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Mapping of four major rice blast resistance genes from 'Lemont' and 'Teqing' and evaluation of their combinatorial effect for field resistance

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Abstract A framework linkage map was developed using 284 F_{10} recombinant inbred lines (RILs) from a 'Lemont'×'Teqing' rice cultivar cross. Evaluation of a subset of 245 of these RILs with five races of the rice blast pathogen permitted RFLP mapping of three major resistance genes from Teqing and one major gene from Lemont. All mapped genes were found to confer resistance to at least two blast races, but none conferred resistance to all five races evaluated. RFLP mapping showed that the three resistance genes from Teqing, designated *Pi-tq5*, *Pi-tq1* and *Pi-tq6*, were present on chromosomes 2, 6 and 12, respectively. The resistance gene from Lemont, *Pi-lm2*, was located on chromosome 11. *Pi-tq1* is considered a new gene, based on its reaction to these five races and its unique map location, while the other three genes may be allelic with previously reported genes. Lines with different gene combinations were evaluated for disease reaction in field plots. Some gene combinations showed both direct effects and non-linear interaction. The fact that some of the lines without any of the four tagged genes exhibited useful levels of resistance in the field plots suggests the presence of additional genes or QTLs affecting the blast reaction segregating in this population.

Keywords *Oryza sativa* L. · Gene mapping · *Magnaporthe grisea* · *Pyricularia grisea* · Disease resistance · Complete resistance · Resistance genes · Pyramiding · RFLP

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Introduction

Blast resistance is one of the major objectives in rice (*Oryza sativa* L.) breeding in both tropical and temperate countries. The causal organism, *Pyricularia grisea*, is known for its genetic instability, allowing it to overcome the genetic resistance of host plants (Shull and Hamer 1994). In spite of this, plant resistance has still been the most-effective and economical control of the disease. Several cultivars, such as 'IR36', 'Moroberekan' and 'Orysica Llanos 5', were found to be durably resistant (Roumen 1994; Wang et al. 1994; Correa-Victoria and Zeigler 1995), showing that, in some cases, genetic resistance can provide effective long-lasting protection against blast. Some major genes, such as *Pi-1*(*t*) and *Pi-2*(*t*), have broad resistance spectra (Levy et al. 1995; Chen et al. 1996), and can prevent disease development by most lineages of blast in several countries. The presence of multiple genes, conferring resistance to different races of blast, has hindered the progress of blast resistance studies (Yu et al. 1987).

Extensive genetic studies were performed in Japan where 13 major blast genes were identified (Ou 1985), and a set of lines with single genes that can differentiate races based on reaction patterns (differentials) were developed (Inukai et al. 1996a). With the development of near-isogenic lines (NILs, Mackill and Bonman 1992), several genes from tropical cultivars like 'Tetep', 'Pai-kan tao', '5173', 'LAC23' and 'Apura' were identi-

fied and mapped using RFLPs (Yu et al. 1991; McCouch et al. 1994; Miyamoto et al. 1996; Rybka et al. 1997). Allelism of these genes with those of the Japanese differentials is known (Inukai et al. 1994). Molecular mapping and classical genetic analysis showed a clustering of the major blast resistance genes to particular chromosomal regions (McCouch et al. 1994). Recent reports identified at least four clusters, with five to eight loci each, located on chromosomes 4, 6, 11 and 12 (Roumen et al. 1994; Kawasaki et al. 1996; Wu et al. 1996; Zheng et al. 1996; Rybka et al. 1997). Any of these clusters may be a gene family, such as the *Xa-21* family reported for rice bacterial blight (Wang et al. 1995) which has a broad spectrum of resistance.

Several approaches have been used to study the complex nature of the durable resistance. Mackill and Bonman (1992) created and used multiple sets of NILs to separate, and thereby identify, several resistance genes from Tetep, Pai-kan tao, LAC23 and 5173. Molecular genetic techniques applied to permanent (pure breeding) populations of doubled-haploid (DH) and recombinant inbred lines (RILs) have allowed the location of major resistance genes and quantitative trait loci (QTLs) to specific chromosomal regions (Yu et al. 1991; Wang et al. 1994). However, severely distorted segregation affected the accuracy of linkage determination (Wang et al. 1994) and, in some cases, impelled subsequent revision of the published results (Inukai et al. 1996b). Crossing NILs to combine specific resistance genes for the study of gene×gene interactions is a strategy currently being used in IRRI and Japan (JRGP) to study resistance bacterial blight (Huang et al. 1997) and blast of rice. However, like near-isoline development, a number of generations of selection and crossing are needed to develop lines with several major genes in a given background.

In the present study, the RIL population used was derived from two parental genotypes which were each resistant to some races of the blast pathogen. The female parent, Lemont, was considered to be resistant to blast in the Southern U.S. more than a decade after its release (Marchetti 1994). Teqing has exhibited complete resistance to all blast races known to be present in the U.S. Thus, this population provided a unique opportunity to study the genetics of resistance to different races of the pathogen and to pyramid genes into durably resistant new genotypes. Resistance of Teqing and Lemont against five races of blast were estimated, by classical genetic analysis, to be controlled by at least four and two independent genes, respectively (Tabien 1996; Tabien et al. 1996). The population available for this study was a later generation (F_8 in 1993) of the Lemont×Teqing cross shown by Li et al. (1995) to have less skewing than other available rice mapping populations, and so was anticipated to provide a better population for *de novo* mapping.

Materials and methods

Greenhouse phenotyping of RILs

A population of RILs at the F_8 was evaluated using the spray inoculation method of Marchetti et al. (1987). One set of seedlings, composed of 245 lines and arranged in a completely randomized design with three replications, was prepared for each of the five races. The sets of RILs were seeded at weekly intervals in 25×35×10 cm galvanized steel flats filled with field soil. Each flat contained 17 test lines, Lemont, Teqing, and 'M-201' (as a susceptible check). Plants were maintained inside the greenhouse until inoculation.

Five races of blast selected from an historical collection out of the Southern U.S. (international races IC-17, IB-49, IB-54, IG-1 and IE-1, according to Ling and Ou 1969), were used to inoculate all the RILs and control lines. Teqing is completely resistant to all Southern U.S. races of blast; Lemont is resistant to most but is susceptible to IC-17, IB-49 and IE-1. These three races were selected for the study because the progeny RILs were expected to segregate for quantitative or qualitative resistance to them. Because of varietal susceptibility, these races are also presently the most prevalent ones in Texas rice fields. Two additional races, IB-54 and IG-1, were included to allow further evaluation of the resistance genes from Teqing. These five races represent three of the eight lineages of *P. grisea* reported in Southern U.S. rice accessions collected over a 30-year period (Levy et al. 1991; Xia et al. 1993). Though IE-1 was not included in either lineage study, the pathogen accessions used to represent IC-17, IB-49, IB-54 and IG-1 in the present study were the same as those used as reference races by Levy et al. (1991).

