RFLP Mapping of Genes Conferring Complete and Partial Resistance to Blast in a Durably Resistant Rice Cultivar

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ABSTRACT

Moroberekan, a japonica rice cultivar with durable resistance to blast disease in Asia, was crossed to the highly susceptible indica cultivar, CO39, and 281 F_7 recombinant inbred (RI) lines were produced by single seed descent. The population was evaluated for blast resistance in the greenhouse and the field, and was analyzed with 127 restriction fragment length polymorphism (RFLP) markers. Two dominant loci associated with qualitative resistance to five isolates of the fungus were tentatively named *Pi-5(t)* and *Pi-7(t)*. They were mapped on chromosomes *4* and *11*, respectively. To identify quantitative trait loci (QTLs) affecting partial resistance, RI lines were inoculated with isolate PO6-6 of *Pyricularia oryzae* in polycyclic tests. Ten chromosomal segments were found to be associated with effects on lesion number ($P < 0.0001$ and LOD > 6.0). Three of the markers associated with QTLs for partial resistance had been reported to be linked to complete blast resistance in previous studies. QTLs identified in greenhouse tests were good predictors of blast resistance at two field sites. This study illustrates the usefulness of RI lines for mapping a complex trait such as blast resistance and suggests that durable resistance in the traditional variety, Moroberekan, involves a complex of genes associated with both partial and complete resistance.

THE use of resistant cultivars is the most economical and effective way of controlling rice blast, an often devastating disease that occurs in most rice-growing areas worldwide (Ou 1985). However, the useful life span of many cultivars is only one or a few years in disease-conducive environments (*e.g.*, Lee and Cho 1990; Kiyosawa 1982) due to the breakdown of resistance in the face of high pathogenic variability of *Pyricularia oryzae* Cavara (Ou 1979; Bonman *et al.* 1986). Breeding for more durably resistant cultivars, therefore, has become a priority in rice improvement.

Resistance is considered durable when it remains effective in a cultivar despite widespread cultivation in an environment favoring the disease. In different pathosystems, durable resistance is variously controlled by single genes, multiple genes with cumulative effects, polygenes, and the resistance may be either complete or incomplete (partial) (Johnson 1983; Parlevliet 1988). Several rice cultivars with durable blast resistance have been identified (Lee *et al.* 1989; Bonman and Mackill 1988). For example, some upland cultivars such as the traditional African cultivars Moroberekan and OS6 have been cultivated for many years in large areas in West Africa without high losses from blast (Notteghem 1985; Bonman and Mackill 1988). These cultivars have been widely used as resistance donors in breeding programs.

Blast resistance is generally classified into two types based on the way the gene(s) affect pathogen reproduction: qualitative (complete) and quantitative (partial). Qualitative resistance conditions incompatibility of the host and pathogen strain, preventing reproduction of the fungus, while partial resistance reduces the extent of pathogen reproduction within the context of a compatible interaction. Genetic studies of qualitative resistance to rice blast were started when Goto established the differential system for races of *P. oryzae* in Japan in the early 1960s (Ou 1985). Since then, the inheritance of resistance has been extensively studied (Kiyosawa 1981; Atkins and Johnston 1965; Mackill and Bonman 1992; Mackill *et al.* 1988) and several genes for complete resistance have been mapped relative to restriction fragment length polymorphism (RFLP) markers. Yu *et al.* (1991) identified RFLP markers linked to *Pi-2(t)* and *Pi-4(t)*; Tohme *et al.* (1991) identified markers linked to a resistance gene from IRAT13; and L. Zhu, (personal communication, Academia Sinica, Beijing, 1991) identified markers linked to *Pi-zh(t)*.

Aside from genes for qualitative resistance to *Pyricularia oryzae*, varieties with durable resistance also have a high level of partial resistance. In 1968, Toriyama *et al.* reported high levels of field resistance in the variety

The results of this analysis and the RFLP data set associated with the CO39/Moroberekan RI population will be accessible in the Rice Genome Database ("RiceGenes") through the National Agricultural Library in Washington, D.C., or through Gopher.

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Chugoku 31. Inheritance of partial resistance was found to be polygenic in studies involving the cultivars IRAT13, IAC25, IAC47 and Dourado Precose (NOTTEGHEM 1985) and Moroberekan (LOUVEL 1985). Similar results were obtained by LIN (1986), whose study showed that inheritance of field resistance to rice blast was quantitative, with additive and partially dominant effects of minor genes. The broad-sense heritabilities ranged from 38 to 83% depending on how resistance was measured. WANG *et al.* (1989) found that inheritance of partial resistance in IR36 was most likely polygenic with very low narrow-sense heritability. ROUMEN (1993) observed a consistent and high level of partial resistance in IR36 and IR64 in both greenhouse and field tests. Though considerable progress has been made, the genetic basis of resistance in durably resistant cultivars is still not well understood and attempts to transfer the character into different genetic backgrounds have not been widely successful due to the complexity of the trait and limitations of the research methodologies used.

The recent development of RFLP techniques makes it possible to investigate the inheritance of complex traits and to locate and manipulate individual genetic factors associated with these traits (TANKSLEY 1993). Recent studies in tomato (PATERSON *et al.* 1988, 1990, 1991; TANKSLEY and HEWITT 1988; TANKSLEY *et al.* 1989), maize (BURR *et al.* 1988; GRANT *et al.* 1989; BEAVIS *et al.* 1991; STUBER *et al.* 1992), and soybean (KEIM *et al.* 1990) have demonstrated that RFLP mapping is a powerful approach for identifying quantitative trait loci (QTLs) controlling agronomically important characters. Although several single-gene characters have been located via linkage to mapped RFLP markers in rice (MACKILL *et al.* 1993; RONALD *et al.* 1992; MCCOUCH and TANKSLEY 1991; MCCOUCH *et al.* 1991; YU *et al.* 1991; YOSHIMURA *et al.* 1992), this is the first published report of a QTL analysis for this crop.

This study was undertaken to gain insight into the genetic basis of blast resistance in a durably resistant rice cultivar, to locate resistance genes on the molecular map of rice, to detect differences among QTLs in their relative effects on components of quantitative resistance, and to provide a permanent mapping population and associated RFLP data set for future QTL studies in rice.

MATERIALS AND METHODS

Development of RI lines: Moroberekan, a West African japonica cultivar with durable blast resistance, was used as the pollen parent in a cross with the very susceptible indica cultivar, CO39, in the 1988 wet season at IRRI. About 50 F_1 seeds were obtained from the cross. Fifteen F_1 seeds were randomly chosen and grown in a greenhouse to obtain an F_2 population. About 300 F_2 seeds were randomly selected and planted in the Rapid Generation Advance (RGA) (VERGARA *et al.* 1982) greenhouse from F_2 to F_6 using single seed descent (SSD). Early flowering of rice plants in the RGA greenhouse was encouraged by short day treatments (10 hr of daylight) and temperatures of 30–36° starting at the 5-leaf stage (around 30 days). Plants were grown in small pots (volume approximately 250 cc soil), with only one tiller per plant. All panicles were bagged at each generation in the RGA. F_7 seeds from bagged panicles were used for genotype analysis and F_7 open-pollinated seeds were used for blast evaluations.

