Theor Appl Genet (2002) 105:313–324
DOI 10.1007/s00122-002-0940-2

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Mapping QTLs for field resistance to the rice blast pathogen and evaluating their individual and combined utility in improved varieties

Received: 9 October 2000 / Accepted: 11 October 2001 / Published online: 14 June 2002

Abstract Lines from a Lemont × Teqing recombinant inbred population were evaluated for dilatory resistance to rice blast disease using: (1) the Standard Evaluation System (SES) for rating leaf blast, (2) the percentage diseased leaf area (%DLA), and (3) the area under a disease progress curve (AUDPC). RFLP mapping using 175 well-distributed loci revealed nine QTLs, one each on chromosomes 1, 2, 3, 4, 6, 7 and 9, with two loci on chromosome 12. All nine putative QTLs were associated with AUDPC, six with both a %DLA and a SES rating. Teqing contributed the resistance allele for all these loci except for the one located on chromosome 4. Individual QTLs accounted for 5–32% of the observed phenotypic variation, and combined QTL models accounted for 43–53%. Three QTLs were located near three of the four major resistance genes previously identified in this population. The resistances of both Lemont and Teqing were attributable to a combination of both major genes capable of inducing hypersensitive reactions and minor genes causing less-distinctive phenotypic differences. Interactions were noted between QTLs and major genes. Our findings are in support of the strategy of pyramiding major genes and QTLs in carefully selected combinations to develop improved varieties with resistance to the blast fungus that is both broad in spectrum and durable.

Keywords *Oryza sativa* L. · Rice blast (*Pyricularia grisea* Sacc.; *Pyricularia oryzae* Cav.; *Magnaporthe grisea*) · Disease resistance · Partial resistance · Horizontal resistance · Gene mapping · Quantitative trait loci (QTLs) · Restriction fragment length polymorphism (RFLP)

Communicated by P. Langridge

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Introduction

Blast disease, caused by *Pyricularia grisea* (Cooke) Sacc., has been a common problem in most rice growing areas in the world (Ou 1985). The fungal pathogen can cause severe leaf necrosis and impede grain filling (blasting), resulting in decreased grain numbers and weight. Severe rice blast epidemics have been reported in several countries particularly in Asia, resulting in 10–50% yield losses. Several major resistance genes which can provide complete host-plant resistance to a specific subset of races through a hypersensitive reaction are employed by rice breeders. Most blast epidemics can be attributed to the breakdown of a major gene's effectiveness due to the emergence of new and highly virulent races or strains of the pathogen, coupled with favorable environmental conditions conducive to blast development.

In addition to complete resistance of the simply inherited hypersensitive type discussed above, partial resistance to blast has been characterized (Yeh and Bonman 1986). This quantitative resistance has been also referred to as field (Ezuka 1972), "slow-blasting" (Villareal et al. 1981) and dilatory (Marchetti 1983a, b) resistance. Partial

resistance is characterized by lesions that are typically spindle-shaped but may be fewer in number, reduced in size, slower to develop, or shorter-lived than those produced in the absence of partial resistance. The net effect is a reduced inoculum potential and a lower probability of a blast epidemic (Marchetti 1983a, b; Wang et al. 1989; Katsar 1993; Roumen 1994; Correa-Victoria and Zeigler 1995). Partial resistance is more difficult than complete resistance for breeders to utilize because it is quantitatively inherited, usually oligo- or poly-genic, and sensitive to environmental factors such as temperature, leaf wetness duration, N-fertilization, soil type and water stress (Ou 1985; Roumen 1994).

Rice cultivars with durable resistance have been reported in several countries. 'Moroberekan' and 'OS6' were found durable in West Africa, 'IR36' in Asia, 'CICA 7' in South America (Notteghem 1993) and 'Lemont' in the U.S. (Lee 1994). The durability of resistance to rice blast has been associated with polygenic partial resistance which lacked race specificity (Bonman 1992). Durable resistance in other crops has been associated in some cases with major genes, in other cases with multiple genes with additive effects, and still other cases with a suite of polygenes, or combinations thereof (Johnson 1983; Parlevliet 1983). QTL analysis of Moroberekan attributed its durable resistance to a combined effect of major genes and QTLs (Wang et al. 1994).

