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Development of Co-Dominant Amplified Polymorphic Sequence Markers in Rice that Flank the *Magnaporthe grisea* Resistance Gene *Pi7(t)* in Recombinant Inbred Line 29

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ABSTRACT

Campbell, M. A., Chen, D., and Ronald, P. C. 2004. Development of co-dominant amplified polymorphic sequence markers in rice that flank the *Magnaporthe grisea* resistance gene *Pi7(t)* in recombinant inbred line 29. Phytopathology 94:302-307.

Pi7(t), a dominant blast resistance gene derived from the rice cultivar Moroberekan, confers complete resistance against the fungal pathogen *Magnaporthe grisea. Pi7(t)* previously was positioned on chromosome 11 by restriction fragment length polymorphism (RFLP) mapping of a recombinant inbred line population. One derivative of this population, recombinant inbred line (RIL)29, was designated as the representative line for *Pi7(t).* A segregating F_2 population was created from RIL29 in order to determine the location of *Pi7(t).* The new mapping data indicate a position for *Pi7(t)* 30 centimorgans distal to the original location. *Pi7(t)* shares a common position with the previously mapped *Pi1 M. grisea* resistance gene. RIL29 carries DNA not derived from either parent used to create the RIL population at the newly assigned *Pi7(t)* locus. RFLP analysis has identified a possible donor source.

The ascomycete *Magnaporthe grisea* is present in nearly all areas of rice cultivation and is a primary biotic source of yield loss worldwide (15). The utilization of resistant cultivars is the most cost-effective means to mitigate disease losses. However, monogenic resistance commonly is rendered ineffective if frequency of corresponding virulence alleles increases in the pathogen population (15). Strategies that would create more durably resistant cultivars presently are focusing upon the pyramiding of resistance loci with differing resistance spectra into a single cultivar through marker-assisted selection (1). In order to successfully generate resistance (*R*)-gene pyramids, an accurate map position and tightly linked markers are required for efficient marker-assisted selection strategies. This report details the genetic characterization of a major dominant resistance gene.

Over 30 *M. grisea* resistance genes have been positioned onto the rice classical genetic map (9). One of those resistance genes, *Pi7(t),* was identified through screening with *M. grisea* and restriction fragment length polymorphism (RFLP) mapping of a recombinant inbred line (RIL) population derived from a cross between the rice cvs. Moroberekan and CO39. *Pi7(t)* was positioned on the long arm of chromosome 11 near the bacterial blight resistance locus *Xa21* (19). *Pi7(t)* was evaluated with diverse *M. grisea* isolates to determine the effective reaction spectrum. *Pi1*, another blast resistance locus present in C101LAC, has a blast reaction spectrum identical to that of *Pi7(t)* but *Pi1* and *Pi7(t)* were not mapped to the same position (7,14,19,21). C101LAC was derived from a cross between rice cvs. LAC23 and CO39. *Pi1* was mapped in two independent reports using segregating populations (14,21). In both of these reports, *Pi1* was linked with markers near the telomere on the long arm of chromosome 11 (14,21). Allelism tests performed using C101LAC carrying *Pi1* and RIL29 carrying *Pi7(t)* (14,21) indicate tight linkage of *Pi1* and *Pi7(t)* (7).

Because the positioning of *Pi7(t)* by RFLP mapping and the allelism tests are in disagreement, we decided to map *Pi7(t)* using a segregating F_2 population. This report provides conclusive data that *Pi7(t)* is tightly linked to *Pi1*. Furthermore, our data demonstrate that RIL29, the representative line for *Pi7(t),* carries non-parental DNA (i.e., derived from neither Moroberekan nor CO39) at the genomic interval spanning the *Pi1/Pi7(t)* locus. LAC23, the donor for *Pi1*, is the likely source.

MATERIALS AND METHODS

Plant materials. All seed were obtained from the International Rice Research Institute (IRRI). The RILs RIL29 and RIL206 were derived from an initial cross between Moroberekan and CO39. Fifteen F_1 hybrids from a Moroberekan and CO39 cross were allowed to self to produce 300 F_2 individuals. The F_2 through F_6 generations were created by single-seed descent from the original selected F_2 individuals The F_2 lines were scored for complete resistance with the PO6-6 isolate of *M. grisea* (19). RIL29 was identified as a representative line containing the locus *Pi7(t)* (7). RIL206 was used as a positive control because this line has amplified fragment length polymorphisms (AFLPs) based Moroberekan markers near the *Pi1* locus (3; M. A. Campbell, *unpublished data*). RIL29 was backcrossed to CO39. The F_1 hybrid was allowed to self-pollinate. Fifty F_2 progeny were isolated from the F_1 hybrid and inoculated with isolate PO6-6. These inoculated F_2 individuals were advanced to the F_3 generation for phenotypic analysis with the Philippine blast isolate PO6-6.