At 17–21 days after seeding (DAS), a 20-ml single-race spore suspension with a cell count of 10^4 to 10^5 was sprayed over each seeded flat. Inoculated plants were kept inside the dew chamber for 16–18 h and then transferred to the greenhouse until scoring time. The reaction of the RILs to each race was evaluated 8 days after inoculation (DAI) following Mackill and Bonman (1992). Average reaction scores of 0–3 were considered incompatible (–) and indicative of resistance in the host plants, while RILs with average scores of 4–5 were considered to exhibit compatible (+) reactions indicative of the susceptibility of the RIL for the studied race.

Field phenotyping of RILs

A separate set of RILs was planted in outdoor blast nursery plots following the procedure of Marchetti (1983) adapted as described in Tabien (1996). All 245 entries were seeded in single-row plots, 60-cm in length, and arranged in a randomized complete block design with three replications. Every two entries were separated by the susceptible check M-201, while Lemont or Teqing was seeded every 20th row. Spreader rows were planted along the windward side to enhance the spread of spores to nearby lines. Plots, which were not inoculated with specific races but were allowed to be wind inoculated with a natural mix of races, were misted during morning and evening hours to maximize leaf wetness, which maximizes natural inoculation. Percentage diseased leaf area (%DLA) was estimated visually at 19 days after seeding (DAS) and every week thereafter for 5 weeks, while a weekly Standard Evaluation System (SES) rating for blast following Marchetti et al. (1987) was started at 20 DAS and continued for 5 weeks. Areas under disease progress curves (AUDPCs) were computed from the serial %DLA data using the formula of Shaner and Finney (1977).

Genotyping of RILs

F_{10} progeny of the 245 phenotyped lines, plus progeny from 39 additional lines that had not previously provided sufficient seed for both phenotyping and further population development (total 284 RILs), were grown in the greenhouse for genotyping. Genom-

ic DNA was isolated from fresh leaf material harvested approximately 30 days after planting. Seedlings were grown in six-inch pots lined with plastic bags and filled with a mix of field soil, peat moss and vermiculite at a ratio of 4:3:1. DNA extraction followed the protocol of Li et al. (1995). DNA digestion using *Eco*RI, *Eco*RV, *Xba*1 and *Hin*dIII, electrophoresis, and Southern blotting followed standard procedures (Chittenden et al. 1994).

Forty one (out of 101) probes previously used by Li et al. (1995) in the F_2 of this pedigree were employed for hybridization, along with 37 Cornell anchor probes (Causse et al. 1994) and 54 landmark probes from Japan (H. Kurata et al. 1994). Additionally, two sorghum cDNA probes ("HHU", provided by P. Westhoff, Heinrich Heine University, Dusseldorf, Germany), and 25 maize cDNA probes ("CSU" provided by M. McMullen and E. Coe, USDA-ARS, Columbia, Mo.) were also evaluated. Probes used primarily for the comparative alignment of chromosomes were evaluated in a subset of 60 RILs (indicated with asterisks in Fig. 1).

Probes were labeled with [^{32}p] d CTP by the random primer method (Feinberg and Vogelstein 1983). After hybridization for 12–18 h, filters were washed three times using wash solutions of 2× SSC+0.1% SDS, 1× SSC+0.1% SDS, and 0.5× SSC+0.1% SDS, respectively. Autoradiography was as previously described (Li et al. 1995).

Data analysis

Discrete greenhouse phenotypic data on resistance (average score 0–3) or susceptibility (average score 4–5) to each of the five races were analyzed for association with the 217 marker loci using the Pearson chi-square and Fisher exact tests combined, while field data were analyzed using PROC GLM (SAS Institute 1988). The normality of data obtained from plants grown in plots in the blast nursery was checked using PROC UNIVARIATE PLOT (SAS Institute 1988) and subsequent square-root transformation was done for %DLA and AUDPC.

The DOS version of Mapmaker Version 3.0 (Lander et al. 1987) was used to establish the framework map. Linkage between markers was determined by the group command with a LOD score greater than 6.0 and a recombination fraction of 0.25. All map distances were in Kosambi centimorgans (cM).

Classical genetic studies suggested that "discrete" phenotypes in this experiment were influenced by multiple genes (Tabien 1996; Tabien et al. 1996). Marker-phenotype combinations were analyzed using PROC FREQUENCY/ CHISQUARE (SAS Institute 1988) to determine significant deviation from an expected 1:1:1:1 ratio for a RIL population, which would suggest the association of the marker with a discrete race-specific phenotype. Because skewing of the phenotypic data toward resistance was noted along with skewing of some marker loci toward either the Teqing or Lemont allele, the data were also analyzed using Fisher's exact test (two-tailed) which calculates the probability that the observed data would arise by chance if the null hypothesis (plants with the Lemont allele have the same proportion of susceptible plants as those with the Teqing allele) is true. This test is more rigorous than chi-square when skewing has significantly reduced the number of individuals in one or more classes in the contingency table. When a probe-race combination had both a χ^2 >10.00 ($P < 0.001$) and a Fisher's exact probability ≤0.0001 it was considered indicative of linkage between the probe and a major gene affecting resistance to the studied race. Interval mapping, which can allow the fine mapping of genes, is too sensitive to miscoring (Wright et al. 1998) for it to be appropriate for this analysis of blast resistance.

RFLP markers flanking the genes were used to estimate the presence or absence of the resistance gene(s) in each inbred line. This information was used to select subsets of the RILs estimated to have one, two, three and four major resistance genes. Field resistance data for each of these subsets of RILs were analyzed using PROC GLM (SAS Institute 1988). Means were compared using Fisher's LSD at 5%. Possible digenic interactions were evaluated by two-way ANOVA using PROC GLM, while higher interaction was evaluated using PROC RSQUARE and PROC STEPWISE regression functions (SAS Institute 1988).