'Lemont', a tropical *japonica* variety, has been considered durably resistant in the U.S. since its release in 1983 (Marchetti et al. 1995). Lemont's blast resistance, presumably derived from it's resistant ancestor 'Dawn', provides complete protection from most blast races existent in the southern U.S. Lemont does not exhibit a hypersensitive response to IC-17, IB-49 and IE-1; however, Lemont does express partial resistance when exposed to these races in the field (Marchetti 1983a; Marchetti et al. 1995). 'Teqing' was found to be highly resistant to these and all other races of rice blast known to be present in the southern U.S. (Marchetti, unpublished). RFLP-mapping research using discrete, race-specific data revealed the location of three major resistance genes from Teqing and one major gene from Lemont (Tabien et al. 2000). Classical genetic analysis of F_2 plants had estimated the presence of at least six genes, four in Teqing and two in Lemont (Tabien 1996; Tabien et al. 1996). The present study utilized quantitative measures of field resistance to locate additional loci contributing to field resistance to blast disease, and to evaluate the variously mapped loci in terms of their individual and combined effectiveness at providing resistance to rice varieties designed for commercial production in the southern U.S.

Materials and methods

Rice linkage map and study population

This study used the same marker data and framework linkage map previously used to locate four major blast resistance genes (Tabien et al. 2000). This map was composed of 173 RFLP-tagged loci plus two morphological markers distributed at an average of 10 cM (Kosambi). Although the map represents approximately 80% of the rice genome, gaps larger than 30 cM occur on nine of the 12 chromosomes, and chromosome 5 is not well represented by these markers. The framework map was formed using genotypic data from 284 recombinant inbred lines (F_8 generation) derived from Lemont × Teqing. The 280 RILs that provided sufficient seed were also grown in the outdoor blast nursery for further evaluation of disease resistance.

Field blast nursery evaluation

F_8 seed of 280 RILs was planted in single-row plots, 60 cm in length, in blast nursery beds in 1993. Entries were arranged in a completely randomized block design with three replications. Each two entries were separated by a susceptible check 'M-201', while Lemont and Teqing were seeded alternately every 20th row. Spreader rows were planted along the windward side of each nursery bed to enhance the spread of spores. Above-canopy sprinklers ran for 6 s every 6 min from 4:30 to 8:30 p.m. and from 9:00 to 10:00 a.m. to extend leaf-wetness duration and enhance infection and disease development. Plots and spreader rows were not inoculated with specific races, but were allowed to be wind-inoculated with a natural mix of races. The primary source of inoculum at the blast nursery was likely to be the nursery bed of highly susceptible 'M-201' located on the windward side of the experimental plots which was inoculated with the blast race IC-17. The most-prevalent race in production fields in Texas is regularly monitored, and in this same year (1993) the predominant races were IC-17, followed by IB-49 and IE-1 (Marchetti, unpublished). It was anticipated, and later indicated during the course of data interpretation, that races in addition to these three would be present in the blast nursery. Among the 108 RILs that were previously determined to contain major gene resistance to IC-17, IB-49 and IE-1 (Tabien et al. 2000), many exhibited significant levels of susceptibility in the blast nursery (e.g., SES ratings of 7–8), suggesting the presence of additional blast races in the present study.

Percent diseased leaf area (%DLA) and Standard Evaluation System (SES) ratings for leaf blast (IRRI 1996) were taken 19 days after seeding and 20 days after seeding, respectively, and every week thereafter for a total of 5 weeks. Areas under disease progress curves (AUDPCs) were estimated using the sequential %DLA data collected from each plot employing the formula of Shaner and Finney (1977).

Data analysis

Analysis of variance indicated that replication effects were insignificant; therefore, trait data were averaged over the three field replications and evaluated for normality. QTL mapping was based on SES ratings collected at 40 days after seeding, and on square root transformations of the %DLA (also observed at 40 days after seeding) and the calculated AUDPC.