Pathogen isolates and inoculation methods. The PO6-6 isolate of *M. grisea,* which has been utilized previously in the initial characterization of the RIL and near isogenic lines (NILs), was used in this study (11). All resistance characterization using PO6-6 of the F_2 and F_3 plants was conducted at IRRI. Fifty F_2 plants were grown in a plastic tray with Moroberekan, CO39, and RIL29 as controls. For F_3 family resistance phenotype characteri-

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zation, plastic trays (37 by 26 by 11 cm) were divided into seven equal rows. The first six rows contained 20 F_3 seedlings from each of the F_3 families. The seventh row contained 10 CO39 and 10 RIL29 seed. Plants were fertilized with ammonium sulfate at a rate of 6 g/kg of soil. Seedlings were grown in the greenhouse for 21 days before inoculation. Inoculum was prepared as described previously (4). Seedlings then were inoculated with 5×10^4 conidia/ml with 0.02% Tween 20. Inoculum suspension (50 ml) was sprayed onto each tray and then held in a dew chamber at 25°C for 24 h. Seedlings then were moved to greenhouses at IRRI. DNA was extracted from the individuals and brought to the University of California (UC)-Davis for molecular characterization. The PO6-6 isolate cannot be imported into California due to U.S. Department of Agriculture restrictions and no inoculations were possible at UC-Davis.

Disease evaluation. Disease reactions were scored 7 days postinoculation using the following scale: 0 = no evidence of infection; 1 = brown speckling (<1 mm); 2 = brown specks (1 to 2 mm); 3 = round to elliptical lesions (2 to 4 mm) with gray center and brown margins; 4 = spindle-shaped lesions with necrotic centers, capable of sporulation; and 5 = coalesced type 4 lesions that have killed the majority of the leaf blade. Plants with scores of 0 to 3 are considered resistant and scores of 4 to 5 indicate susceptibility.

DNA manipulations. Total rice DNA from each of the F_2 and F_3 individuals, Moroberekan, CO39, RIL29, RIL206, LAC23, and C101LAC were extracted following the protocols previously described (13). Of the 50 F_2 individuals, three F_2 DNA samples were found to be degraded upon transport to UC-Davis and discarded from further molecular characterization. The F_3 DNA was used to verify the F_2 genotype using co-dominant amplified polymorphic sequence (CAPS) markers. Nipponbare × Kasalath RFLP markers were obtained from specific primers using polymerase chain reaction (PCR) except C10150S, C481S, C105, C10295S, and R543. These cDNA based markers were obtained directly from the Rice Genome Research Project (RGP) as purified plasmids. These plasmids were transformed into DH5α chemically competent cells and amplified from liquid cultures with appropriate universal primers. For RFLP analysis, 6 µg of genomic DNA was digested in a total volume of 60 µl for 12 h. One-tenth of the reaction volume was run on a mini-gel to verify digestion. The remaining DNA was separated via electrophoresis in 0.9% agarose gels and transferred to Hybond N+ membranes (Amersham Pharmacia Biotech, Piscataway, NJ) (without EtBr staining) using 0.4 N NaOH. RFLP probes were generated from gel-purified PCR amplicons (Qbiogene, Montreal, Canada) by the random hexamer labeling method using γ-[^{32}P]dCTP. The membranes were hybridized and washed by manufacturer's protocols (Amersham). The washed filters were visualized using Phosphoimager screens (Molecular Dynamics, Mountain View, CA).