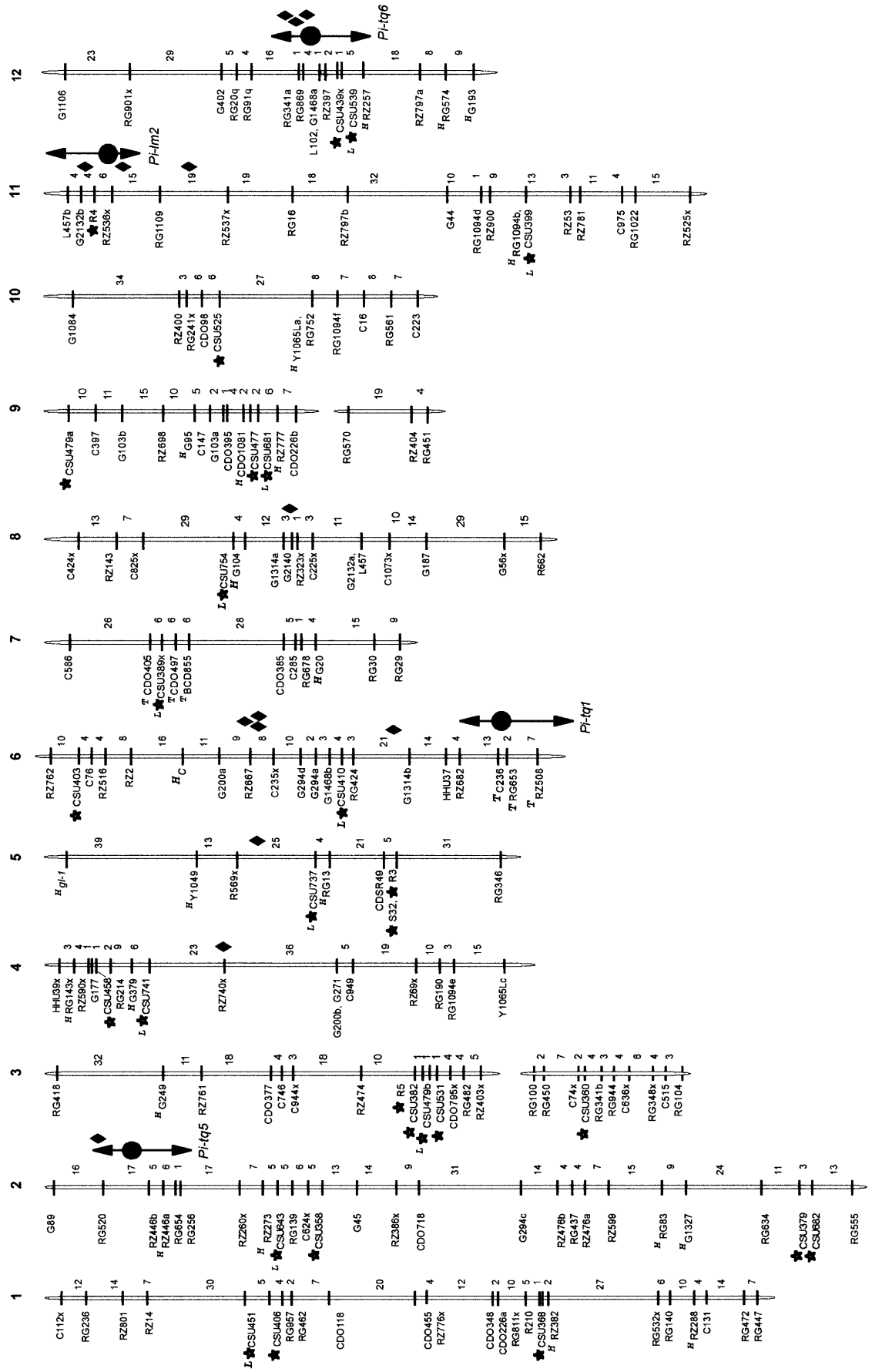
Results and discussion

The framework linkage map

The genetic map (Fig. 1) of 284 RILs and 205 loci has an average interval length of 10 cM between markers and covers a total of 1829 cM. Although this is 338 cM longer than the saturated interspecific map developed at Cornell (Causse et al. 1994), this map does not represent a greater chromosomal length. Probes that are on the ends of the chromosomes in this map are generally at or near the ends of the chromosomes on the Cornell map, except for chromosomes 4, 5, 9, and 12 which have one or both ends poorly represented in the present map. The increase in genetic distance in the Lemont×Teqing map is largely due to a general increase in the estimated distance between probes contained in the two maps, reflecting increased recombination rates in this "narrower" (inter-subspecific) cross, and perhaps also influenced by lower levels of segregation distortion.

Marker segregation ratios in this population were closer to Mendelian expectations than in many previous inter-specific rice populations (Fig. 2), with less bias favoring the *indica* (Teqing) allele than that reported in the Moroberekan×CO39 population (Wang et al. 1994). For only seven (3%) marker loci were Teqing (*indica*) alleles found in more than 70% of the RILs (expected=50%) and only one of these exceeded 80% (CDO405 at 84%). The seven loci skewed toward Teqing were all clustered in two regions, one on chromosome 6 and one on chromosome 7 (Fig. 1). Eleven probes were skewed (>70%) toward Lemont (*japonica*) alleles, the most severe of which was CSU643 on chromosome 2 which contained 74% Lemont alleles. The probes skewed toward Lemont were scattered among the chromosomes (Fig. 1) and were not mapped using data from the entire population, but were each mapped based on a subset of the population. This skewing, then, is characteristic of the subset of the RILs used for comparative alignment purposes but is not characteristic of the population as a whole.

The framework map was a nearly perfect match with that determined from an earlier generation of the same inter-subspecific Lemont×Teqing cross (Li et al. 1995), and the order of markers along the chromosomes was similar to that in an interspecific rice map (Causse et al. 1994). The primary difference between this map and the interspecific Cornell map was the separation by less than 10 cM of probes that were previously reported to be at the same locus. Out of 93 markers in common between this and the Cornell map, the possible re-location of six markers (RG190, RG29x, CDO395, RZ53, RG20q and RG91q) was confounded by the fact that two (RG29 and CDO395) of these probes exhibited multiple bands in one of the two populations, suggesting the presence of multiple loci. Four possible inversions of a maximum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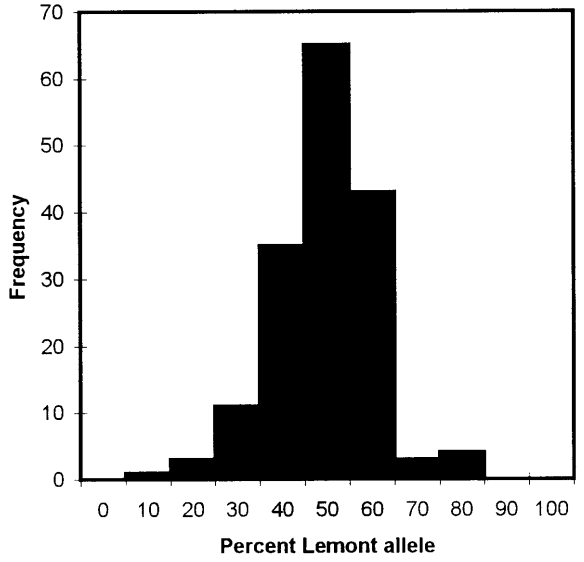

Fig. 2 Frequency distribution of the percent Lemont allele at 205 loci within a population of 284 recombinant inbred lines from a Lemont×Teqing cross

11-cM in length might have occurred; between CDO118 and RG957 on chromosome 1, RZ260x and RZ273 on chromosome 2, RG100 and RG450 on chromosome 3, and RG678 and CDO385 on chromosome 7. Several probes that had not been previously mapped (Causse et al. 1994) segregated in this population, including RG447 (chromosome 1), RG634 (chromosome 2), RZ777 (chromosome 9), CDO1081 (chromosome 9) and CDO226b (chromosome 9). Among the 52 markers from Kurata et al. (1994) that were mapped, ten were located on different chromosomes than previously reported. Five of the ten noted differences were with probes that gave multiple bands in either the Lemont/Teqing or the (Kurata et al. 1994) population.

