberekan' is considered to have durable resistance to blast disease. This durable resistance has been attributed to a combination of complete and partial resistance factors (Ikehashi and Khush 1979; Wang et al. 1994). Four major loci conferring complete resistance to blast and nine QTLs for partial resistance were previously reported in 'Moroberekan' (Wang et al. 1994; Naqvi et al. 1995, 1996). The work reported here was undertaken to allow mapping of an additional and intriguing resistance gene identified in a recombinant inbred line derived from 'Moroberekan'. The gene was mapped and tagged with AFLP markers. However, non-parental alleles were detected at marker loci linked to the gene.

While it is possible that the two marker loci (microsatellite marker RM224 and AFLP-derived marker AF$_{348}$) were both legitimately inherited from 'Moroberekan' and subsequently mutated, it seems more probable that pollen contamination occurred during the development of RIL276 or that the parental strain of 'Moroberekan' (or CO39) was heterozygous for this chromosomal region. Thus, while a novel gene has been mapped, on the basis of the present investigation it is not possible to conclude that the gene was derived from 'Moroberekan'.

The resistance locus Pi44(t) in RIL276 was different in its resistance spectrum from the two previously reported 'Moroberekan' resistance loci, Pi5(t) and Pi7(t) (Wang et al. 1994). Both Pi5(t) and Pi7(t) condition complete resistance to blast isolate PO6-6, while RIL276 carrying Pi44(t) was susceptible to isolate PO6-6. RIL276 was susceptible to the blast pathogen populations present in two field trials conducted in the Philippines and Indonesia, while lines carrying Pi5(t) or Pi7(t) were highly resistant (Wang et al. 1994). In

extensive greenhouse inoculations, RIL276 was susceptible to all the isolates from different lineages tested except for lineage 44 isolates, while lines carrying *Pi5(t)* and *Pi7(t)* were resistant to some of the isolates tested (Inukai et al. 1996; Wang et al. 1994).

The chromosome location of *Pi44(t)* is distinct from the four major blast resistance loci previously identified in 'Moroberekan' (Wang et al., 1994; Naqvi et al. 1996). *Pi44(t)* is located on chromosome 11, linked to AFLP markers AF_{349} and AF_{348}, and bracketed by RFLP markers RZ536 and CDO520 in the region where *Pi1* is located (Yu et al. 1996). Although *Pi7(t)* was also localized on chromosome 11, it is in the region flanked by RFLP markers RG103 A and RG16 (Wang et al. 1994).

The use of combinations of resistance genes with complementary resistance spectra has been suggested as a method for increasing the utility of major gene resistance (Zeigler et al. 1995). Though the resistance provided by single genes is usually short-lived in blast-prone environments, it is likely that combinations of loci with different resistance spectra against different lineages would provide an effective barrier to the pathogen infection. *Pi2(t)*, which is probably allelic to *Piz* (Inukai et al. 1994), showed the broadest resistance spectrum among two sets of near-isogenic lines tested in greenhouse inoculations in the Philippines and in the International Blast Nursery trials (Chen et al. 1996). It was, however, overcome by lineage 44 isolates (Chen et al. 1996). The resistance spectrum of *Pi44(t)* identified in this study is complementary to that of *Pi2(t)*. The combination of *Pi2(t)* and *Pi44(t)* together with other genes conditioning qualitative and quantitative resistance would provide a broad spectrum and possibly durable resistance (Zeigler et al. 1995; Chen et al. 1996).

The identification of DNA markers linked to the blast resistance locus *Pi44(t)* should facilitate the development of a near-isogenic line for this gene (Inukai et al. 1996). In this study, RIL276 was backcrossed to CO39. The F_2 genotypes were inferred from the inoculation of F_3 families. Candidates for near-isogenic lines carrying *Pi44(t)* could be easily selected from the lines homozygous resistant to lineage 44 isolates. With a few more cycles of backcrossing and selection, near-isogenic lines carrying *Pi44(t)* could be achieved.

Although *Pi44(t)* and *Pi1* were both located in the same region on chromosome 11, the resistance spectrum of *Pi44(t)* is much more narrow than that of *Pi1*. The multi-allele locus *Pi-k* is also present on chromosome 11 near *Pi1*. The allelism relationship between *Pi44(t)* and the alleles of the *Pi-k* locus needs to be determined. Interestingly, in addition to *Pi44(t)*, *Pi1* and *Pi7(t)*, six other loci for blast resistance (*Pik*, *Pif*, *Piis-1*, *Pise-1*, *Pikur-2*, *Pia*) and six loci for bacterial blight resistance (*Xa3*, *Xa4*, *Xa10*, *Xa21*, *Xaa*, *Xah*) are located on chromosome 11 (Kinoshita 1995), making this the most densely populated chromosome with resistance genes. The evolutionary relationship among these resistance genes will be uncovered once these genes are cloned and analyzed at the molecular level.

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