PCR amplifications. CAPS markers were amplified using *Taq* polymerase buffer, 200 µM dNTP, 1 mM primer, 100 to 200 ng of DNA, and *Taq* polymerase (Promega Corp., Madison, WI). CAPS marker development and use have been described previously (8). All amplifications were run on a Tetrad thermocycler (MJ Research Inc., Waltham, MA). All CAPS markers had the following profile: preheat 94°C for 2 min, 40× (94°C for 45 s, variable annealing temps [Table 1] for 45 s, and 1 min and 45 s extension for 72°C). Amplification cycles were completed with a 10-min exten-

TABLE 1. Name, position, accession number, and primer sets of all rice markers developed and evaluated in this study

Marker	Position origin[a]	Accession, anneal temperature (°C)[b]	Primer sets[c]
C950	110 cM, RGP(YAC)	TIGR TC56715, For, 60	5′AGAGCTCTAGGGTTTCCGCTGCCG
		Rev	5′GAGTACTTAGGTTATTAGGCCTTC
22A14	110–112.9 cM, CUGI(STC)	OSJNBa22A14f, For, 55	5′TTACAGTTCTTTTTATAAAGTAAG
		Rev	5′TTGATACAAATCGTAATTACACAT
R251	112.9 cM, RGP(N/K)	TIGR TC62909, For, 55	5′GATCAGGAAGATGCTGGAGAAGCT
		Rev	5′CTCCTGGTCTCGTCGCTGTACAGCC
C30662a	112.9 cM, RGP(YAC)	TIGR TC62275, For, 61	5′GTGCCATCCACGTATCCAACGAACG
		Rev	5′AGATATATAACCATGTGTACACTAT
C30662b	112.9 cM, RGP(YAC)	TIGR TC 66850, For, 58	5′TTCAAACGATCGATCGACAGGCATC
		Rev	5′GAAATCACTGGAGCGGAGAGCTTC
E50658	112.9 cM, RGP(YAC)	GB AU030126, For, 58	5′CTCATTGGGCGTCGATGATCGAGA
		Rev	5′AATACAGCATTGTATTCGATGACTG
E50301S	116.1 cM, RGP(YAC)	TIGR TC61223, For, 58	5′TCAAGTCTGTATCTGACAGCCCTTC
		Rev	5′CATGGAACAGCTGTTCATATTCAGG
R1506	116.1 cM, RGP(N/K)	TIGR TC51900, For, 61	5′CTTGGAAGGTGCTAATCAGCATGTC
		Rev	5′GTTATCTCGTGAGCTAGAAAGAGA
61M17	116.1–117.0 cM, CUGI(STC)	OSJNBb61M17f, For, 58	5′CTGCAAGATGGAGTACTTACTGATA
		Rev	5′GCCAACCTGATATGAATATTGTGGG
S12886	117.0 cM, RGP(N/K)	5′ GB D47425, For, 58	5′CAAGGTAGAAGTGAACAAGGTTAG
		3′ GB AU082171, Rev	5′GTAAGTCACACAAGCTATGTTGCAC
C10150[d]	117.0 cM, RGP(N/K)	GB D21990, For, N/A	N/A
		Rev	N/A
C481S[d]	117.0 cM, RGP(N/K)	GB D15341, For, N/A	N/A
		Rev	N/A
C105[d]	117.3 cM, RGP(N/K)	GB D28177, For, N/A	N/A
		Rev	N/A
C10295S[d]	117.3 cM, RGP(YAC)	TIGR TC63186, For, N/A	N/A
		Rev	N/A
R543[d]	117.6 cM, RGP(N/K)	TIGR TC56778, For, N/A	N/A
		Rev	N/A
S10640	117.9 cM, RGP(YAC)	TIGR TC58996, For, 56	5′GTGATAATGTTCTTGACACTGGAG
		Rev	5′GTAAACCAACATTTTACGATGTAG

[a] Position in centimorgans (cM). RGP(N/K) = restriction fragment length polymorphism (RFLP) marker derived from the Rice Genome Research Project (RGP) Nippobare × Kasalath-based genetic map; CUGI(STC) = Clemson University Genomics Institute bacterial artificial chromosome (BAC) end sequence, which is referred to as a sequence tagged connector (STC); RGP(YAC) = cDNAs which were found to hybridize to a single yeast artificial chromosome (YAC). An ordered YAC array previously was developed and positioned onto the Nipponbare × Kasalath map.

[b] For = forward, Rev = reverse, N/A = not applicable.

[c] N/A = not applicable.

[d] These RFLP markers were obtained directly from RGP in plasmids. No primer sets were developed.

sion at 72°C. All primers were obtained from Operon Technologies, Inc., Alameda, CA.