More than 50 marker loci were mapped for the first time in rice. Some of these involved probes from other sources (e.g. two HHU probes from sorghum and 25 CSU probes from maize). Others, like RG447 on the bottom of chromosome 1, were due to the ability to map probes representing monomorphic loci in previous mapping populations. Other new loci were due to reportedly single-copy probes having multiple loci in the present population. RG1094, previously reported to be single copy, had six distinct polymorphic bands and four of these were mapped to chromosomes 11 (two genes), 4 and 10. This is contrary to previous reports of single copy clones remaining single-copy in other rice populations (Causse et al. 1994).

◀ **Fig. 1** Framework rice linkage map consisting of 203 RFLP loci plus two morphological loci based on 284 recombinant inbred lines (RILs) from a Lemont×Teqing cross. The orientation of each chromosome follows Causse et al. (1994); *numbers* are map distances in cM (Kosambi). Markers with *asterisks* had data from less than 100 RILs and were not used to determine resistance gene positions. Loci marked with *H* were heterozygous in ≥10% of the RILs. Loci marked with *T* or *L* had the Teqing or Lemont allele in more than 70% of the RILs, respectively. The markers that exhibited the most-significant linkage to each of the four major genes for blast resistance (*Pi-tq1*, *Pi-tq5*, *Pi-tq6* and *Pi-lm2*) are indicated with a *circle*. *Arrows* emanating from the circles indicate additional probes that exhibited statistically significant linkage. *Diamonds* indicate the estimated location of major blast resistance genes mapped in various other populations (Yu et al. 1991; McCouch et al. 1994; Hittalmani et al. 1995; Inukai et al. 1996b; Miyamoto et al. 1996; Naqvi and Chattoo 1996; Pan et al. 1996; Rybka et al. 1997)

Mapping blast resistance genes

Based on the chromosomal locations of molecular markers determined from chi-square and Fisher's exact tests to be associated with disease resistance, four major resistance genes were identified. These genes were each effective against two or more races of blast and were located on chromosomes 2, 6, 11 and 12 (Table 1).

A resistance gene located on chromosome 2 (*Pi-tq5*) conferred resistance to four of the five races: IC-17, IB-49, IG-1 and IE-1 (Table 1). For five neighboring probes located on the bottom half of chromosome 2, nearly all (81 out of 87) of the plants homozygous for the Teqing allele were classified as resistant to each of these four races, and 70% (81 out of 116) of the resistant plants were homozygous for the Teqing allele, indicating that Teqing was the donor of this resistance gene. Based on the chi-square values (χ^2) and the Fisher exact probabilities for these four races (Table 1), the gene resides between RG520 and RG446b (Fig. 1). The location of this gene was very near the estimated location of *Pi-b* (Miyamoto et al. 1996).

Four markers on chromosome 6 were linked to a gene effective against IC-17, IB-49 and IE-1 (Table 1, Fig. 1). This gene, originating from Teqing and designated *Pi-tq1*, was located between markers C236 and RZ508. Six major genes were previously reported to be on this chromosome; however, each of them was located near RG64 at the top-most segment of chromosome 6 (McCouch et al. 1994; Fig. 1). Because the *Pi-tq1* gene falls in the bottom half of chromosome 6, it appears to be a new gene for blast resistance. All the markers associated with *Pi-tq1* on chromosome 6 were skewed. The most-skewed locus in this region was C236 with 80% Teqing alleles. Based on an average of 150 RILs and this level of skewing, the longest distance that can be estimated with precision is 7.3 cM (Kosambi 1944; Manly 1994). The three probes most-closely associated with this gene were spaced at intervals of 2.2 and 7.2 cM. Although this is sufficiently within the threshold distance to assert linkage between these probes and *Pi-tq1*, caution is still warranted in interpreting the chromosomal location of these probes and this gene. At least one instance exists where major genes tagged with skewed markers were later found associated with another marker or re-located on another chromosome (Inukai et al. 1996a).

Table 1 Ratios of susceptible: resistant phenotypes[a] observed in RILs inoculated with five individual races of rice blast, and chi-square values and Fisher exact probabilities for statistically significant associations[a] between molecular markers and disease response data

Susc.: res.[a]	IC-17 60:181		IB-49 34:206		IB-54 68:174		IG-1 49:192		IE-1 42:196	
	χ^2-value	Exact *P*	χ^2-value	Exact *P*	χ^2-value	Exact *P*	χ^2-value	Exact P	χ^2-value	Exact P
Chromosome 2										
RG520	48.2	<0.0001	40.3	<0.0001	–	–	22.6	<0.0001	55.3	<0.0001
RZ446b	28.8	<0.0001	18.7	<0.0001	–	–	20.0	<0.0001	31.1	<0.0001
RZ446a	35.6	<0.0001	27.1	<0.0001	–	–	16.4	0.0008	41.3	<0.0001
RG654	42.9	<0.0001	33.8	<0.0001	–	–	23.9	0.0006	37.9	<0.0001
RG256	34.6	<0.0001	31.3	<0.0001	–	–	18.9	0.0037	36.0	<0.0001
Chromosome 6										
RZ682	12.3	0.0014	14.1	0.0005	–	–	–	–	17.4	0.0001
C236	33.7	<0.0001	35.8	<0.0001	–	–	–	–	33.7	<0.0001
RG653	10.1	0.0190	15.2	0.0020	–	–	–	–	23.4	0.0005
RZ508	28.8	<0.0001	26.2	<0.0001	–	–	–	–	28.8	<0.0001
Chromosome 11										
L457b	–	–	–	–	19.9	<0.0001	14.5	<0.0001	–	–
G2132b	–	–	–	–	19.5	<0.0001	13.1	0.0029	–	–
RZ536x	–	–	–	–	37.3	<0.0001	22.5	0.0009	–	–
RG1109	–	–	–	–	20.8	0.0170	12.5	0.0690	–	–
Chromosome 12										
RG341a	33.0	<0.0001	20.6	<0.0001	25.1	<0.0001	–	–	27.5	<0.0001
RG869	34.7	<0.0001	17.1	0.0001	27.9	<0.0001	–	–	27.5	<0.0001
L102	38.9	<0.0001	27.9	<0.0001	19.7	<0.0001	–	–	31.8	<0.0001
G1468a	35.3	<0.0001	24.4	<0.0001	18.4	0.0001	–	–	29.3	<0.0001
RZ397	38.5	<0.0001	30.3	<0.0001	12.1	0.0051	–	–	30.0	<0.0001
RZ257	25.4	<0.0001	16.0-	0.0010	–	–	–	–	27.6	<0.0001

[a] Segregation for susceptibility/resistance was noted for some RILs in the single-race inoculation studies; phenotypic segregation within the RILs varied depending on the race. The above segregation ratios and correlation analyses excluded lines determined to be heterozyous to a given race

Table 2 Classification of rice plants for genotype (allele type) and disease reaction for four probes located on chromosome 11 and two blast races