Parental polymorphism survey and RFLP analysis of RI lines: To identify probe-enzyme combinations revealing polymorphism between Moroberekan and CO39, DNA was extracted from the leaves of the two parents as described by DELLAPORTA *et al.* (1984) and digested with the restriction enzymes *Dra*I, *Eco*RI, *Eco*RV, *Hin*dIII and *Sca*I. The digested DNAs were subjected to electrophoresis on 0.9% agarose gels and transferred to Hybond N+ membranes (Amersham Corp., Chicago) according to the manufacturer's instructions. Two hundred eighty DNA clones distributed throughout the 12 chromosomes of rice (184 rice genomic clones, coded RG; 62 rice cDNAs, coded RZ; and 28 oat cDNAs, coded CDO) (MCCOUCH *et al.* 1988; TANKSLEY *et al.* 1992) were linearized and labeled with [^{32}P]dCTP by the random hexamer method (FEINBERG and VOGELSTEIN 1984). Hybridized filters were washed once in 1.5 × SSPE and once in 0.5 × SSPE at 65° for 15–20 min. Filters were exposed to X-ray film at −80° with one intensifying screen for 1–4 days. One hundred twenty-seven informative probes were used for segregation analysis of the RI lines using the procedures outlined above.

Evaluation of blast resistance of RI lines: *Monocyclic test:* Genes conditioning partial resistance can only be detected in the absence of effective qualitative resistance genes. Moroberekan often shows no disease even in highly conducive environments, and therefore it was inferred to contain qualitative resistance gene(s) (MACKILL *et al.* 1985). In an attempt to uncover partial resistance factors, a systematic search for isolates capable of infecting Moroberekan was undertaken. More than 300 isolates collected from many cultivars in different regions of the Philippines were used to inoculate Moroberekan. No isolate capable of infecting this cultivar was found (A. CALVERO and J. M. BONMAN, unpublished). Therefore, five isolates representing diverse regions of the Philippines were used to map qualitative resistance gene(s). Based on phylogenetic analysis of these strains using the repetitive probe, *MGR 586* (HAMER *et al.* 1989), they represent three distinct clonal lineages of the pathogen (D. CHEN and R. NELSON, unpublished).

The disease reaction of each RI line was scored 6–7 days after inoculation using a modified scoring system based on BONMAN *et al.* (1986). A score of 3+ was added between scores 3 and 4 on the 0–5 scale. Lines were given a score according to the most susceptible lesion type that was abundantly represented. Roundish lesions of about 1–2 mm in diameter with gray centers surrounded with brown margins and capable of sporulation were classified as lesion type 3. Those with roundish to elliptical lesions of about 2–3 mm in diameter with gray centers surrounded with brown margins and capable of sporulation were classified as type 3+.

Polycyclic test: A single isolate, PO6-6, was selected from the five used in monocyclic tests for mapping partial resistance genes in the RI population. PO6-6 has a broad spectrum of virulence, is genetically stable, and is routinely used for studies at IRRI. Because quantitative resistance could only be evaluated in the absence of genes governing qualitative resistance to PO6-6, RI lines showing complete resistance (*i.e.*, those with no disease symptoms in the monocyclic test when inoculated with PO6-6) were excluded from the polycyclic evaluation. The remaining 131 lines showing susceptible reactions to this isolate were used in polycyclic tests. The polycyclic test was developed in order to measure cumulative disease development on a population of test plants after several cycles of pathogen reproduction. This test is simple and can be readily used on

many lines. It is useful because small differences in the degree of susceptibility are amplified, making phenotypic evaluation more informative and reliable.

About 100 seeds of each RI line were sown in 34 × 27 × 12-cm plastic trays (5 rows with 20 seeds/row) and grown in the greenhouse. Fourteen days after sowing, seedlings were moved to polyethylene chambers (0.5 × 1.0 × 1.0-m^3 or larger) for polycyclic disease evaluation. The plants were inoculated by spraying 15 ml of spore suspension (5 × 104 conidia/ml) in each chamber and were subsequently sprayed with a mist of water 4–5 times during the day. The cages were covered with moist jute sacks and polyethylene at night. Plants were scored 14 days after inoculation. Diseased leaf area (DLA) was visually estimated for 10 randomly selected plants from each line. Lesion number and lesion size were measured for 12 leaves from four plants (three leaves/plant) of each line and means of the four replications were used in data analysis. All three parameters were standardized prior to analysis by dividing the average measurement per RI line by the average measurement of the susceptible CO39 parent.

Field tests: All RI lines were grown in a randomized complete block design at an IRRI upland screening site in Cavinti, Laguna, Philippines. Two trials with two replications each were evaluated during July-October 1991 using a miniplot technique (MARCHETTI 1983). Test entries were drilled in two-row miniplots 60 cm long with 10 cm row spacing, using approximately 5 g of seeds per entry. Test entries were separated by two rows of a resistant cultivar (IRAT13) to minimize the interferences between adjacent rows. Three rows of susceptible cultivars (50% IR50 and 50% C22) were planted around the blocks to enhance the natural inoculum. The percentage of diseased leaf area was visually estimated at 3, 4, 5 and 6 weeks after sowing. In collaboration with ZULKIFLI ZAINI and SYAHRIL DARWIS of Sukarami Research Institute for Food Crops (SARIF), Indonesia, a similar experiment was conducted at Sitiung Substation, SARIF, West Sumatra, Indonesia, during January-February, 1992. The disease levels of RI lines were evaluated in one trial with two replications.

Statistical analysis: The programs Mapmaker (LANDER *et al.*, 1987) and Map Manager (MANLY 1993) were used to establish an RFLP map. Linkage groups were inferred based on the existing consensus RFLP map of rice (TANKSLEY *et al.* 1992), but marker order and map distances were derived using the RI algorithm in Version 3.0 of the Mapmaker program and in Version 2.4b16 of the Map Manager program, based on the segregation data in the F_7 RI lines. Summary statistics regarding allele frequencies at each locus, single locus double crossovers, and missing data points per interval were assembled using Map Manager. The PROC GLM procedure in the Statistical Analysis System (SAS) was used to determine associations between molecular markers and the three parameters of quantitative resistance (lesion number, lesion size, and DLA). Mapmaker/QTL (LANDER and BOTSTEIN 1989) was also used to identify putative loci affecting quantitative resistance based on point and interval analysis. Results from the different analytical approaches were compared.

RESULTS

Parental polymorphism survey: Of the 288 RFLP markers tested, 171 were polymorphic between Moroberekan and CO39 with one or more of the five enzymes tested. The level of polymorphism revealed by each enzyme ranged from 30.2 (*Dra*I) to 37.4% (*Eco*RV). The overall level of polymorphism detected by rice genomic (RG) clones between the two parents was 68.3%. This is slightly less than the 78% previously reported for indica/japonica crosses using the same library (MCCOUCH and TANKSLEY 1991). Levels of polymorphism using the rice cDNA (RZ) and oat cDNA (CDO) clones were 48.3 and 57.0%, respectively, with *Eco*RI having the highest level of polymorphism (20%) compared to 17% for *Hin*dIII and *Dra*I, and only 13% for *Eco*RV. These results are consistent with those from other experiments and suggest that the RG clones are more efficient at detecting polymorphism in indica/japonica crosses than are the cDNA clones used in this experiment.

Segregation of polymorphic markers in F_7 RI lines and map construction: One hundred twenty-seven markers showing polymorphism between the two parents were chosen for mapping and analysis in the RI lines. Most of the markers showed a single hybridizing band, but five multiple-copy markers were included in order to provide more uniform genome coverage. Because allelism could not be assumed for multiple-copy probe bands, scoring was dominant/recessive for individual bands in these cases and markers were coded with an A suffix. For 23 probes, nonparental band mobilities were observed and the average frequency of non-parental alleles was 1.21%. Nonparental bands were coded as missing data. In the case of *RG103*, a multiple copy marker located on chromosome 11, a non-parental allele of consistent molecular weight was observed in 10% of the lines and null alleles for all bands (complete lack of signal) was observed in 26% of the lines. Further experiments are underway to determine whether the nonparental allele can be traced to heterogeneity in either of the parental lines, or whether the null alleles may be the result of transposon activity during RI line development. Null alleles were also coded as missing data.