Polymorphism screens for CAPS markers. Each primer set was used to amplify genomic DNA from the parents (i.e., CO39 and RIL29) in 10 separate 50-µl reactions. The 10 reactions then were pooled for polymorphism surveys. In all, 20 to 30 common restriction enzymes were used for each marker. From each parent, PCR amplification product (15 µl) was digested in a 40-µl total reaction volume for 3 h at the manufacturer's specified temperature and buffer conditions. Digestion products were separated via electrophoresis in a 1.5% agarose gel in Tris-acetate EDTA (TAE) buffer and visualized with ethidium bromide under UV illumination.

F_2 and F_3 analysis using CAPS markers. All amplifications from F_2 individuals with primer sets for CAPS markers had a 0-µl reaction volume. Ten microliters of all F_2 PCR amplifications were separated via electrophoresis on a 0.8% agarose test gel in TAE buffer to ensure amplification quality. The F_2 PCR reaction (20 µl) was digested for 3 h at the manufacturer's specified temperature and buffer conditions in a total reaction volume of 50 µl. Digestion products were separated via electrophoresis in a 1.5% agarose gel in TAE buffer and visualized after staining with ethidium bromide. Four to six F_3 progeny from each F_2 parent were genotyped by CAPS markers to ensure consistency.

RESULTS

Phenotypic analysis of 50 F_2 derived from the CO39–RIL29 cross. RIL29 is the representative line for *Pi7(t). Pi7(t)* confers complete resistance to blast isolate PO6-6 and RIL29 has a reaction score of 0. CO39 showed complete susceptibility, with reaction scores of 4 or 5. Moroberekan was completely resistant to PO6-6 with a reaction score of 0. RIL29 was backcrossed to CO39 to generate an F_2 mapping population. The F_2 population of 50 individuals was scored directly with PO6-6 and found to follow the predicted 3:1 phenotypic segregation ratio for a single dominant gene. In the F_2 phenotypic analysis, there were 41 resistant and 9 susceptible individuals. The χ_2 sum for this F_2 analysis was 1.4 ($P = 0.25$). Each of the F_2 individuals was advanced to F_3 for further resistance phenotype confirmation. F_3 progeny from each of the F_2 progenitors were scored with PO6-6 and used to verify the disease scores obtained from the F_2 inoculation. The segregation ratios for the three genotypes in the F_3 families were 19 homozygous resistant (RR), 22 heterozygous (RS), and 9 homozygous susceptible (SS). The χ_2 sum for a 1:2:1 ratio was 4.8 ($P = 0.09$). Within each heterozygous F_3 population, the resistance and susceptible ratios fit the predicted 3:1 segregation ratio and the χ_2 analysis was consistent within all heterozygous F_3 families. These results supported the hypothesis that RIL29 possesses a single dominant locus for resistance to the blast isolate PO6-6.

Resistance to *M. grisea* isolate PO6-6 in RIL29 is located on chromosome 11. Both *Pi1* and *Pi7(t)* initially were mapped using a chromosome marker set derived from the mapping population developed at Cornell in the late 1980s and early 1990s (17). However, all markers used in this report are derived from the high-density RFLP map developed by the Nipponbare and Kasalath cross (5). Subsequent to the creation of the Nipponbare × Kasalth map, a tiled bacterial artificial chromosome (BAC) array was developed using Nipponbare (22). *Pi1* is tightly linked with the Cornell markers *G181* (derived from the RFLP marker *XNpb181*) and *RZ536* (21). *G181* was incorporated as an RFLP marker into the Nipponbare × Kasalath map at position 111.8 centimorgans (cM). Sequence analysis showed that the Cornell-based marker *RZ536* shares exact sequence identity with the Nipponbare × Kasalth RFLP marker *S10003. S10003* is found at 117.9 cM on the Nipponbare × Kasalath-based map (Fig. 1).