Probe	Allele type	Blast reaction[a]	Number of plants	
			IB-54	IG-1
RZ536a	Lemont	+	3	4
		–	59	58
	Teqing	+	29	25
		–	69	72
RG2132b	Lemont	+	2	2
		–	52	52
	Teqing	+	29	27
		–	71	72
L457b	Lemont	+	1	9
		–	48	50
	Teqing	+	27	24
		–	65	73
RG1109	Lemont	+	9	7
		–	50	52
	Teqing	+	24	22
		–	73	76

[a] (–)=Incompatible reaction or host resistance, (+)=compatible reaction or host susceptibility

For the four probes mapped to chromosome 11, chi-square values, Fisher exact probabilities (Table 1) and analyses of genotype-phenotype classification data (Table 2) all indicate a major gene conferring resistance to both IB-54 and IG-1. Determining whether the resistance allele was from Lemont or Teqing is less straightforward than it was for the genes on chromosomes 2 and 6 because the susceptible (+): resistant (–) ratios were significantly different from 1:1 due to a statistically significant reduction of the susceptible individuals for both the homozygous-Lemont and the homozygous-Teqing portions of the RIL population. Among the homozygous-Lemont portion of the population, plants susceptible to these two races were especially rare (2–15%) compared to the homozygous-Teqing portion of the population (25–30%)(Table 2). Thus, we concluded that the resistance allele for this gene originated from the Lemont parent, and designated the gene as *Pi-lm2* accordingly. However, the severity of the reduction of the +: – ratio for the RILs homozygous for the Teqing allele (Table 2) makes it appear as if the Teqing alleles at these four loci might also be associated with resistance to IB-54 and IG-1, suggesting the possibility of a second gene from Teqing also effective against these two races being nearby on the same chromosome.

Previous reports identified two major genes for blast resistance linked with RZ536 (Yu et al. 1991; Inukai et

al. 1994; Fig. 1). *Pi-1*(*t*) was found to be 14 cM from RZ536 and *Pi-k* was reportedly only 4.7 cM from *Pi-1*(*t*). The gene located by us on chromosome 11 and designated *Pi-lm2* was effective against IB-54 and IG-1, which would be as expected if *Pi-lm2* was indeed the same as *Pi-k*^{h}, an allele of *Pi-k*. 'Dawn' reportedly contains the *Pi-k*^{h} allele (Kiyosawa 1974) and is an ancestor of Lemont (Bollich et al. 1985). It cannot yet be ruled out, however, that *Pi-lm2* might be allelic to *Pi-1*(*t*) which was identified from Tetep (Yu 1991), a parent of Teqing. However, the race specificity of *Pi-lm2* detected by the Fisher exact test is narrower than that of *Pi-1*(*t*) (Chen et al. 1995) so that this would represent a newly reported allele of this gene. Allelism tests will be necessary to provide conclusive identity of the gene(s) identified on chromosome 11.

Although the data cannot prove or disprove the possibility of a second resistance gene being associated with the Teqing alleles in this region of chromosome 11, there are other possible, and even more likely, explanations for the observed skewing towards resistance among these RILs. Of the 44 RILs that had the Teqing allele at all four of these markers and were resistant to IB-54, 21 also had *Pi-tq6* which is also effective against IB-54 but on chromosome 12. Likewise, 15 of the 44 plants contained *Pi-tq5* on chromosome 2 which is effective against IG-1. The significant reduction in the susceptible class among the RILs homozygous-Teqing for the gene on chromosome 11 can be explained, at least in part, by the presence of these two other genes conferring resistance to these same two races.

In fact, the classification data for all probes and allele types were skewed with varying degrees toward resistance. Unlike the data shown in Table 2, the skewing usually was not severe and the numbers did not differ significantly from the expected 1:1 ratio. While much of this can be explained by multiple genes affecting the response to the same race(s), the possible loss of highly susceptible individuals during population development cannot be ruled out. The F_3 generation was grown in panicle rows in the winter nursery in Puerto Rico. Much sterility due to neck blast was noted during harvest (Pinson et al. 1996), and care was taken to avoid selection for resistance by taking panicles from random plants. However, some plants had no seed, whether due to neck blast or other causes (i.e., very late heading, extremely poor vigor, shattering, etc.). Poor plant stands were also observed in this generation, which could have had multiple causes, including rice blast disease. Skewing toward the resistance allele was strongest for *Pi-tq1* on chromosome 6 (78% Teqing alleles). Unintentional selection during population development may have contributed to this bias. Interestingly, chromosome 7 also had a region with a strong bias toward the Teqing allele, but was not found to have a resistance gene. Co-segregation of unlinked markers associated with fitness was reported previously in this cross (Li et al. 1997).

Six markers on chromosome 12 showed association with a gene (*Pi-tq6*) effective against all five blast races except IG-1 (Table 1). RILs containing Teqing alleles had susceptible:resistant ratios ranging from 2:57 to 10:58 (0.03 to 0.14) suggesting that the resistant allele for *Pi-tq6* was from Teqing. Similar to the situation previously discussed on chromosome 11, however, the RILs containing Lemont alleles were also skewed toward resistance with nearly twice as many resistant lines as susceptible ones when classified for reaction to IB-49 and IB-54 (data not shown). Re-evaluation of these lines relative to *Pi-tq2* on chromosome 6 and *Pi-lm2* on chromosome 11 showed that the resistance reaction was most likely due to the presence of these other resistance genes rather than for an additional gene from Lemont located on chromosome 12. Relative to the framework map and the chi-square and exact *P* values with the adjacent markers, the Teqing gene (*Pi-tq6*) was located between RG869 and L102.

Pi-tq6 is likely to represent the *Pi-4*(*t*) locus which was found closely linked to RG869 and RZ397 at 5.4 cM and 3.3 cM (Mew et al. 1994, Fig. 1). The linear order of these closely linked markers was different in the two maps, thus a direct comparison cannot be made. *Pi-tq6* could also be similar to the gene from 'Hong Jiao-Zhan', which also was reportedly linked to RG869 and RZ397 (Zheng et al. 1996), or to *Pi-62*(*t*) from 'Yashiro-mochi' reportedly linked to RG869 (Wu et al. 1996). If *Pi-tq6* is allelic to *Pi-4*(*t*), this gene most likely originated from Tetep, a progenitor of Teqing which has *Pi-4*(*t*) (Inukai et al. 1994). Another possible source of *Pi-4*(*t*) was 'Pai-kan-tao' (McCouch et al. 1994), also a Chinese variety. Additional allelism tests can clarify the identity of *Pi-tq6*.