Interval mapping used MapMaker-QTL version 1.1 (Lander and Botstein 1989) and a LOD threshold value of 2.4 to infer the presence of a QTL. For each trait, the QTL having the largest LOD score was "fixed", then the genome was re-scanned to test for the presence of additional QTLs with smaller effects (Lin et al. 1995; Wright et al. 1998). This process of "fixing" the largest QTL and re-scanning the genome continued to use a LOD threshold of 2.4 over the background LOD and was repeated until no new putative QTLs were detected. When a scan of the data resulted in multiple peaks in a chromosomal region, the peak with the larger LOD was "fixed" and the genome was re-scanned. An increase of more than 2.00 in LOD value was considered indicative of a second, linked QTL. The weight of each QTL was calculated from an interval analysis model that simultaneously included all the QTLs identified for a particular trait. Absolute values of QTL weights are equivalent to additive effect estimates (though expressed here in the context of transformed rather than original %DLA and AUDPC

Table 1 Interval[a] and stepwise regression[b] parameters for QTLs for AUDPC. Putative QTLs were identified through agreement between ANOVA and interval analysis, and are listed below according to their LOD significance in the interval analysis

QTL	Nearest marker[b]	Interval analysis			Stepwise regression			
		LOD value	Percent variance explained	Weight[c]	Order entered into model	Partial R^2	*F*-value	*P* > *F*
qBLASTads-2	RG520	9.69	21.2	–0.83	1	0.156	21.5	0.000
qBLASTads-12-1	RZ397	5.49	8.7	–0.54	7	0.027	5.6	0.020
qBLASTads-3	RZ474	5.36	12.9	–0.49	6	0.023	4.6	0.035
qBLASTads-6	C236	3.77	7.3	–0.35	3	0.074	12.3	0.001
qBLASTads-9	G103b	3.25	7.4	–0.35	5	0.035	6.7	0.011
qBLASTa-7[d]	RG678	2.71	4.4	–0.27	Not incl.[d]	–	–	–
qBLASTads-4[e]	RZ69x	2.36[e]	4.8	0.56	4	0.074	13.7	0.000
qBLASTa-1[e]	RZ14	1.99[e]	5.5	–0.29	2	0.089	13.6	0.000
qBLASTa-12-2[f]	RG574	5.21[f]	1.9	–0.35	8	0.014	3.0	0.048
Entire model	Nine loci	26.77	49.2		Eight loci[d]	0.490		

[a] Interval analysis employed by MapMaker-QTL version 1.1

[b] Stepwise regression considered all marker loci that had exhibited significance in either interval analysis or single factor ANOVA of any of the three traits measured here, as well as all loci that had been associated with major resistance genes (Tabien et al. 2000)

[c] Weights reported are from an interval analysis model that included all nine QTLs. Esitmated QTL weights are equivalent to additive effects within the context of the transformed AUDPC data, and are expressed here in terms of the estimated change in $(AUDPC)^{1/2}$ that one would expect from incorporating an allele from Teqing into Lemont. Thus, a negative weight reported in the table indicates that Teqing contains the resistant allele for that QTL

[d] *qBLASTa-7* did not meet requirements for significance in the stepwise regression for inclusion into that model. It also did not exhibit a LOD > threshold after fixing *qBLASTads-2*, the most significant QTL in the interval analysis and the first QTL "fixed" in that analysis

[e] *qBLASTads-4* and*qBLASTa-1* acquired LOD > 2.4 in the interval analysis after fixing one QTL (*qBLASTads-2)*

[f] *qBLASTa-12-2* exhibited a LOD increase of 2.9 after "fixing" the larger, neighboring locus *qBLASTads-12-1*

data) and are presented here in terms of the change in phenotype one would expect from the substitution of the Teqing allele into Lemont. Thus, a negative weight indicates that Teqing is the donor parent of the resistance allele. Single-factor ANOVA was performed using the SAS PROC GLM (SAS Institute 1988) with a *F*-test probability level set conservatively at ≤0.002 to minimize the occurrence of the type-1 error. Stepwise regressions were conducted in SAS to further evaluate the putative QTLs identified through agreement between interval analysis and single-factor ANOVA. Independent variables considered in the stepwise regressions were the same for the analysis of each of the three traits and included all the marker loci that had: (1) exhibited a LOD peak > 2.0 in the open and "fixed" interval analyses for any of the three traits, (2) been significantly associated with a trait by single-factor ANOVA, or (3) been previously associated with a major resistance gene (Tabien et al. 2000).

Two-way and three-way ANOVAs were conducted (SAS PROC GLM) to evaluate digenic and trigenic epistasis among the putative QTLs and to determine the best two- and three-QTL combinations for conferring useful levels of field resistance to blast in the southern U.S.