To verify that *Pi7(t)* was indeed on chromosome 11, a CAPS marker was developed using the cDNA-based RFLP marker *S12886. S12886* is positioned on the Nipponbare × Kasalath map at 117.0 cM. Primers were designed from the two ends of the cDNA sequence (GenBank entries AU082171 and D47425 for the 5′ and 3′ ends, respectively) for *S12886*. The amplicon from genomic DNA was approximately 1,200 bp in length for the parental cvs. Moroberekan and CO39 as well as their derivative, RIL29. *Hpa*II was found to digest the CO39 into two fragments and left both the Moroberekan and the RIL29 alleles undigested. If the allelism test results for tight linkage of *Pi1* and *Pi7(t)* are accurate, then the *S12886* CAPS marker should show tight linkage with the F_2 PO6-6 resistance genotype. Exact co-segregation was found between the CAPS marker derived from *S12886* and the resistance locus *Pi7(t)* for the 47 F_2 individuals from the CO39 × RIL29 population. Four to six F_3 progeny of these 47 F_2 parents also were genotyped with the *S12886* CAPS marker and confirmed the F_2 genotype. Therefore, (i) *Pi7(t)* is on chromosome 11, (ii) the original chromosomal assignment of *Pi7(t)* is incorrect, (iii) *Pi7(t)* maps to the same location as the resistance locus *Pi1*, and (iv) the conflict between the molecular and genetic maps is resolved.

To refine this analysis, a new set of CAPS markers was developed at the *Pi1* locus. The markers were derived from two sources:

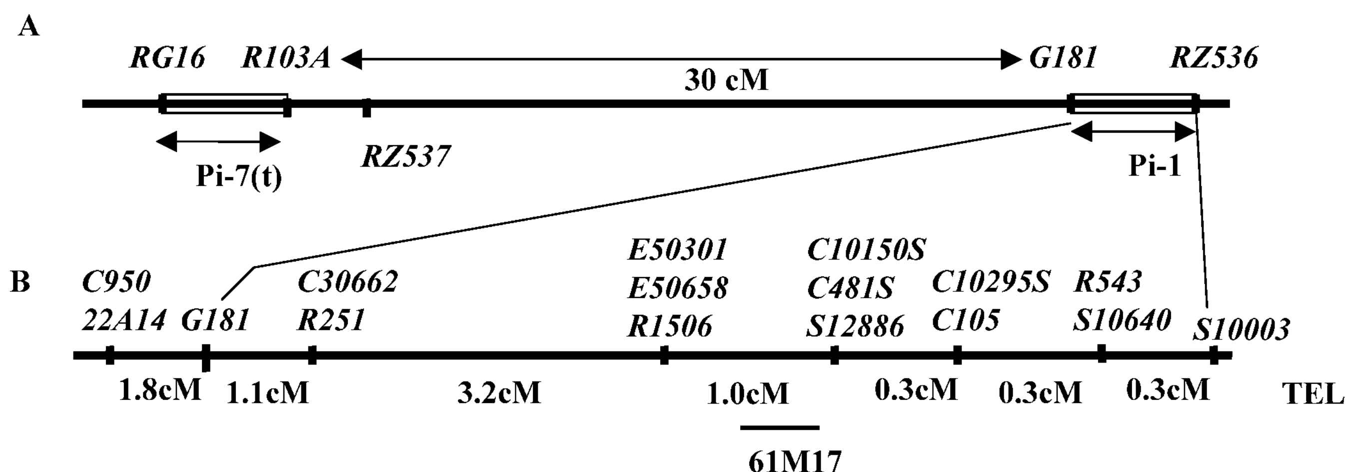

Fig. 1. A, Relative map positions of *Pi1* and *Pi7(t)* on the long arm of chromosome 11 using the Cornell markers from the 1992 high-density map. **B,** Map of the *Pi1* locus using the Rice Genome Research Project (RGP) markers from the Nipponbare × Kasalath high-density classical map, yeast artificial chromosome (YAC) positive restriction fragment length polymorphism (RFLP) markers, and the bacterial artificial chromosome (BAC)-end sequence markers. *61M17* represents a BAC that was positioned on the ordered BAC array between the RGP markers *R1506* and *S12886*. Note that *RZ536* from the Cornell-based map has exact sequence identity with the Nipponbare × Kasalath RFLP marker *S10003*.

(i) sequence of the RFLP markers from the Nipponbare × Kasalath map (5) and (ii) the sequence tagged connector (STC) markers derived from the end sequence of a tiled Nipponbare BAC assembly (22). The chromosomal arrangement of all markers utilized in this study are illustrated in Figure 1 and detailed in Table 1.