Skewed segregation favoring indica alleles was observed for most of the markers (Figure 1). At only 6 marker loci were there at least 50% japonica alleles represented in the RI population. Segregation was most distorted for *RG1094*; the japonica allele was present in only seven (2.5%) of the lines at this locus. Among the 281 lines, 14 had japonica alleles at only 2/127 loci.

The degree of skewing in the RI population (overall average of 80% CO39 alleles and 20% Moroberekan alleles) affected our ability to map the markers *de novo* based on the segregation data of RI lines alone. Calculations of linkage in most analyses assume a 50:50 allele ratio in the population, and the null hypothesis (nonlinkage or random assortment) is accepted when the observed number of recombinants is compatible with an expected recombination fraction of 0.5. Calculations demonstrate that for a population where one allele is present in only 20% of the progeny, the expected frequency of recombination for unlinked markers is 0.32 rather than 0.5. This shift in probability affects the accuracy of linkage determinations by making a smaller number of recombinants in a population compatible

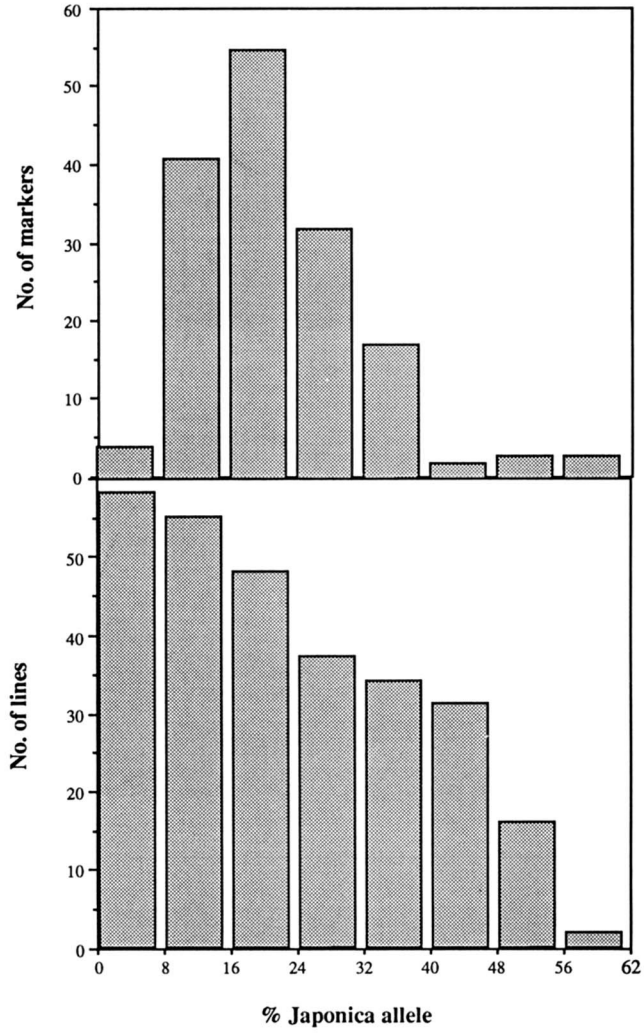

FIGURE 1.—Distribution of percent japonica alleles for RFLP markers and recombinant inbred lines.

with random assortment, or nonlinkage. Thus, when our RI data was analyzed under the assumption of a 50:50 allele distribution, many unlinked loci appeared linked, distorting our ability to accurately assemble linkage groups. (See APPENDIX 1 for details.) For these reasons, we used information assembled during previous mapping experiences (MCCOUCH *et al.* 1988; TANKSLEY *et al.* 1992) to help us define our primary linkage groups, and included only single copy probes in these preliminary groups. Once linkage groups had been tentatively defined based on previously constructed maps, the order of those markers derived from the RI data obtained during this study (based on either Mapmaker or Map Manager) showed no major disagreement with the order derived based on the interspecific BC population described by TANKSLEY *et al.* (1992). Map distances are expected to fluctuate among crosses, and as they do not affect our ability to detect QTLs (KNOTT and HALEY 1992), we were not concerned with how those reported here might differ from map distances observed between the same markers in other populations.

In summary, the map shown in Figure 2 was derived by first assigning markers to linkage groups based on a previously established consensus map of rice (TANKSLEY *et al.* 1992) and subsequently establishing the most likely order and recombination fraction on the chromosomes based on data from the RI population developed during this study. The recombination fractions between markers were estimated using both Mapmaker (LANDER *et al.* 1987) and Map Manager, Version 2.4b16 (MANLY 1993), and were in good agreement. The map distances reported here are based on the algorithm appropriate for calculating centiMorgan distances for RI lines in Mapmaker/EXP, Version 3.0 (E. LANDER and S. LINCOLN, Whitehead Institute, Cambridge, Massachusetts, personal communication). Markers on the framework map were placed with a LOD > 2.0. An RFLP map containing 127 loci was established.

Mapping genes for complete resistance to blast: Data from 11 of the 277 lines tested were eliminated due to variation in disease reaction among plants within a line, indicating possible heterozygosity at one or more resistance loci. One hundred seventeen lines were classified as resistant and 149 lines were classified as susceptible. Lines showing resistant scores of 0–2 or susceptible scores of 4–5 showed consistent reactions to all five isolates. However 41 lines that had intermediate scores of 3 (13 lines) and 3+ (28 lines) showed inconsistent reactions in different inoculation tests. Subsequent analysis indicated that most of these lines had the CO39 allele (susceptible) at one or both of the loci associated with genes for complete resistance, and had the Moroberekan alleles (resistant) at many loci associated with genes for partial resistance. Because of the epistatic effects of genes for partial and complete resistance, the 41 lines (15%) with intermediate disease reactions (scored 3 and 3+) were eliminated from the dataset used for mapping of complete resistance. Of the remaining 225 RI lines, 104 were resistant and 121 were susceptible. They all showed consistent disease reactions to the five isolates tested, indicating that the resistance genes in Moroberekan confer complete resistance to all five isolates.

The location of complete resistance genes on the RFLP map of rice was analyzed using both SAS/GLM and Mapmaker/QTL software. A total of 16 markers showed a significant correlation with complete resistance using SAS/GLM ($F > 30.0$), and 15 markers were significant based on Mapmaker/QTL analysis (LOD score > 6.0). The same 15 markers were detected using both analytical approaches.

Markers significantly associated with complete resistance defined 5 chromosomal regions. The marker with the highest F value was *RG788* ($F > 95.8$ and LOD > 15.5), with an $R^2 = 0.27$ (SAS/GLM), and accounting for 25.4% of the phenotypic variance for complete resistance (Mapmaker/QTL). Two other significant markers also mapped to the same region of chromosome *4*