Nomenclature

QTLs were named following McCouch et al. (1997) with a slight modification. A '*qBLAST*' initiates the name to indicate a QTL for blast resistance. This is followed by one or more letters to indicate which measure(s) of dilatory resistance the locus was associated with: '*a*' for AUDPC, '*d*' for %DLA and '*s*' for SES ratings. The chromosome onto which the QTL was mapped follows after a dash. When multiple QTLs for the same trait were located on the same chromosome, an additional dash and number was added to the name to clearly distinguish between them. For example, a locus name of '*qBLASTads-12-1*' means a QTL for blast resistance associated with AUDPC, %DLA and SES data, and located higher on chromosome 12 (Fig. 1) than a second QTL on that same chromosome.

Results and discussion

QTL locations

Nine putative QTLs based on both single-marker ANOVA (data not shown) and interval mapping (Tables 1, 2 and 3) were identified on eight of the 12 rice chromosomes (Fig. 1). The weights calculated from interval analysis indicated that resistance was associated with the Teqing allele for eight of the nine QTLs; Lemont provided the resistant allele for *qBLASTads-4* (Tables 1, 2 and 3). The presence of major genes with a large effect in the population complicated the ability to detect QTLs of smaller effect. For example, the relatively small QTL on chromosome 4 did not acquire a LOD score ≥ 2.4 until after "fixing" the largest QTL (*qBLASTads-1*) for both SES and AUDPC (Tables 1 and 3). The QTL located on chromosome 1 and associated with AUDPC alone, *qBLASTa-1*, also did not acquire a significant LOD until after "fixing" *qBLASTads-2* in the interval analysis. Stepwise regression detected an association between *qBLASTa-7* and %DLA (Table 2) even though this locus met significance requirements to be named as a QTL only for AUDPC. In addition to the nine named QTLs, stepwise regression of SES data also detected association with a region on chromosome 5 (Table 3). This region did not meet significant requirements for either interval analysis or single-factor ANOVA, so it is not being named as a QTL from the present data.

Table 2 Interval[a] and stepwise regression[b] parameters for QTLs for %DLA. Putative QTLs were identified through agreement between ANOVA and the interval analysis, and are listed below according to their LOD significance in the interval analysis

QTL	Nearest marker[b]	Interval analysis			Stepwise regression			
		LOD value	Percent variance explained	Weight[c]	Order entered into model	Partial R^2	*F*-value	*P* > *F*
qblasTads-2	RG520	6.49	15.3	–1.21	3	0.088	15.1	0.000
qBLASTads-12-1	RZ397	5.10	8.1	–0.93	6	0.019	3.9	0.050
qBLASTads-3	RZ474	4.78	10.9	–0.79	Not incl.[d]	–	–	–
qBLASTads-6	C236	4.75	8.2	–0.82	1	0.142	19.5	0.000
qBLASTads-9	G103b	3.38	7.2	–0.73	5	0.051	10.3	0.002
qBLASTads-4	RZ69x	2.70	5.6	1.06	2	0.095	14.6	0.000
qBLASTa-7[e]	RG678[e]	2.26[e]	3.7	–	4	0.064	12.1	0.001
Pi-lm2 on chr. 11[e]	RZ536x[e]	1.31[e]	2.7	–	7	0.013	2.8	0.049
Entire model	Six loci[e]	25.77	42.6		Seven loci[d,e]	0.462		