Mapping major genes using RILs

Based on classical genetic analysis of F_2 plants (Tabien 1996; Tabien et al. 1996), Teqing was thought to have four major genes for blast resistance, two genes each for resistance to IC-17, IG-1 and IE-1, and one each for IB-49 and IB-54, while Lemont was thought to have two major genes, one conferring resistance to IB-54 and IG-1, the other conferring resistance to IB-49 alone (Table 3). Mapping results using RILs detected most, but not all, of the genes suggested previously from classical genetic analysis (Table 3). The classical estimate of genes in Lemont and Teqing (Tabien 1996; Tabien et al. 1996) was based on the evaluation of F_2 plants from Teqing×'Rosemont' as well as F_2 plants and RILs (F_8) from Lemont×Teqing. Rosemont is known to be susceptible to all the observed races (Bollich et al. 1993), so it was deduced that all the genes estimated to be segregating in this population were from Teqing. The two additional genes then estimated to be segregating in the Lemont×Teqing progeny were presumed to be from Lemont rather than from Teqing. The limited number of F_2 plants (80 plants) evaluated in the Teqing×Rosemont cross may have, however, simply prevented the classical analysis from identifying these resistance genes found from RFLP mapping to be from Teqing. As observed

Table 3 Reaction of genes identified from Teqing and Lemont rice cultivars using classical genetic analysis[a] and gene mapping in a recombinant inbred population using five races of blast

Gene identification	Source	Gene	Race[b]				
			IC-17	IB-49	IB-54	IG-1	IE-1
Classical[a] genetic analysis	Teqing	*Pi-tq1*	–	–	+	+	–
	Teqing	*Pi-tq2*	–	+	–	–	+
	Teqing	*Pi-tq3*	+	+	+	+	–
	Teqing	*Pi-tq4*	+	+	+	–	+
	Lemont	*pi-b1*	+	–	+	+	+
	Lemont	*Pi-lm2*	+	+	–	–	+
Mapping using RILs	Teqing	*Pi-tq1*	–	–	+	+	–
	Teqing	*Pi-tq5*	–	–	+	–	–
	Teqing	*Pi-tq6*	–	–	–	+	–
	Lemont	*Pi-lm2*	+	+	–	–	+

[a] From Tabien 1996 and Tabien et al. 1996. Genes from Teqing were estimated from an F_2 population of Rosemont×Teqing. Genes from Lemont were estimated from an F_2 plus a RIL population of Lemont×Teqing

[b] – =Incompatible reaction or host resistance, + =compatible reaction or host susceptibility

previously (Mackill and Bonman 1992), the presence of multiple genes affecting disease response greatly complicates genetic analysis.

Unmapped Teqing genes may have also been identical with a gene(s) from Lemont. The genes from Teqing were identified in a cross with susceptible Rosemont but not with Lemont. If Lemont and Teqing each contained the resistance allele, the Lemont×Teqing RIL population would not have segregated for this locus.

Field performance of some gene combinations

Marker data allowed genotype estimation at the four mapped resistance genes in 122 RILs; inconsistent allelotypes (e.g., from cross-over events) and occasional missing marker data prevented the genotype estimation of other lines. Of these 122 RILs, all but 21 were estimated to contain the resistance allele of *Pi-tq1*, the major gene located in a skewed region of chromosome 6. Of the 12 RILs estimated to contain a single resistance gene, six contained *Pi-tq1*, three contained resistance alleles only for *Pi-tq5* (chromosome 2), three contained resistance from *Pi-lm2* (chromosome 11), and none contained resistance from *Pi-tq6* (chromosome 12) alone. These subsets of RILs containing single genes were not large enough to study the effects of each gene individually. Two- and three-gene combinations that did not include *Pi-tq1* were also rare, precluding a study of epistasis among the resistance genes. It was noted, however, that all lines having at least one gene exhibited some field resistance in that they all had very small AUDPC, %DLA, and a low SES rating compared to M-201. Furthermore, the average AUDPC, %DLA, and SES ratings decreased as the number of estimated genes per RIL increased (Table 4), although the differences between these groups were not statistically significant. Thus, we conclude that the major genes are impacting field resistance, but they appear to do so in different ways. *Pi-q6* significantly affected AUDPC and SES ratings but not %DLA, while *Pi-q1* contributed to variation in AUDPC and %DLA, but not SES ratings. *Pi-tq5* was an important determinant of all three traits but *Pi-lm2* was not associated with any of the nursery measured traits. While some of the phenotypic variation not attributable to direct effects of the identified major genes could be attributed to gene interactions (Tabien et al., submitted), the data also strongly suggest the presence of genetic factors in addition to these four major resistance genes. For example, the lines estimated to contain four major genes had highly significant variation for all traits (Table 4). Furthermore, some of the eight lines having susceptible alleles for all of the four identified major genes exhibited useful levels of resistance, particularly in terms of AUDPC (Table 5).

Some gene combinations may not significantly increase resistance, as reported in cotton (El-Zik and Bird 1970) and barley (Brown et al. 1996). In the present study, average AUDPC, %DLA, and SES ratings decreased as the number of estimated genes increased from one to three (Table 4), suggesting that gene-pyramiding may increase resistance. Increasing the gene number to four, however, did not appear to further improve resistance. Negative interaction was noted for blast resistance genes studied in a different rice population (Wang et al. 1994), and was also reported in cotton (El-Zik and Bird 1970) where F_1 plants having a combination of three genes were more susceptible than those having only two major genes for resistance to bacterial blight of cotton.

Major genes identified using single races in the greenhouse may not perform well under field conditions. The major genes identified both from Lemont and Teqing were not completely effective in the blast nursery. The same observation was noted for two genes from Moroberekan found effective against five races of blast pathogen during greenhouse tests. Lines estimated to have two major genes had different resistance expression in two nurseries. The differential response was attributed to a possible wide array of genes from the resistant parent effective against complex populations of pathogen. Differential response of a major gene was also reported on apple scab (Gardiner et al. 1996) where a reduction in resistance was attributed, instead, to the absence of resistance modifying genes.

Breeding implications

Major blast genes can be combined, as is being done for three genes [*Pi-1*(*t*), *Pi-2*(*t*), and *Pi-4*(*t*)] being pyramided at IRRI (Mew et al. 1994), and as was done for the US variety ‘Jefferson’ (McClung et al. 1997). Jefferson’s su-

Table 4 Mean[a] and standard deviation of AUDPC, %DLA and SES rating of parents, control lines, all 245 RILs considered together, and various subsets of RILs determined from flanking marker data to contain one, two, three and four major genes for rice blast resistance

Line/group	AUDPC		%DLA		SES rating	
	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation
Lemont	5.6	0.5	24.3	2.6	5.7	0.5
Teqing	2.7	0.1	13.0	0.8	2.7	0.5
M-201	24.5	0.3	100.0	0.0	9.0	0.0
All 245 RILs	4.2	3.9	21.1	19.3	4.9	2.3
min–max range:	0.0–19.1		0.0–85.3		1.3–8.3	
Various subsets[b] of RILS containing:						
No genes	4.9	3.6	25.0	21.2	6.1	0.9
One gene	4.3	2.7	21.1	14.9	4.9	1.9
Two genes	4.0	3.6	20.6	18.6	4.7	1.9
Three genes	3.5	2.7	16.9	13.2	4.6	1.8
Four genes	4.4	4.4	22.3	20.0	4.9	2.0

[a] Averaged over three field-plot replications
[b] Each subset contains a mixture of all possible genoytpes. For example, lines containing just *Pi-tq5*, *Pi-tq1*, *Pi-lm2*, or *Pi-tq6* would all be included in the subset of RILs containing one gene