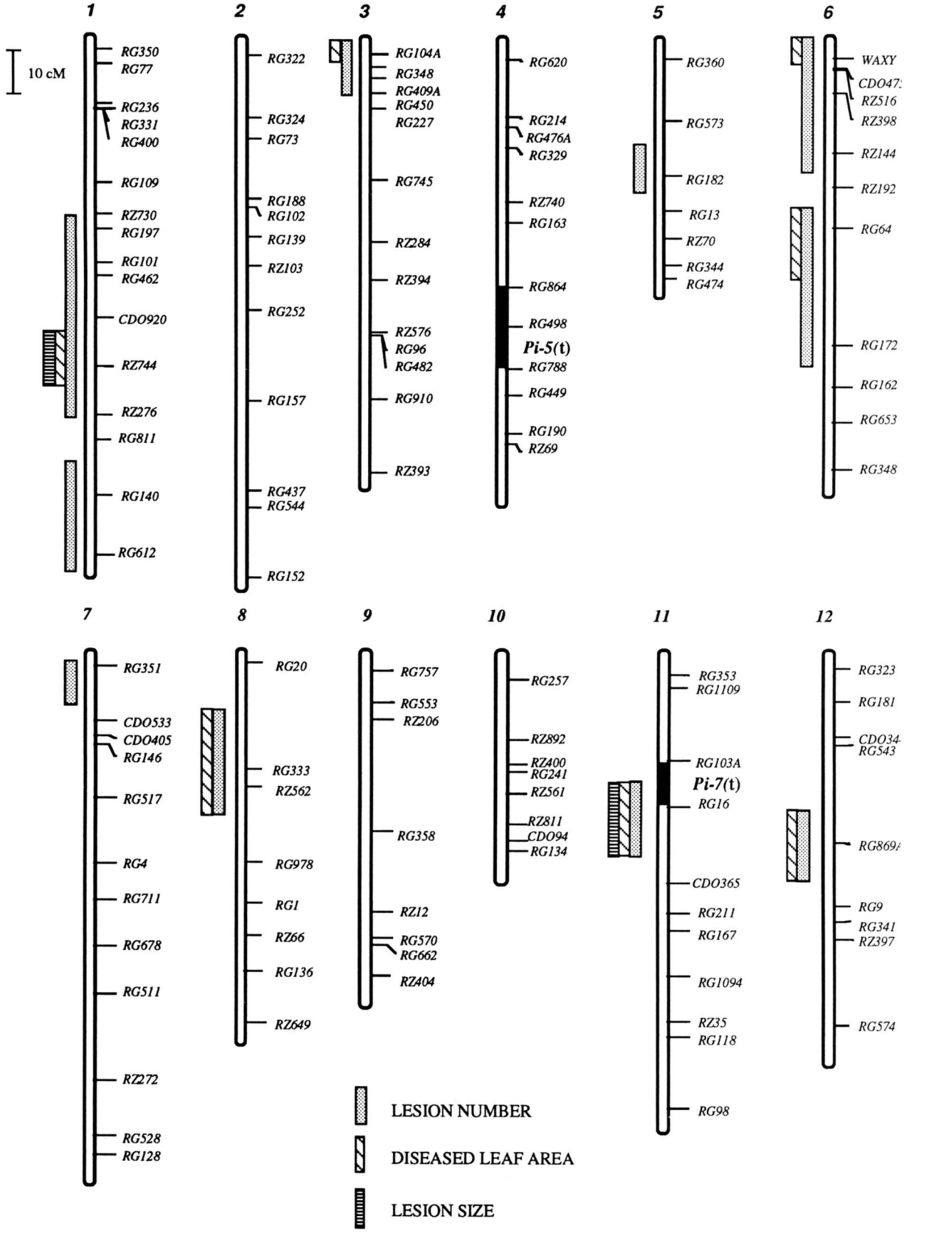

FIGURE 2.—RFLP map derived from segregation data of the Moroberekan/CO39 recombinant inbred (RI) lines. The map distances depicted here are calculated in centiMorgans using the RI algorithm in Mapmaker/EXP, Version 3.0. Solid-filled areas on chromosome *4* and *11* represent supporting intervals around the complete resistance loci *Pi-5(t)* and *Pi-7(t)*. Open bars represent chromosomes; designations to the right represent marker names; stippled bars to the left represent supporting intervals around the chromosomal regions associated with partial resistance. Significant associations ($P < 0.0001$ and LOD > 6.0) between markers and the three individual parameters of partial resistance (lesion number, lesion size and diseased leaf area) are as indicated.

TABLE 1

Markers bracketing ten QTLs associated with lesion number in polycyclic tests (*F* value >29.0 and LOD >6.0) are listed by chromosome

QTL[a]	Chromosome no.	*F* value	R^{2b}	LOD[c]	Percent variation	Allele effect[d]
RZ744-RZ276	*1*	155.0	0.52	22.5	58.8	0.46
RG612-RG140	*1*	41.1	0.25	11.9	32.5	0.38
RG104A-RG348	*3*	50.4	0.28	9.7	28.8	0.46
RG182-RG573	*5*	30.4	0.24	6.1	19.4	0.45
RZ744-waxy	*6*	58.9	0.32	10.4	30.8	0.44
RG64-RG172	*6*	49.0	0.28	13.7	60.0	0.34
RG351-CDO533	*7*	29.4	0.19	6.8	23.6	0.34
RG333-RZ562	*8*	51.8	0.29	10.0	30.5	0.34
RG16-CDO365	*11*	40.4	0.24	8.8	28.7	0.40
RG869B-RG9	*12*	50.0	0.28	9.8	32.5	0.43

Markers were identified using SAS/GLM (*F*-values and R^2) and Mapmaker/QTL (LOD and % variation).

[a] Markers bracketing the QTL are listed in order of significance—the first marker had higher *F* value and R^2 (SAS/GLM).

[b] Fraction of the total variation explained by the first marker of the interval (SAS/GLM).

[c] LOD scores ($\log_{10}$-likelihood ratio) offer an indication of the strength of the data supporting the existence of a QTL in a defined interval (Mapmaker/QTL).

[d] Mean differences for lesion number between the two genotypic groups carrying CO39 and Moroberekan alleles at the first marker.

(*i.e.*, *RG498* and *RG864*) (Figure 2). The second highest *F* value was for *RG16* on chromosome 11 ($F \geq 69.0$, LOD > 11.5), with an $R^2 = 0.22$ (SAS/GLM) and accounting for 19.9% of the phenotypic variance (Mapmaker/QTL). The putative resistance gene was bracketed by *RG16* and *RG103A*. In addition to these loci, Moroberekan alleles at *RG188* and *RG102* on chromosome *2*, at *RG323* on chromosome *12*, and at *RG528* on chromosome *7* were significantly associated with the resistant phenotype.

Among the 104 lines showing complete resistance in the population, 77 lines had the Moroberekan allele at *RG788*, *RG498* and/or *RG864* (chromosome *4*), while 58 lines had the Moroberekan allele at *RG103A* and/or *RG16* (chromosome *11*). When considered together, the resistance of 96 of the 104 lines could be explained by genetic factors associated with marker loci in these two areas of the genome. Of the 8 lines showing resistance to blast but containing susceptible (CO39) alleles at these loci, 6 had resistant (Moroberekan) alleles at candidate loci in the other 3 areas of the genome. We have no explanation for the remaining 2 lines at this time. Based on these results, we suggest that at least one locus on chromosome *4* and one on *11* are involved in conferring complete resistance to the five blast isolates tested in this study. The loci on chromosome *4* and *11* were tentatively named *Pi-5(t)* and *Pi-7(t)*, respectively, after Yu *et al.* (1991), Mackill and Bonman (1992) and Tohme *et al.* (1991).

When markers on all five chromosomes associated with complete resistance were tested for interaction among each other (two-way ANOVA, SAS), significant interactions were detected (Table 2). To distinguish between epistasis and pseudolinkage among the putative resistance loci on chromosomes *2*, *7* and *12*, and *Pi-5(t)* and/or *Pi-7(t)*, we further tested the significance of each marker locus once individuals carrying the Moroberekan (resistant) allele associated with *Pi-5(t)* and/or *Pi-7(t)* had been removed from the dataset. This analysis demonstrated that *Pi-5(t)* and *Pi-7(t)* were independent and epistatic, and that pseudolinkage with *Pi-5(t)* or *Pi-7(t)* could not explain the significance of the other three loci. Further crossing and purification of near-isogenic lines from this population is required to fully characterize the effects of these putative factors conferring complete resistance to five blast isolates.