The original cross to generate the RILs utilized upland *japonica* cv. Moroberekan and susceptible *indica* cv. CO39. The intersubspecies cross was predicted to possess sufficiently high rates of polymorphism to efficiently generate CAPS markers. Eight CAPS markers, including *S12886*, were found at the *Pi1* locus. Table 2 details the CAPS polymorphisms.

In addition to *S12886*, three CAPS markers were selected for mapping *Pi7(t).* The markers *22A14*, *R251*, and *C30662b* were applied to the segregating F_2 population and spanned a 7-cM interval on chromosome 11. *R251* and *C30662* are cDNA-based RFLP markers from the Nipponbare × Kasalath map at 112.9 cM (Fig. 1) STC sequence from BAC clone OSJNBb0089D23 is identical to the Nipponbare × Kasalth RFLP marker *C950*. The marker *22A14* has been shown to hybridize to a single digestion fragment using an *Eco*RI digestion of the BAC OSJNBb0089D23 (data not shown). Therefore, *C950* and *22A14* are shown at the same location on the genetic map.

The CAPS markers *22A14* (110.0 cM), *R251* (112.9 cM), and *C30662b* (112.9 cM) showed no recombinants when compared with one another when applied to the CO39–RIL29 F_2 population of 47 individuals. Each of these markers showed the same five recombination events for 94 meiotic events (47 F_2 individuals) when compared with the F_2 genotype for *Pi7(t)* obtained from inoculation of the F_3 progeny. *S12886* showed no recombination events with the F_2 genotype for *Pi7(t)* (Table 3). These results lead to the conclusion that the *Pi7(t)* resistance locus is positioned between the RGP markers *R251* and *C30662* (112.9 cM) and the telomere (Fig. 1).

The origin of *Pi7(t)*. The resistance in RIL29 should be derived from the Moroberekan parent. However, the three nonparental CAPS polymorphisms in Table 2 would be difficult to explain if RIL29 was indeed the product of solely Moroberekan and CO39. RIL29 amplicons of *22A14*, *R251*, and *C30662a* all show nonparental polymorphisms with respect to the two parental cultivars (Moroberekan and CO39). Thus, during the development of RIL29, nonparental DNA appears to have been incorporated. To assess the source of the nonparental DNA, Southern blots using 12 markers linked with the *Pi1/Pi7(t)* locus were performed on six cultivars: CO39, Moroberekan, RIL29, RIL206, LAC23, and C101LAC. LAC23 and the representative NIL for *Pi1*, C101LAC, were incorporated due to the identical blast reaction profiles of *Pi1* and *Pi7(t)* to a diverse set of *M. grisea* isolates (6). RIL206 was incorporated as a positive control. RIL206 has Moroberekan alleles at the region flanking *Pi1* (3; M. A. Campbell, *unpublished data*). The relative order of these RFLP markers on the genetic map is provided in Figure 1 and the results of the RFLP analysis are provided in Table 4. A representative Southern blot for the marker *C30662* is provided in Figure 2.

The results of the RFLP analysis are consistent with the hypothesis that the RIL29 genome is not exclusively derived from the parents Moroberekan and CO39. The RFLP patterns observed in RIL29 indicate the presence of nonparental alleles (e.g., the hybridization patterns are polymorphic when compared with Moroberekan and CO39). For a subset of the markers, the nonparental hybridization patterns in RIL29 and the hybridization patterns in LAC23 are monomorphic. In evaluating the RFLP markers *22A14*, *C30662*, *C10150S*, *C481S*, and *S10640*, all show monomorphism between RIL29 and LAC23. Further, RIL29 is polymorphic for these markers with respect to its parents CO39 and Moroberekan. These results suggest that, during the creation of RIL29, nonparental DNA was incorporated into the genome where the RIL29-derived resistance locus was mapped. The most likely origin for this genetic material is cv. LAC23.