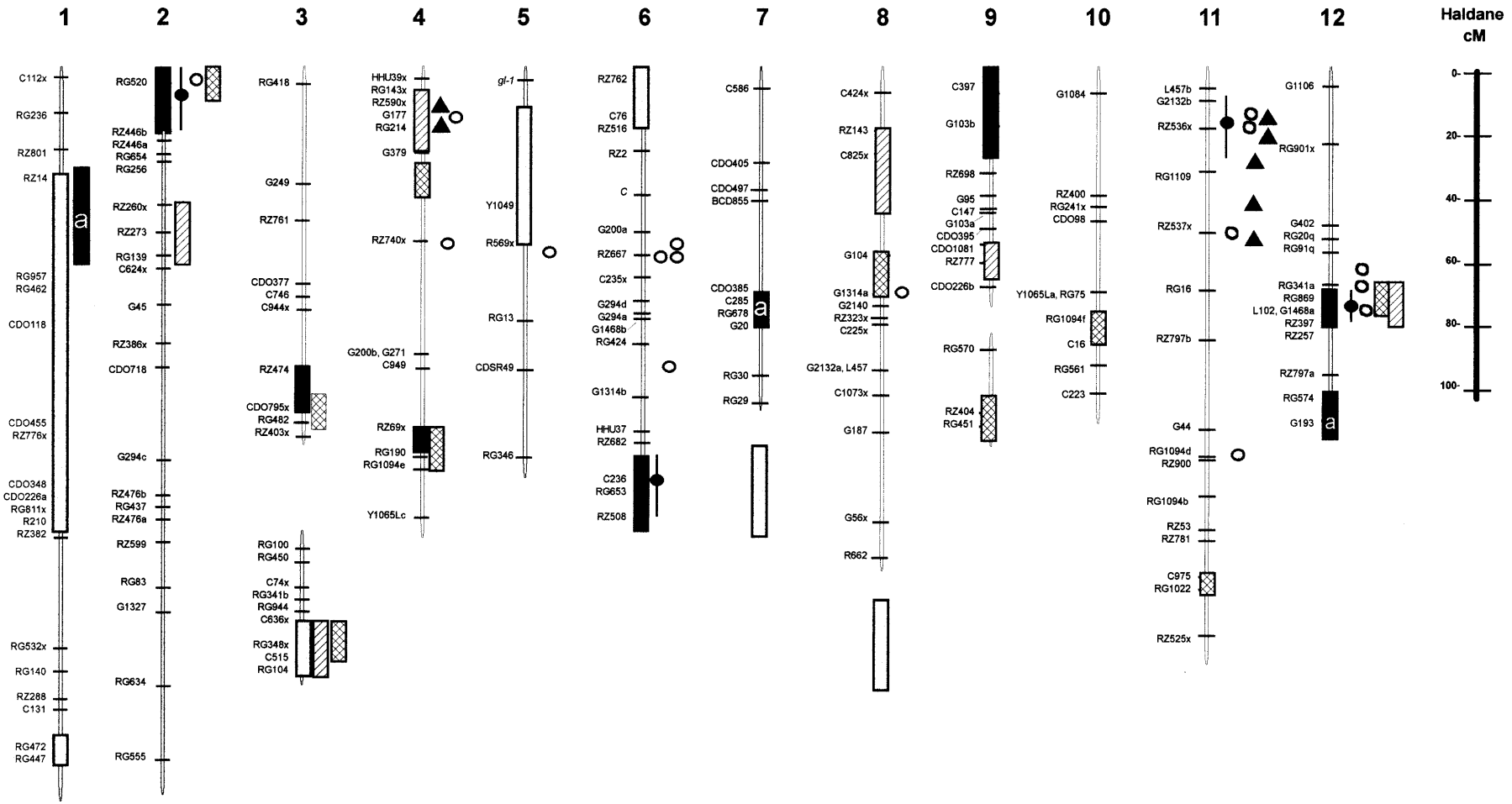
[a] Interval analysis employed by MapMaker-QTL version 1.1
[b] Stepwise regression considered all marker loci that had exhibited significance in either interval analysis or single factor ANOVA of any of the three traits measured here, as well as all loci that had been associated with major resistance genes (Tabien et al. 2000)
[c] Weights reported are from an interval analysis model that included all six QTLs. Esitmated QTL weights are equivalent to additive effects within the context of the transformed %DLA data, and are expressed here in terms of the estimated change in $(\%DLA)^{1/2}$ that one would expect from incorporating an allele from Teqing into Lemont. Thus, a negative weight reported in the table indicates that Teqing contains the resistant allele for that QTL
[d] *qBLASTads-3* did not meet the requirements for significance in the stepwise regression for inclusion into that model. It also did not exhibit a LOD > threshold after fixing *qBLASTads-2*, the most significant QTL in the interval analysis and the first QTL "fixed" in that analysis
[e] Loci represented by RG678 on chromosome 7 and and RZ536 on chromosome 11 were selected into the stepwise regression model, but were not named as %DLA QTLs because they had subthreshold LOD scores throughout the interval analysis

Table 3 Interval[a] and stepwise regression[b] parameters for QTLs for SES. Putative QTLs were identified through agreement between ANOVA and interval analysis and are listed below according to their LOD significance in the interval analysis