Because there was more than one locus associated with complete resistance in this population, the precise map distances between these genes and linked RFLP markers could not be established using Mapmaker. However, analysis using both SAS/GLM and Mapmaker/QTL suggest that *Pi-5(t)* falls in a 12-cM interval bracketed by *RG788* and *RG498* and that *Pi-7(t)* falls in a 16-cM interval bracketed by *RG103A* and *RG16* (Figure 2).

Mapping genes that control partial resistance: Lesion number, lesion size and DLA are three important components of partial resistance (Yeh and Bonman 1986). The three parameters were measured for 131 lines in polycyclic tests using isolate PO6-6 with four replications. All three parameters had a roughly normal distribution among the 131 lines. The means of the four replications of each of the three parameters measured were used in SAS/GLM and Mapmaker/QTL analysis. The analysis of variance indicated significant differences among the lines tested for all parameters. Though the components were significantly correlated, lesion number had the largest variance among the lines.

At an *F* value > 29.0 and a LOD > 6.0, 20 markers defining 10 chromosomal regions were found to be associated with lesion number (Table 1). Eight markers distributed in seven different regions of the genome were associated with effects on DLA at a threshold of *F* value > 29.0 and a LOD > 6.0, and two markers in two regions were found to be associated with effects on lesion size (*F* value > 29.0 and LOD > 6.0) (Figure 2).

Despite the fact that Mapmaker/QTL is not designed for recombinant inbred populations, it appears robust to violations of population structure, as evidenced by the similarity of results from linear modeling and interval analysis in this study. This observation is consistent with results of STUBER *et al.* (1992) and KNOTT and HALEY (1992).

Most of the markers identified affected all three parameters of blast resistance. This suggests that genes located in the same chromosomal regions may affect the three components of partial resistance, and is consistent with previous observations that the traits are correlated (YEH and BONMAN 1986; ROUMEN 1993). Both the LOD scores and the percent of variance explained were generally higher for QTLs associated with lesion number. This is probably because lesion number could be measured more accurately than lesion size or diseased leaf area. Further, differences in lesion number but not lesion size would be amplified after 2–3 cycles of infection, giving a larger variance for lesion number among the lines in the polycyclic assay.

The individual contribution of each putative QTL to variation in lesion number was analyzed. Using SAS/GLM, the proportion of the phenotypic variation explained by markers, R^2, ranged from 52.0 to 19.0% among the top 10 markers (Table 1). Point analysis was also performed using Mapmaker/QTL (by setting the distances between markers to 0 cM), and the percent of phenotypic variance explained ranged from 51.1 to 18.9% (data not shown). As expected, these estimates are almost identical because, by setting marker intervals equal to zero, we have effectively constrained Mapmaker to perform linear regression. When interval analysis was performed using Mapmaker/QTL, the percent of phenotypic variance explained by the markers flanking the most significant resistance factors ranged from 60.0 to 19.4% (Table 1). The allele effect at each significant marker locus was computed based on the mean phenotypic difference between the two genotypic groups. These differences ranged from 0.46 to 0.30 (standardized lesion number/mm^2). The correlation between allele effect and R^2, or percent variance explained, for the 10 loci analyzed was not significant (r = 0.41 NS).

To assess the proportion of additive *vs.* epistatic effects among loci associated with partial resistance, putative QTLs were tested for two-way interaction in all pairwise combinations. Ten significant interactions were detected (see Table 2). In addition, multiple regression analysis (stepwise procedure) showed that the optimum combination of five markers accounted for a total of 76.3% of the variance for lesion number. The markers included in the model were *RZ744* (56.43%), *RG333* (10.30%), *RG612* (2.97%), *RG16* (2.54%) and *RG64* (1.34%). The percent of phenotypic variance explained in this 5 variable model contrasted to that explained by the individual one variable models tested previously (Table 1) and provided additional evidence of epistatic interactions among the QTLs associated with partial resistance.

TABLE 2

Significance of two-way interactions among QTLs associated with complete and partial resistance to blast

Pair of QTLs	*F* value	Probability
Complete resistance		
RG788 × *RG528*	33.35	0.0001
RG788 × *RG16*	14.54	0.0002
RG788 × *RG323*	12.69	0.0005
RG788 × *RG102*	9.02	0.003
RG16 × *RG323*	30.50	0.0001
RG103A × *RG528*	10.46	0.001
RG102 × *RG528*	17.25	0.0001
RG102 × *RG323*	8.31	0.004
Partial resistance		
RG64 × *RG333*	21.41	0.0001
RG351 × *RG104A*	14.63	0.0002
RG104A × *waxy*	11.27	0.001
RZ744 × *RG869B*	11.01	0.001
RG351 × *RG869B*	10.97	0.001
RG612 × *RG869B*	10.34	0.002
RG351 × *RG16*	9.15	0.003
RG351 × *RZ7744*	8.99	0.003
waxy × *RG869B*	8.54	0.004
RG351 × *RG612*	8.13	0.005
RZ744 × *RG16*	7.16	0.009
RG351 × *RG64*	7.14	0.009

To rule out the possibility that putative QTLs were correlated due to pseudolinkage (rather than epistasis) in this highly skewed population, QTLs located on different chromosomes were tested for their individual contribution to phenotype after individuals that carried the resistant (*i.e.*, Moroberekan) allele at a locus showing correlated scores for lesion number had been removed from the data set. The remaining data, drawn from lines with CO39 alleles at the correlated loci, were then analyzed to investigate whether significant differences could be detected between groups carrying the Moroberekan and the CO39 alleles at the locus in question. Out of 10 pairs of correlated loci tested in this manner, significant contributions to phenotype were confirmed for each putative QTL. For example, although phenotypic scores for individuals carrying resistant or susceptible alleles at *RG333* (chromosome *8*) and *RG64* (chromosome *6*) were highly correlated, a significant difference in lesion number was found between groups carrying alleles from the resistant or susceptible parent at the *RG64* locus, when the data set contained only individuals with the susceptible allele at *RG333*. We therefore reject the hypothesis that the interactions observed among the putative QTLs in this study are due to pseudolinkage and conclude that these QTLs are epistatic and associated with genes for partial resistance.

Two linked QTLs were identified on chromosome *6* (Figure 2 and Table 1). These were tested for independence of effect using Mapmaker/QTL by fixing the variance associated with *RG64* and testing for an additional

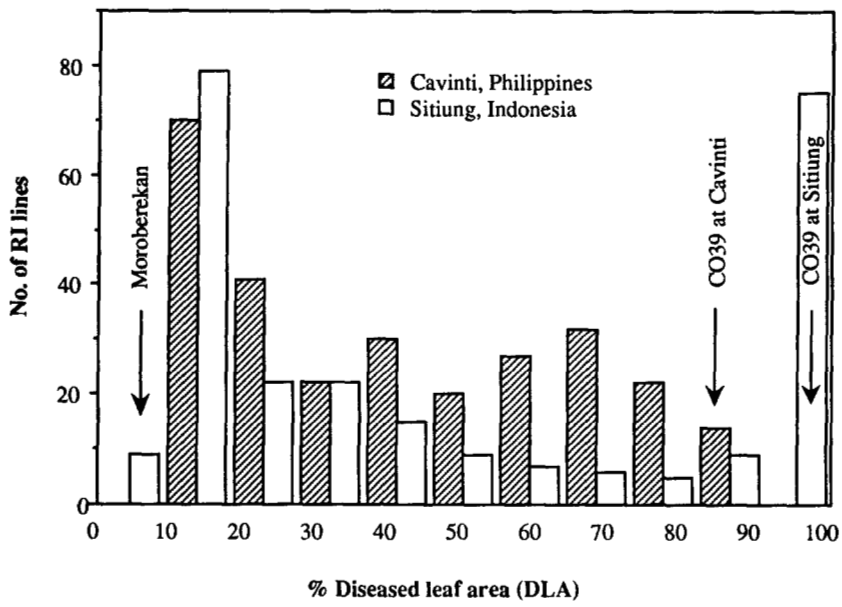

FIGURE 3.—Distribution of diseased leaf area (DLA) of RI lines tested at Cavinti, Philippines, July-October 1991, and at Sitiung, Indonesia, January-February 1992.

effect of *waxy*. This analysis demonstrated that these putative QTLs are not purely additive. However, the interaction term was nonsignificant when analyzed using a two-way ANOVA (SAS). Additional marker data and genetic analysis is required to clarify the behavior of loci in this region.