Table 5 Several of the eight rice lines estimated to have no major genes for blast resistance based on the absence of RFLP markers for *Pi-tq1*, *Pi-tq5*, *Pi-tq6*, and *Pi-lm2* that showed useful levels of disease resistance in the blast nurser

Line/cultivar	AUDPC[a]	%DLA[b]	SES rating[c]
TQ72	18.60	63.00	6.33
TQ77	8.67	28.33	5.67
TQ167	21.51	56.33	6.67
TQ176	3.13	15.00	5.00
TQ226	9.57	24.00	6.00
TQ234	14.75	20.67	8.00
TQ267	17.48	72.33	7.67
TQ289	10.58	26.33	7.00
Lemont	5.64	24.33	5.67
Teqing	2.70	13.33	2.67
M-201	24.50	100.00	9.00

[a] Critical LSD=2.70
[b] Critical LSD=13.83
[c] Critica

perior disease resistance in test plots supports the hypothesis that pyramided lines will perform better than lines with single genes. This hypothesis might hold true if the major genes act in an additive fashion as is often expected. Gene interaction was significant in our field resistance data, however. Negative interaction wherein some combinations of resistance alleles actually favored susceptibility, was also noted in RILs of rice (Wang et al. 1994) and in F_1 plants of cotton (El-Zik and Bird 1970). Gene interactions should not be ignored when selecting genes to include in a pyramiding scheme.

Screening for resistance involving major genes is being conducted at the earliest generation possible in order to minimize the number of lines in subsequent screening. However, some recombinant types might not occur at this time, especially those involving genes that are masked by other genes, or genes that are linked in repulsion. Additional selfing, as in RIL development, or even intermating (Liu et al. 1996), may reveal additional desirable recombinant types. Late-generation screening is being practiced only for traits with low heritability or those controlled by minor genes. However, this practice will be important in identifying minor genes and novel recombinants of major genes, both implicated in durable resistance.

References

Bollich CN, Webb BD, Marchetti MA, Scott JE (1985) Registration of 'Lemont' rice. Crop Sci 25:883–885

Bollich CN, Webb BD, Marchetti MA, Scott JE (1993) Registration of 'Rosemont' rice. Crop Sci 33:877

Brown AHD, Garwin DF, Bundon JJ, Abott DC, Read BJ (1996) The effect of combining scald resistance genes on disease levels, yield and quality of barley. Theor Appl Genet 93:361–366

Causse CA, Fulton FM, Cho YG, Ahn SW, Chunwongse J, Wu K, Xia J, Yu Z, Ronald PC, Harrington SE, Second G, McCouch SR, Tanksley SD (1994) Saturated molecular map of the rice genome based on an interspecific backcross population. Genetics 138:1251–1274

Chen DH, Zeigler RS, Leung H, Nelson RJ (1995) Population structure of *Pyricularia grisea* at two screening sites in the Philippines. Phytopathology 85:1011–1020

Chen DH, Zeigler RS, Ahn SW (1996) Phenotypic characterization of the rice blast resistance gene *Pi-2*(*t*). Plant Disease 80:52–56

Chittenden LM, Schertz KF, Lin YR, Wing RA, Paterson AH (1994) A detailed RFLP map of *Sorghum bicolor*×*S. propinquum* suitable for high-density mapping suggests ancestral duplication of sorghum chromosomes or chromosomal segments. Theor Appl Genet 87:925–933

Correa-Victoria FJ, Zeigler RS (1995) Stability of partial and complete resistance in rice to *Pyricularia grisea* under upland conditions in eastern Colombia. Phytopathology 85:977–982

El-Zik KM, Bird LS (1970) Effectiveness of specific genes and gene combinations in conferring resistance to races of *Xanthomonas malvacearum* in upland cotton. Phythopathology 60: 441–447

Feinberg AP, Vogelstein B (1983) A technique for radiolabelling DNA restriction fragments to high specific activity. Anal Biochem 132:6–13

Gardiner SE, Bassett CM, Noiton DAM, Bus VG, Hafstee ME, White AG, Ball RD, Forster RLS, Rikkerink EHA (1996) A

detailed map around an apple scab resistance gene demonstrated that two disease resistance classes both carry the Vf gene. Theor Appl Genet 93:485–493

Hittalmani S, Foolad M, New T, Rodriguez RL, Huang N (1995) Development of a PCR-based marker to identify rice blast resistance gene, *Pi-2*(*t*), in a segregating population. Theor Appl Genet 91:9–12

Huang N, Angeles ER, Domingo J, Magpantay G, Singh S, Zhang G, Kumaravadivel N, Bennett J, Khush GS (1997) Pyramiding of bacterial blight resistance genes in rice: marker-assisted selection using RFLP and PCR. Theor Appl Genet 95:313–320

Inukai T, Nelson RJ, Zeigler RS, Sarkarung S, Mackill DJ, Bonman JM, Takamure I, Kinoshita T (1994) Allelism of blast resistance genes in near-isogenic lines of rice. Phytopathology 84:1278–1283

Inukai T, Nelson RJ, Zeigler RS, Sarkarung S, Mackill DJ, Bonman JM, Takamure I, Kinoshita T (1996a) Genetic analysis of blast resistance in tropical rice cultivars using near-isogenic lines. In: Khush GS (ed). Rice Genetics III. Proc 3rd Int Rice Genet Symp, Oct. 16–20 1995, Manila, The Philippines, pp 447–450

Inukai T, Zeigler RS, Sarkarung S, Bronson M, Dung LV, Kinoshita T, Nelson RJ (1996b) Development of pre-isogenic lines for rice blast resistance by marker-aided selection from a recombinant inbred line population. Theor Appl Genet 93:560–567

Kawasaki S, Rybka K, Miyamto M, Tanaka Y, Kataoka M, Saito A, Ando I (1996) A trial positional cloning of rice blast resistance genes. In: Khush GS (ed). Rice Genetics III. Proc 3rd Int Rice Genet Symp Oct 16–20 1995, Manila, The Philippines, pp 675–678

Kosambi DD (1944) The estimation of map distances from recombination values. Ann Eugen 12:172–175

Kurata N, Nagamura Y, Yamamoto K, Harushima Y, Sue N, Wu J, Antonio BA, Shomura A, Shimizu T, Lin SY, Inoue T, Fukuda A, Shimano T, Kuboki Y, Toyama T, Miyamoto Y, Kirihara T, Hayasaka K, Miyao A, Monna L, Zhong HS, Tamura Y, Wang ZX, Momma T, Umehara Y, Yano M, Sasaki T, Minobe Y (1994) A 300-kilobase interval genetic map of rice including 883 expressed sequences. Nature Genet 8:365–372

Lander ES, Green SP, Abrahamson J, Barlow A, Daly MJ, Lincoln SE, Newbury L (1987) MAPMAKER: an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genome 1:174–181

Levy M, Romao J, Marchetti MA, Hamer JE (1991) DNA Fingerprinting with a dispersed repeated sequence resolves pathotype diversity in the rice blast fungus. Plant Cell 3:95–102