QTL	Nearest marker[b]	Interval analysis			Stepwise regression			
		LOD value	Percent variance explained	Weight[c]	Order entered into model	Partial R^2	*F*-value	*P* > *F*
qBLASTads-2	RG520	13.80	31.7	–1.86	7	0.016	3.3	0.045
qBLASTads-12-1	RZ397	4.52	7.2	–0.99	1	0.241	38.5	0.000
qBLASTads-3	RZ474	5.19	12.5	–0.83	2	0.064	11.1	0.001
qBLASTads-6	C236	2.66	4.6	–0.39	3	0.034	6.0	0.016
qBLASTads-9	G103b	3.26	7.7	–0.74	5	0.023	4.7	0.032
qBLASTads-4[d]	RZ69x[d]	1.52[d]	3.3	0.86	4	0.032	6.3	0.013
region on chr. 5[e]	CDSR49[e]	0.40[e]	–	–	6[e]	0.030	5.7	0.019
Entire model	Six loci[e]	30.31	52.8		Seven loci[e]	0.440		

[a] Interval analysis employed by MapMaker-QTL version 1.1
[b] Stepwise regression considered all marker loci that had exhibited significance in either interval analysis or single factor ANOVA of any of the three traits measured here, as well as all loci that had been associated with major resistance genes (Tabien et al. 2000). Not all loci named as QTLs met the significance requirements to be included into the final stepwise model
[c] Weights reported are from an interval analysis model that included all six QTLs. Esitmated QTL weights are equivalent to additive because SES data were not transformed prior to analysis and are expresed here in terms of the estimated change in $(AUDPC)^{1/2}$ that one would effects expect from incorporating an allele from Teqing into Lemont. Thus, a negative weight reported in the table indicates that Teqing contains the resistant allele for that QTL
[d] *qBLASTads-4* acquired a LOD > 2.4 in interval analysis after fixing one QTL (*qBLASTads-2*)
[e] A locus represented by CDSR49 on chromosome 5 was selected into the stepwise regression model, but had a maximal LOD peak of 2.2 (below the threshold of 2.3) after fixing two QTLs and so was not named a QTL

Fig. 1 Estimated location of major genes and QTLs conferring resistance to rice blast, bacterial blight and sheath blight diseases. *Solid black rectangles* indicate QTLs presently determined to be associated with data from all three methods of evaluating field resistance to blast disease; *black rectangles marked with an* '*a*' indicate QTLs that were associated with AUDPC but not with %DLA or SES; *black circles with lines* indicate the probes that exhibited statistically significant linkage with major blast resistance genes that were identified using the same mapping population (Tabien et al. 2000); *gray circles* indicate major blast resistance genes that were mapped in various other populations (Yu et al. 1991; McCouch et al. 1994; Hittalmani et al. 1995; Inukai et al. 1996; Miyamoto et al. 1996; Naqvi and Chattoo 1996; Pan et al. 1996; Rybka et al. 1997; Chen et al. 1999); *gray rectangles* indicate blast resistance QTLs mapped from Moroberekan (Wang et al. 1994); *triangles* indicate major resistance genes for bacterial blight mapped in various populations (Abenes et al. 1993; Yoshimura et al. 1995; Lin et al. 1996; Zhang et al. 1996; Li et al. 1999); *rectangles with a diamond pattern* indicate bacterial blight QTLs that were mapped using a related set of Lemont × Teqing RILs (Li et al. 1999); and *rectangles with diagonal lines* indicate QTLs for resistance to sheath blight disease that were identified and mapped in an earlier Lemont × Teqing generation (Li et al. 1995) ▶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R210
RZ382
RG532x
RG140
RZ288
C131
RG472
RG447
RG520
RZ446b
RZ446a
RG654
RG256
RZ260x
RZ273
RG139
C624x
G45
RZ386x
CDO718
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RG437
RZ476a
RZ599
RG83
G1327
RG634
RG555
RG418
G249
RZ761
CDO377
C746
C944x
RZ474
CDO795x
RG482
RZ403x
RG100
RG450
C74x
RG341b
RG944
C636x
RG348x
C515
RG104
HHU39x
RG143x
RZ590x
G177
RG214
G379
RZ740x
G200b, G271
C949
RZ69x
RG190
RG1094e
Y1065Lc
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Y1049
R569x
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CDSR49
RG346
RZ762
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RZ516
RZ2
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RZ667
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G294d
G294a
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RG424
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C236
RG653
RZ508
C586
CDO405
CDO497
BCD855
CDO385
C285
RG678
G20
RG30
RG29
C424x
RZ143
C825x
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G2140
RZ323x
C225x
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C1073x
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G56x
R662
C397
G103b
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RG451
G1084
RZ400
RG241x
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C223
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RZ537x
RG16
RZ797b
G44
RG1094d
RZ900
RG1094b
RZ53
RZ781
C975
RG1022
RZ525x
G1106
RG901x
G402
RG20q
RG91q
RG341a
RG869
L102, G1468a
RZ397
RZ257
RZ797a
RG574
G193
LMNT/TQNG QTL for blast resistance - associated with % DLS, AUDPC and SES
QTL associated with AUDPC but not with %DLS or SES for blast resistance
Major gene from LMNT/TQNG conferring resistance to specific blast races
Major gene from other population(s) for conferring resistance to blast races
Moroberekan QTL for blast resistance
Gene for resistance to bacterial leaf blight
QTL for resistance to bacterial leaf blight mapped in related LMNT/TQNG RILs
QTL for sheath blight resistance mapped in an early LMNT/TQNG generation