Three putative QTLs for partial resistance identified in this study, *i.e.*, those linked to *RG64*, *RG869B* and *RG333*, have been reported to be linked to genes conditioning complete resistance to the same or different isolates in previous studies (YU *et al.* 1991; L. ZHU, personal communication, Academia Sinica, Beijing, 1991). The allelic relationships between loci associated with partial and complete resistance, between QTLs and major gene loci, will be analyzed in future studies.

Disease reactions of RI lines under field conditions: To evaluate the performance of *Pi-5(t)*, *Pi-7(t)* and the putative QTLs identified in response to isolate PO6-6 under greenhouse conditions, the RI lines were tested under field conditions at two blast screening sites in different countries. Diseased leaf area was evaluated and the distribution of disease levels is shown in Figure 3. Analysis of variance of the data showed that there were significant differences among the lines 6 weeks after sowing at both sites.

The DLA data from both sites were used to detect RFLP markers affecting the field disease reactions of RI lines using both SAS/GLM and Mapmaker/QTL. Forty three and 62 markers were found to be correlated with DLA at Cavinti and Sitiung, respectively, using SAS/GLM ($P < 0.0001$). When Mapmaker QTL was used to perform point analysis, 46 and 68 markers associated with DLA at both Cavinti and Sitiung were detected. The same 12 markers were associated with the largest effects in both analyses (Table 3). Among those 12 markers, 8 were associated with complete resistance to the 5 isolates tested in monocyclic experiments, and 4 were identified to condition partial resistance to isolate PO6-6 in polycyclic tests. *RG214*, associated with complete resistance in greenhouse tests, showed a highly significant association with DLA at Sitiung, but no correlation with DLA at Cavinti. Of the QTLs identified in greenhouse tests using isolate PO6-6, only the one associated with *RG104A* and *RG409A* had a significance value greater than 0.0001 at both field sites. These results suggested that the genes identified under greenhouse conditions were the main genetic factors controlling resistance to the two different field populations of *P. oryzae* and provides strong evidence for the validity and usefulness of QTLs governing partial resistance that were identified in response to a single isolate in the greenhouse.

More markers showed high levels of significance at Sitiung than at Cavinti. Further, most markers also had higher allele effects at Sitiung. This is probably due to the higher disease pressure at Sitiung. Markers associated with decreased DLA demonstrated a mean effect ranging from 49.8 to 32.9% at Sitiung and 28.5 to 19.8% at Cavinti.

In contrast to other correlated markers, lines carrying the CO39 allele at *RG574* (chromosome *12*) had lower DLA at both field sites than those carrying the Moroberekan allele at this locus. This marker was associated with a decrease of 49.8 and 28.4% in DLA at Sitiung and Cavinti, respectively, indicating that a resistance gene from the susceptible parent, CO39, conferred resistance to some field isolates at the two screening sites. This result agreed with our observations from other inoculation tests (R. ZEIGLER and R. NELSON, IRRI, Philippines, unpublished data). It was also found that RG574 was correlated with resistance to PO6-6 ($P < 0.0001$) in greenhouse tests and ranked 29th among the markers identified. The mean score of lines carrying the Morob-

TABLE 3

Twelve markers most significantly associated with diseased leaf area (DLA) at two blast field screening sites; Cavinti, Philippines and Sitiung, Indonesia

Marker	Chromosome no.	Cavinti		Allele effect[a] (% DLA)	Sitiung		Allele effect[a] (% DLA)
		F value	LOD[b]		*F* value	LOD[b]	
Complete resistance							
RG788	*4*	40.1	7.4	20.4	55.0	10.4	37.5
RG214	*4*	NS	2.4	8.0	80.5	13.7	49.8
RG864	*4*	36.3	7.2	19.8	44.1	16.9	46.7
RG103	*11*	34.7	11.8	27.3	37.9	13.6	46.4
RG188	*2*	36.0	7.2	20.1	42.8	10.4	37.2
RG102	*2*	23.9	5.0	16.7	41.8	8.5	34.6
RG574	*12*	56.4	10.5	-28.5	44.5	8.5	-41.4
RG323	*12*	53.3	10.4	24.9	47.9	9.5	38.3
Partial resistance							
RZ744	*1*	43.9	8.1	20.4	55.2	10.0	36.1
RG612	*1*	39.0	8.1	20.4	49.9	10.1	36.3
RG16	*11*	60.8	11.5	25.4	76.2	14.1	44.3
RG869B	*12*	51.5	10.3	23.9	38.9	7.9	34.1

[a] Mean differences of diseased leaf area (DLA) between the two genotypic groups carrying CO39 and Moroberekan alleles.
[b] Log_{10}-likelihood ratio, estimated by Mapmaker/QTL using point analysis (accomplished by setting map distances between all markers equal to 0).

erekan allele was 4.50 and the mean score of lines carrying the CO39 allele was 2.96.

Lines carrying the identified qualitative resistance genes, *Pi-5(t)* and *Pi-7(t)*, generally had lower disease levels than those without them. At both Cavinti, Philippines, and Sitiung, Indonesia, the RI lines with Moroberekan alleles at the markers linked to *Pi-5(t)* and *Pi-7(t)* had significantly lower levels of disease than the lines with the CO39 alleles at those loci.

A significant correlation was found between disease reactions and the number of positive QTLs in individual RI lines ($r = 0.64^{**}$ at Cavinti, Philippines, and $r = 0.67^{**}$ at Sitiung, Indonesia). As the number of QTLs per RI line increased, the percent DLA decreased (Figure 4). At both screening sites, the RI lines having more QTLs showed relatively slow disease progress compared with the susceptible parent, CO39.

There was no significant correlation between number of positive QTLs and DLA in the lines with *Pi-5(t)* and *Pi-7(t)* ($r = 0.21$ ns at Cavinti and $r = 0.22$ ns at Sitiung). This result indicates that the effects of QTLs could not easily be detected in the presence of genes conditioning complete resistance, as would be expected. At both sites, some lines inferred to carry *Pi-5(t)* and *Pi-7(t)* had significant amounts of disease (>20% DLA) (Figure 4). This may be due to the presence of compatible races in the field. Studies are now underway to analyze the virulence spectra of isolates collected from lines carrying *Pi-5* and *Pi-7*. Isolates representing a greater pathogen diversity are also being used in inoculation tests to look for genes not identified in this study.

DISCUSSION

Certain upland cultivars have remained resistant while being cultivated under severe blast pressure in upland rice fields for many years (ITO 1965). It has been suggested that this stable resistance may be due to a combination of complete and partial resistance factors (IKEHASHI and KHUSH 1979). Using RFLP techniques, we identified and located two dominant genes for complete resistance and several genes for partial resistance, including one in the susceptible parent. The genes governing partial resistance were distributed in 10 regions of the rice genome. This preliminary mapping effort supports the view that a combination of complete and partial resistance factors may confer stable resistance, and provides a first step in allowing a more efficient utilization of these particular genes in future breeding efforts.