DISCUSSION

In order to accurately position the resistance locus derived from RIL29, a new segregating population and new PCR-based CAPS markers were developed. The recombination data from these CAPS markers applied to the population support the positioning

TABLE 2. Co-dominant amplified polymorphic sequence markers developed for the mapping of *Pi7(t)* in a rice F_2 population derived from a cross between CO39 and Moroberekan[a]

Marker	Length[b]	Lengths of digestion products for cultivars[b]			Enzyme
		CO39	RIL29	Moroberekan	
C950	2,000	2,000	1,100 + 900	1,100	*Cla*I
22A14	500	350 + 150	500	350 + 150	*Bcl*I
R251	1,000	700 + 300	1,000	700 + 300	*Hae*III
C30662a	800	800	600 + 200	800	*Ava*I
C30662b	1,200	800 + (200 × 2)	1,000 + 200	1,000 + 200	*Dra*I
E50301	1,000	1,000	800 + 200	800 + 200	*Nde*I
61M17	1,000	1,000	700 + 300	n.d.[c]	*Pvu*II
S12886	1,200	800 + 400	1,200	1,200	*Hpa*II

[a] Approximate size of the amplicon is given (which was the same for all three cultivars) and the resultant digestion products producing the polymorphism.
[b] All lengths are in base pairs.
[c] The Moroberekan amplicon was not tested for digestion with *Pvu*II.

TABLE 3. Genotypes of the five F_2 recombinants and the number of recombinations displayed for each marker for the small F_2 population (47 F_2 individuals)

Marker	F_2 individual[a]					Marker[b]				
	13	22	29	30	32	*22A14*	*R251*	*C30662*	*S12886*	*Pi7(t)*
22A14	CC	RC	RC	RC	RC	n/a	0	0	5	5
R251	CC	RC	RC	RC	RC	…	n/a	0	5	5
C30662	CC	RC	RC	RC	RC	…	…	n/a	5	5
S12886	RC	RR	RR	CC	RR	…	…	…	n/a	0
Pi7(t)[c]	RC	RR	RR	CC	RR	…	…	…	…	…

[a] R and C indicate the alleles from the resistant (RIL29) and susceptible (CO39) parent, respectively.
[b] n/a = Not applicable.
[c] *Pi(t)*is the genotype of the F_2 individual determined by the phenotypic inoculation screen of the F_3 progeny.

TABLE 4. Restriction fragment length polymorphism (RFLP) hybridization patterns and microsatellite scores are given for six cultivars[a]

Marker	Enzyme[c]	Cultivars used for RFLP analysis[b]					
		CO39	Moroberekan	RIL29	RIL206	LAC23	C101LAC
22A14	*RI,H3*	CO39	CO39	LAC23	CO39	LAC23	N.S.P.
C30662	*RI,H3,RV*	CO39	CO39	LAC23	CO39	LAC23	LAC23
E50658	*RI,H3*	CO39	CO39	CO39	CO39	CO39	CO39
R1506	*SacI*	CO39	Moroberekan	N.S.P.	N.D.	N.D.	N.D.
S12886	*H3*	CO39	Moroberekan	Moroberekan	N.S.P.	Moroberekan	N.S.P.
C10150S	*H3*	CO39	Moroberekan	LAC23	Moroberekan	LAC23	N.S.P.
C481S	*H3*	CO39	Moroberekan	LAC23	Moroberekan	LAC23	N.D.
C105	*RI,H3*	CO39	CO39	CO39	CO39	CO39	CO39
C10295S	*H3*	CO39	Moroberekan	Moroberekan	Moroberekan	Moroberekan	Moroberekan
R543	*RI*	CO39	Moroberekan	CO39	CO39	Moroberekan	Moroberekan
S10640	*H3,RV*	CO39	CO39	LAC23	CO39	LAC23	LAC23

[a] Any patterns that were monomorphic between cultivars were listed as the first cultivar to show that pattern.
[b] N.S.P. = no similar polymorphism; the cultivar possessed a unique RFLP pattern when compared with the other five cultivars in this RFLP analysis. N.D. indicates that an RFLP analysis was not performed.
[c] Enzymes used for this RFLP polymorphism analysis were *Eco*RI (*RI*), *Hin*dIII (*H3*), and *Eco*RV (*RV*).

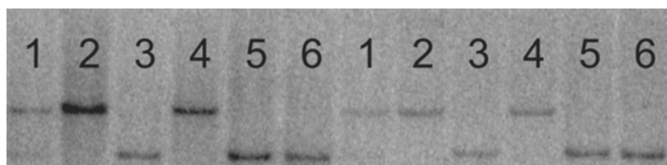

Fig. 2. Representative restriction fragment length polymorphism (RFLP) analysis using the RFLP marker *C30662*. Six cultivars were digested with *Eco*RI (left side) and *Hin*dIII (right side). Cultivars: 1 = CO39, 2 = Moroberekan, 3 = RIL29, 4 = RIL206, 5 = LAC23, and 6 = C101LAC.

of *Pi7(t)* on chromosome 11. *Pi7(t)* shares a common position with the previously mapped *Pi1* blast resistance gene. This assignment of the *Pi7(t)* locus confirms the results from previous allelism tests using the representative lines C101LAC and RIL29 (7).