Correlation between QTLs for AUDPC, %DLA and SES

Six of the nine putative QTLs were significantly associated with AUDPC, %DLA and SES ratings, while three QTLs were associated with AUDPC alone. Although AUDPC is the most-difficult and labor-intensive method of estimating disease resistance, it also appears to be the most comprehensive.

Correlation between quantitative, classical and major-gene analyses

The classical (Tabien 1996; Tabien et al. 1996) and major-gene mapping analyses (Tabien et al. 2000) conducted prior to this study both utilized qualitatitve blast resistance data from single-race inoculations. The present study involved plants exposed to a mixture of pathogenic races, although possibly dominated by IC-17, and quantitative data from three different rating systems. Some 25 races of rice blast have been characterized in Texas since 1960 (Marchetti, unpublished). Although the specific races present in the blast nursery plots were not identified, the three-most prevalent races in Texas in this same year were IC-17, IE-1 and IB-49 (Marchetti, unpublished). Because Teqing is considered completely resistant to blast in Texas (the present SES average was 2.4) and was shown to have three major genes that induced hypersensitive reactions to all three of these common races (Tabien et al. 2000), there was some concern prior to data collection and analysis that a predominance of complete resistance in the RIL population might preclude our ability to study minor genes affecting partial resistance. As was discussed in the Materials and methods section, however, several of the RILs known to express hypersensitive responses (Tabien et al. 2000) to each of the predominant races of blast found in production fields that same year (IC-17, IE-1 and IB49) consistently exhibited significant levels of susceptibility (average SES ratings from 7 to 9) in the nursery plots. This indicates that the present field study involved one or more blast races capable of causing disease even in the combined presence of all four mapped major genes. SES, %DLA and AUDPC data for the 108 "resistant" RILs ranged from 1 to 8, 0 to 63 and 0 to 19, with averages of 4.0, 17.2 and 4.4, respectively. To put these numbers into perspective, among the 24 RILs which were susceptible in race inoculation studies to IC-17, IB-49 and IE-1, the SES, %DLA and AUDPC data ranged from 4 to 8, 4 to 85 and 2 to 25, with averages of 5.6, 35.4 and 11.1, respectively.

The QTL analysis generally corroborated the earlier classical and qualitative analyses. Classical genetic analysis using F_2 and RIL populations had estimated at least six resistance genes to be segregating in this Lemont × Teqing mapping population (Tabien 1996; Tabien et al. 1996). RFLP mapping, using discrete data from inoculation by five individual races of blast, identified four major genes, three of which had race specificities identical to three genes as were predicted by classical analysis (Tabien et al. 2000). This study identified putative QTLs located similarly with three of the four mapped major genes (on chromosomes 2, 6 and 12) and identified six additional QTLs located on chromosomes 1, 3, 4, 7, 9 and 12 (Fig. 1). The differential levels of susceptibility associated with *qBLASTads-2*, *qBLASTads-6* and *qBLASTads-12-1*, as detected here in the presence of virulent races, suggests that these regions contain genetic factors conferring field resistance in addition to their ability to confer complete resistance due to a hypersensitive reaction to other races (Tabien et al. 2000). Some of the apparent field resistance, however, may indeed be due to complete resistance to many of the races present in the southern U.S. (Parlevliet 1983). Teqing itself appears to be completely resistant to all U.S. races of blast disease (Marchetti, unpublished). Screening selected RILs with additional single races would clarify the action(s) of the resistance genes not yet associated with major gene resistance. The development of NILs with a variable disease response to race inoculations would be required to determine that these complete- and partial-resistances are due to multiple linked loci rather than to a single locus. Until such a time, we will consider the similarly mapped QTLs and major genes as single genetic factors.