The genes *Pi-5(t)* and *Pi-7(t)* identified in Moroberekan conditioned complete resistance in greenhouse tests to the five isolates tested. These two genes were also effective, although not completely, under field conditions: RI lines with *Pi-5(t)* generally had low levels of disease in the two testing sites. Some disease was observed in the field on lines carrying both *Pi-5(t)* and *Pi-7(t)* while Moroberekan was highly resistant, suggesting that Moroberekan may carry other resistance genes that were not identified in this study. In addition, the high level of field resistance of some lines, not explained by the loci identified in polycyclic tests, further suggests that Moroberekan carries a wide array of resistance genes.

The 20 markers found to be most significantly associated with partial resistance in Moroberekan were identified in response to a single blast isolate, PO6-6. The ten genomic regions defined by these markers were considered putative QTLs for blast resistance. In this study, the collective action of several genes for partial resistance identified against a single pathogen isolate in the greenhouse was found to provide substantial resistance to a

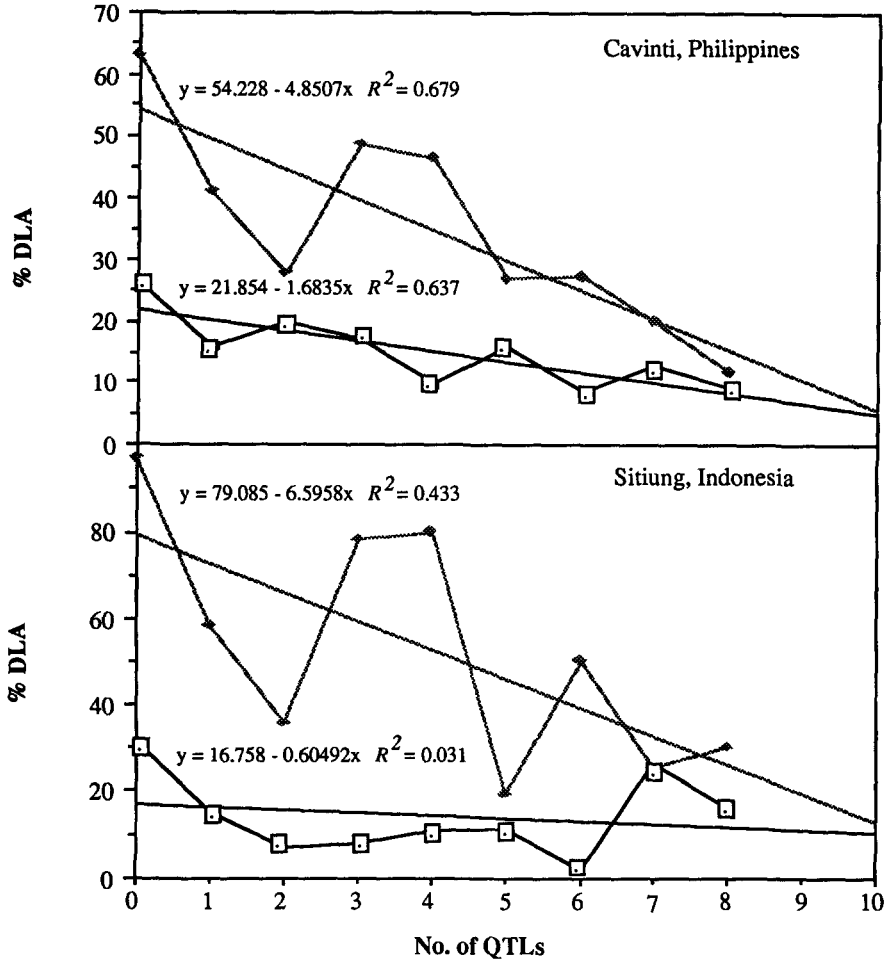

FIGURE 4.—The relationship between disease reaction under field conditions and the number of QTLs per RI line, among the eight most significant QTLs identified in greenhouse tests using isolate PO6-6. Disease reaction data was scored as final diseased leaf area (DLA) measured 6 weeks (Cavinti site) and 5 weeks (Sitiung site) after sowing. The stippled lines represent the field performance of RI lines lacking *Pi-5(t)* and *Pi-7(t)* (those that were partially resistant to isolate PO6-6 in greenhouse tests). The solid lines represent the field performance of RI lines carrying both complete and partial resistance genes (those that were completely resistant to isolate PO6-6 in greenhouse tests).

mixed pathogen population under field conditions. This suggests that many of the loci conferring quantitatively inherited, partial resistance may have race-nonspecific effects, in accordance with VAN DER PLANK's (1968) theory .

Irrespective of which QTLs occurred together, the number of QTLs per line was significantly correlated with the level of disease at both field sites. For breeding purposes, identification of specific loci with large effects is desirable. The combination of loci explaining the largest proportion of phenotypic variation and conferring the largest degree of resistance (evaluated with respect to lesion number and diseased leaf area in greenhouse tests) is predicted to involve genes bracketed by markers *RZ744* and *RZ276* (chromosome *1*), *RG64* and *RG172* (chromosome *6*), *RG333* and *RZ562* (chromosome *8*), *RG16* and *CDO365* (chromosome *11*), and *RG869B* and *RG9* (chromosome *12*). These QTLs will be the focus of future experiments designed to transfer a useful level of partial resistance in combination with qualitative resistance into high yielding rice cultivars.

The presence of race-nonspecific genes conferring partial resistance would be expected to extend the useful life of a gene(s) conferring complete resistance. This is because selection pressure on the pathogen would be minimized when resistance is controlled by more than one gene, especially if those genes affect pathogen reproduction in different ways. Our results suggest that this genetic structure may be a key factor governing durable blast resistance in Moroberekan.

Quantitative or partial resistance (BONMAN *et al.* 1986) or field resistance has been reported to be very sensitive to environmental conditions (OU 1985). An accurate evaluation and screening method is essential for assessing the level of partial resistance to blast. In previous studies (WANG *et al.* 1989; BONMAN *et al.* 1986; ROUMEN 1993), monocyclic tests in temperature-controlled conditions or field tests with natural inocula were used in evaluations. The polycyclic test used in this study is an improved screening method for the genetic study of quantitative resistance to blast (J. M. BONMAN, unpublished data). It allowed a single pathogen isolate to undergo two or three cycles of infection so that small differences in resistance were amplified and could be reliably detected. Further, detailed genetic studies of blast resistance require analysis with multiple blast isolates in replicated tests. Recombinant inbred lines and doubled haploid lines are suitable mapping populations for such traits (BURR *et al.* 1988). In this study, the large quantity of seeds of each RI line made it possible to

replicate controlled inoculation tests and field evaluations using identical genotypes.

ROBERTSON (1989) hypothesized that qualitative mutant alleles that affect quantitative traits (*e.g.*, mutants such as dwarfs and those with defective kernels and narrow leaves) represent one extreme in a spectrum of alleles. The conclusions of BEAVIS *et al.* (1991) also support the hypothesis that qualitative genetic loci are the same loci that affect quantitative traits. In this study, among 20 RFLP markers found to be associated with partial resistance, *RG16* (on chromosome *11*) was also identified with complete resistance to the same blast isolate. The most likely location of the putative QTL for partial resistance was in the interval *RG16-CDO365* (Table 1), while the most likely location of the putative QTL for complete resistance was in the interval *RG103A-RG16* (Figure 2). This suggests that there may be more than one blast resistance gene located in this region of chromosome *11*. In addition, *RG64*, *RG869B* and *RG333*, three marker loci associated with partial resistance in this study, had previously been found to be linked to genes conferring complete resistance in other studies, namely *Pi-2(t)*, *Pi-4(t)* and *Pi-zh*, respectively (YU *et al.* 1991; L. ZHU personal communication, Academia Sinica, Beijing, 1991). The relationship between the QTLs for partial resistance identified in this study and loci associated with complete resistance is not clear at this time. However, the following possibilities are under investigation: (1) the QTLs for partial resistance identified in this study are different loci than the previously mapped genes conferring complete resistance; (2) they are different loci but tightly linked to those previously identified genes; (3) they are different alleles at the same loci; (4) they are the same alleles but show differential reactions to different isolates of the fungus, and are weakly effective against the isolate tested and/or (5) they are the same genes as previously reported but express partial resistance due to gene interactions in the Moroberekan background. One way to test the above hypotheses is to conduct fine mapping (PATERSON *et al.* 1990) and to analyze purified introgression lines (similar to the concept of isolines for qualitative genes) (ESHED *et al.* 1992) for their reaction to a variety of different pathogen isolates in different environments. Using marker-assisted selection to identify progenies from the CO39/Moroberekan RI population that contain different combinations of genes for qualitative and partial resistance, these evaluations are in progress. Further crossing experiments have also been initiated to purify putative QTLs in the CO39 background, and to transfer specific QTLs to different genetic backgrounds for further testing and characterization.