Surprisingly, the assumption of Moroberekan as the donor of *Pi7(t)* in RIL29 has been called into question. The generation of CAPS markers from the Nipponbare × Kasalath RFLP map and Nipponbare BAC end sequence revealed the presence of nonparental polymorphisms for three of the eight markers. Simultaneous mutation of three tightly linked markers in the creation of the RILs seems unlikely. The genetic interval containing the *Pi1/ Pi7(t)* locus has DNA from a nonparental source. A similar result was observed in the mapping of *Pi44(t)* that was identified from this same Moroberekan- and CO39-derived RIL population (2). *Pi44(t)* is loosely linked to *Pi7(t)/Pi1* on the long arm of chromosome 11. For the *Pi44(t)* representative line RIL276, AFLP-derived co-segregating marker AF348 and the microsatellite marker *RM224* showed nonparental banding patterns when compared with Moroberekan and CO39. No other cultivar was identified as the donor of the nonparental DNA in RIL276 containing *Pi44(t)* (2).

In this report, the RFLP-based characterization of the six cultivars confirms the hypothesis that RIL29 has nonparental genetic material at the *Pi7(t)/Pi1* locus. Because RIL29 shares alleles with LAC23 and the NIL derivative C101LAC, LAC23 is the most likely source for the nonparental alleles. The nonparental polymorphisms in RIL29 as well as the work with RIL276 present a common problem in developing pure lines as well as sharing materials. The introduction of foreign (i.e., nonparental) DNA in an inbreeding species, such as rice, means that genotyping of advanced lines must be an essential step before release. In the case of *Pi7(t)* research, NILs were being developed at IRRI concurrently with the CO39- and Moroberekan-derived RILs. LAC23, the donor of *Pi1*, likely was incorporated accidentally into the RIL lines, although LAC23 has not been proven as the source of the nonparental DNA.

The region surrounding the *Pi7(t)/Pi1* locus is known to be a rich source of *R* gene homologues. Several degenerate PCR strategies have independently identified nucleotide-binding site leucine-rich repeat (NBS/LRR) homologue clusters that map to tightly linked positions with respect to *Pi1* (10,12). Further, an ordered analysis, by the authors, of all STC sequences using TIGR BLAST has shown that a large number of NBS/LRR sequences exist in clusters along this interval. For the interval between *C950* and *R1506*, 20 NBS-LRR homologues were identified with homology to family members R2, R3, R6, and R10 (10).

Not only does the telomeric interval on the long arm of chromosome 11 carry a high concentration of NBS-LRR homologous sequences, but it also carries multiple resistance specificities. For example, *Xa4*, a bacterial blight resistance gene, was tightly linked with RGP marker *L1044*, which is positioned between *C950* and *R251* (20). Several other *M. grisea* and bacterial blight resistance loci (*Pi-f*, *Xa3*, and *Pi18(t)*) have been positioned near the *Pi1/Pi7(t)* locus (16,20). Interestingly, the multi-allelic *M. grisea* resistance locus, *Pik*, has been demonstrated by allelism tests to be tightly linked to *Pi1*. *Pik* showed tight linkage with RFLP marker *G181* (7). Using diverse blast isolates from the Philippines, the reaction spectrum for *Pi1* (in C101LAC), *Pi7(t)* (in RIL29), and *Pik* were shown to be identical (18). Therefore, *Pi1* and *Pi7(t)* actually may prove to be alleles or identical to *Pik*.

In the process of mapping *Pi7(t),* new PCR-based CAPS markers quickly were generated. These markers will be useful for subsequent fine mapping of the *Pi7(t)* locus. Furthermore, these CAPS markers are useful for breeders wishing to incorporate this locus into their breeding programs. Although the positioning of the *Pi7(t)* to the *Pi1* locus was successful, the nonparental polymorphisms indicate that the donor cultivar for this resistance gene presently is unclear. Subsequent fine mapping will be required to clearly identify the origin and position of *Pi7(t)* in a genetic interval rich with resistance gene homologues.

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