Levy M, Shahjahan KM, Valent B (1995) Lineage structure, avirulence locus polymorphism and organization of pathotype diversity in rice blast fungus. Abstract 3rd Int Rice Genet Symp Oct 16–20 1995, Manila, The Philippines, poster 81

Li Z, Pinson SRM, Stansel JW, Park WD (1995) Identification of two major genes and quantitative trait loci for heading date and plant height in cultivated rice (*Oryza sativa*). Theor Appl Genet 91:374–381

Li Z, Pinson SRM, Park WD, Paterson AH, Stansel JW (1997) Epistasis for three grain yield components in rice (*Oryza sativa* L.) Genetics 145:453–465

Ling KC, Ou SH (1969) Standardization of the international race numbers of *Pyricularia orpae*. Phytopathology 59:339–342

Liu D, Kowalski SP, Lan TH, Feldmann KA, Paterson AH (1996) Genome-wide high-resolution mapping by recurrent intermating using *Arabidopsis thaliana* as a model. Genetics 142: 247–258

Mackill DJ, Bonman JM (1992) Inheritance of blast resistance in near-isogenic lines of rice. Phytopathology 82:746–749

Manly KF (1994) Establishing genetic linkage using recombinant inbred lines with abnormal segregation ratios. Genetics 136: 1433–1434

Marchetti MA (1983) Dilatory resistance to rice blast in USA rice. Phytopathology 73:645–649

Marchetti MA (1994) Race specific-rate-reducing resistance to rice blast in US rice cultivars. In: Zeigler RS, Leong SA, Teng PS (eds) Rice blast disease. CAB International, Wallingford, England, pp 231–244

Marchetti MA, Lai X, Bollich CN (1987) Inheritance of resistance to *Pyricularia oryzae* in rice cultivar grown in the United States. Phytopathology 77:799–804

McClung AM, Marchetti MA, Webb BD, Bollich CN (1997) Registration of 'Jefferson' rice. Crop Sci 37:629–630

McCouch SR, Nelson RJ, Tohme J, Zeigler RS (1994) Mapping blast resistance genes in rice. In: Zeigler RS, Leong SA, Teng PS (eds). Rice blast disease. CAB International, Wallingford, England, pp 167–186

Mew TW, Parco A, Hittalmani S, Inukai T, Nelson R, Zeigler RS, Huang N (1994) Fine mapping of major genes for blast resistance. Int Rice Res Notes 19:4–5

Miyamoto M., Ando I, Rybka K, Kodama O, Kawasaki S (1996) High-resolution mapping of the *indica*-derived rice blast resistance genes. I. *Pi-b*. Mol Plant-Microbe Interact 9:6–13

Naqvi NI, Chattoo BB (1996) Development of a sequence characterized amplified region (SCAR)-based indirect selection method for the dominant blast resistance gene in rice. Genome 39:26–30

Ou SH (1985) Rice diseases (2nd edn). Commonwealth Mycological Institute, New England

Pan Q, Wang L, Ikehashi H, Tanisaka T (1996) Identification of a new blast resistance gene in the *indica* rice cultivar Kasalath using Japanese differential cultivars and isozyme markers. Genetics 86:1071–1075

Pinson SRM, Li Z, Tabien RE, Paterson AH, Park WD, Stansel JW (1996) RFLP mapping of genes: potential, limits and current status. Proc 26th Rice Technol Work Group Meet, Feb 25–28 1996, San Antonio, Texas, p 56

Roumen EC (1994) The inheritance of host plant resistance and its effect on the relative infection efficiency of *Magnaporthe grisea* in rice. Phytopathology 89:498–503

Rybka K, Miyamoto M, Tanaka Y, Kataoka M, Ando I, Saito A, Kawasaki S (1997) High resolution mapping of the *indica*-derived rice blast resistance genes II. *Pi-ta*^{2} and *Pi-ta* and a consideration of their origin. Mol Plant-Microbe Interact. 10:517–524

SAS Institute Inc (1988) Additional SAS/STAT procedures: release 6.03. SAS Tech Report, SAS Institute Inc, Cary, North Carolina

Shaner G, Finney RE (1977) The effect of nitrogen fertilization on the expression of slow-mildewing resistance in Knox wheat. Phytopathology 67:1051–1056

Shull V, Hamer JE (1994) Genomic structure and variability in *Pyricularia grisea* In: Zeigler RS, Leong SA, Teng PS (eds) Rice blast disease. CAB International, Wallingford, England, pp 65–68

Tabien RE (1996) Utilization of recombinant inbred lines in tagging blast resistance genes for a marker-assisted selection scheme in rice. PhD thesis. Texas A&M University, College Station, Texas 77843, USA

Tabien RE, Pinson SRM, Marchetti MA, Li Z, Park WD, Paterson AH, Stansel JW (1996) Blast resistance genes from Teqing and Lemont. In: Khush GS (ed) Rice genetics III. Proc 3rd Int Rice Genet Symp, Oct 16–20 1995, Manila, The Philippines, pp 451–455

Wang GL, Mackill DJ, Bonman JM, McCouch SR, Champoux MC, Nelson RJ (1994) RFLP mapping of genes conferring complete and partial resistance to blast from durably resistant cultivar. Genetics 136:1421–1434

Wang GL, Song WY, Chen LL, Kim HS, Ruan R, Sideris S, Ronald PC (1995) Spectrum, race specificity and expression of the rice *Xa-21* disease resistance gene family. Abstract 3rd Int Rice Genet Symp. Oct 16–20 1995, Manila, The Philippines, poster 23

Wright RJ, Thaxton PM, El-Zik KM, Paterson AH (1998) D-subgenome bias of *Xcm* resistance genes in tetraploid Gossypium

(cotton) suggests that polyploid formation has created novel avenues for evolution. Genetics 149:1987–1996

Wu KS, Martinez C, Lentini Z, Tohme J, Chumley FG, Scolnik PA, Valent B (1996) Cloning a blast resistance gene by chromosome walking. In: Khush GS (ed) Rice Genet III. Proc 3rd Int Rice Genet Symp, Oct 16–20 1995, Manila, The Philippines pp 669–674

Xia JQ, Correll JC, Lee FN, Marchetti MA, Rhoads DD (1993) DNA fingerprinting to examine microgeographic variation in the Magnaporthe grisea (Pyricularia grisea) population in tow rice fields in Arkansas. Phytopathology 83:1029–1035

Yu ZH, Mackill DJ, Bonman JM (1987) Inheritance of resistance to blast in some traditional and improved rice cultivars. Phytopathology 77:323–326

Yu ZH, Mackill DJ, Bonman JM, Tanksley SD (1991) Tagging genes for blast resistance in rice via linkage to RFLP markers. Theor Appl Genet 81:471–476

Zheng KL, Zhuang JY, Lu J, Qian HR, Lin HX (1996) Identification of DNA markers tightly linked to blast resistance genes in rice. In: Khush GS (ed) Rice genetics III. Proc 3rd Int Rice Genet Symp, Oct 16–20 1995, Manila, The Philippines, pp 565–569