It was previously known that a qualitative resistance gene can obscure the presence of quantitative resistance genes. It was not clear, however, how the presence of genes conferring quantitative resistance would affect the detection of genes for qualitative resistance. In this study, we observed that the presence of genes for partial resistance could affect the classification of lines in relation to the presence or absence of genes for complete resistance to blast. Certain lines showed inconsistent reactions to the isolates tested, and were found to carry the susceptible (CO39) allele at one or both of the RFLP loci associated with the *Pi-5(t)* and *Pi-7(t)* loci, and resistant (Moroberekan) alleles at QTLs associated with partial resistance. The combined effect of genes conferring partial resistance was difficult to distinguish from the effect of a single gene conferring complete resistance. The observation that the presence of partial resistance genes can lead to inconsistent scoring results might explain some of the variable reactions that have frustrated blast researchers for many years (R. ZEIGLER, personal communication, IRRI, 1992). Thus, the level of quantitative resistance should be considered when studies on the genetics of resistance are undertaken.

For many years, rice breeders have tried to combine genes for complete and partial resistance to blast. Procedures for incorporating such an array of genes aimed at providing durable resistance to blast were proposed by rice breeders in Japan (ASAGA and HIGASHI 1973; KIYOSAWA *et al.* 1975). However, it has been difficult to measure the level of quantitative resistance when genes conferring complete resistance were present. Marker-aided selection may now allow genes with overlapping effects to be efficiently combined. The level of quantitative resistance can be estimated based on the presence of markers linked to QTLs in a plant/line. To make this approach truly practical, the genetic behavior of the QTLs in different genetic backgrounds must be further investigated, markers more closely linked to resistance genes must be identified, and efficient and economical breeding procedures utilizing molecular genotyping need to be developed. Research in all of these areas is underway with the immediate goal of facilitating the selection of cultivars with more durable forms of resistance.

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APPENDIX 1

Establishing Genetic Linkage Using Recombinant Inbred Lines With an Abnormal Segregation Ratio

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The analysis of genetic linkage is similar for recombinant inbred strains and for experimental backcrosses. Since all recombination events are defined and can be counted, linkage can be established by rejecting the null hypothesis of non-linkage using a critical number of recombinants based on the binomial distribution. This critical number of recombinants is calculated as the smallest integer x which satisfies (1), where N is the number of informative progeny and a is the significance level.

$$\sum_{i=0}^{x}\left(\frac{N}{i}\right)\left(\frac{1}{2}\right)^{N} \geq \frac{\alpha}{2} \qquad (1)$$

Tables of such critical numbers of recombinants have been published for $a = 0.05$ and $a = 0.01$ (SILVER 1985). (Although these tables were published for use with recombinant inbred strains, the limits for linkage also apply to backcross progeny.) However, as is discussed below, these limits can be used only to suggest possible linkages; they are too low to establish linkage (NEUMANN 1990; OTT 1991). In addition, these tables assume that the expected recombination fraction for unlinked markers is 0.5, a figure which derives from the assumption that maternal and paternal alleles are equally frequent. What happens if maternal and paternal alleles are not equally frequent? The expected recombination fraction is the probability of observing a maternal allele for one locus and a paternal allele for the other. That is,

$$R = P(m)\,P(p) + P(p)\,P(m)$$

where R is the expected recombination fraction for unlinked markers, $P(m)$ is the frequency of maternal alleles, and $P(p)$ is the frequency of paternal alleles. For a population with a normal segregation ratio, $P(m) = P(p) = 0.5$ and $R = 0.5$. For a population with a 20:80 segregation ratio, $R = (0.2)(0.8) + (0.8)(0.2) = 0.32$. In this case, the relationship which provides critical numbers of recombinants for establishing linkage is not [1], but the more general form (2).

$$\sum_{i=0}^{x}\left(\frac{N}{i}\right)[R]^{i}[1-R]^{N-i} \geq \frac{\alpha}{2} \qquad (2)$$

Table 4 shows critical numbers of recombinants calculated from (2) for various numbers of progeny having normal or skewed segregation ratio.

The value a is a conditional probability, the probability that linkage will seem significant when in fact the loci

TABLE 4

Critical numbers of recombinants for establishing linkage in recombinant inbred lines with a skewed segregation ratio

	Significance and segregation ratio			
	0.001		0.0001	
Progeny[a]	50:50	20:80	50:50	20:80
20	3[b]	1	2	—
25	5	1	3	—
30	6	2	5	1
40	10	4	8	3
50	14	6	12	4
75	23	11	21	9
100	34	17	31	15
150	55	30	51	27
200	77	43	73	39
250	99	56	94	52
280	113	65	108	60
300	122	70	116	66

[a] The table gives, for numbers of progeny between 20 and 300, the smallest number of recombinants expected from unlinked markers. That is, numbers of recombinants smaller than those shown are to be interpreted as indicating linkage between the markers. These critical values are given for two levels of significance, 0.001 and 0.0001, and for two segregation ratios, the normal 50:50 ratio and the 20:80 skewed ratio. The — symbol indicates that linkage cannot be established at the 0.0001 significance level with the smallest progeny numbers listed.

[b] The critical values shown in the table were calculated by calculating the smallest value of x which satisfies (2), where N is the number of progeny, a is the significance (0.001 or 0.0001), and R is the probability of recombination between unlinked loci (0.50 for a 1:1 segregation ratio or 0.32 for a 1:4 segregation ratio). These critical values were calculated with a Macintosh microcomputer using a custom program written in Pascal. Selected values were checked by recalculating with the Theorist mathematics program (Prescience Corporation).

are unlinked (type I error). However, *most* pairs of loci are unlinked; that is, the *a priori* probability of linkage is low (as low as a few percent, depending on the organism). Therefore, using a significance of 0.05 will result in a frequency of type I errors comparable to the true frequency of linked markers. That is, when testing a given locus with 100 other loci, using $a = 0.05$ will detect as many apparent linkages among the ~95 loci which are not linked as among the ~5 which are. One solution is to use a more stringent value of a, such as 0.001 or 0.0001, as is done in Table 4.

The tabulated values can be used with backcross data or with recombinant inbred data. However, backcrosses and recombinant inbred lines differ in the relationship between observed number of recombinants and the implied map distance. Using the more stringent level of significance, the greatest number of recombinants which implies linkage is 65 in a 300-progeny backcross. This corresponds to a recombination fraction of about 0.22. This is, therefore, the largest distance between linked markers which can be established by a skewed backcross at this level of significance. For selfed recombinant inbred lines, however, this number of recombinants is equivalent to a distance of $0.22/(2(1 - 0.22)) = 0.14$ (HALDANE and WADDINGTON 1931). This is the largest distance between linked markers which can be established by skewed recombinant inbred lines. Recombinant inbred lines with a severely skewed segregation ratio are therefore limited to detecting linkage among closely linked markers.