Quantitative analysis provides a more-accurate estimation of gene location than does qualitative analysis because it is less-sensitive to even modest numbers of phenotypic mis-scores (Wright et al. 1998). Replication is necessary to minimize such mis-scoring, but escapes and environmental fluctuation are a common problem in greenhouse and nursery evaluations of blast resistance. The quantitative analysis placed *qBLASTads-2*, which correlates with the major gene *Pi-tq5* (Tabien et al. 2000), to the approximate midpoint between RG520 and RZ446b which are separated by 18 cM on chromosome 2, placed *qBLASTads-6* (correlating with *Pi-tq1*) essentially at or slightly above C236, and placed *qBLASTads-12-1* (correlating with *Pi-tq6*) 1 to 2 cM away from RZ397 in the 6-cM region between RZ397 and RZ257.

Only one of the four previously mapped major resistance genes (Tabien et al. 2000) was not detected by QTL analysis, namely *Pi-lm2* located on chromosome 11. This gene from the Lemont parent conferred resistance only to IB-54 and IG-1, but not to IC-17, IB-49 or IE-1 in single-race inoculation studies (Tabien et al. 2000), consistent with this gene being the *Pi-k*^{h} allele known to be in Dawn (Kiyosawa 1974), an ancestor of Lemont (Bollich et al. 1985). It is probable that the two races that are avirulent to *Pi-lm2*, IB-54 and IG-1, were not present during this study because they are no longer prevalent in Texas and, indeed, were not detected at all in the year of the present study (Marchetti, unpublished). A similar instance of quantitative data not detecting a major blast resistance gene was previously reported (Wang et al. 1994). RG241 was associated with complete resistance from Moroberekan and was highly associated with the %DLA data collected in one location but not in another location. The differences observed were

attributed to possible differences of the races prevalent in the test areas (Wang et al. 1994). Bonman et al. (1989) have also reported on race-specific partial resistance.

Because plants containing the resistance allele for *Pi-lm2* were expected to be susceptible to the three prevalent races in Texas, this locus was not expected to play a major role in field resistance unless it conferred partial resistance to other virulent races, or interacted epistatically with other resistance loci. Neither single-factor ANOVA nor interval analysis indicated a QTL on chromosome 11. Stepwise regression detected a weak association between this locus and the %DLA (Table 2), but not with SES or AUDPC data. Significant levels of epistasis were detected between chromosome 11 and the identified QTLs, however. As discussed in more detail in a later section, when paired and triplet combinations of QTLs and genes were evaluated, this locus added significantly to the correlations between other QTLs and field resistance data.

Relative importance of individual QTLs to field resistance

The QTL on chromosome 2, *qBLASTads-2*, controlled the largest portion of variability for all three measures of disease resistance, individually accounting for 21, 15 and 32% of the total variation in AUDPC, %DLA and SES, respectively (Tables 1, 2, 3). This QTL mapped near RG520 (Fig. 1), similar in location to the major gene *Pi-tq5* which conferred resistance to IC-17, IB-49, IE-1 and IG-1 (Tabien et al. 2000). Low levels of cross protection have been reported in rice blast. Ohata and Kozaka (1967) found that disease lesions were reduced in size when virulent strains were inoculated either in a mixture with avirulent strains or 3-days after inoculation with avirulent strains at the same inoculation site. Lesion sizes were not affected by prior or simultaneous inoculations with avirulent races as little as 5 to 7-mm away from the inoculation of virulent races; nor however, by simultaneous inoculation in a mixture with nonpathogenic strains of *P. grisea*, suggesting that the cross protection was directly related to the localized hypersensitive reaction induced by the avirulent infection. Cross protection is considered to have minimal impact on disease response in the rice blast nursery because a plant's primary source of inoculum are the compatible lesions sporulating on that particular plant or on closely spaced plants in the same susceptible plot (Robinson 1976). Once plants containing the *Pi-tq5* gene become infected with a virulent race, cross protection and initial differences in spore inoculum into the blast nursery are not considered significant factors of subsequent disease response and the SES, %DLA and AUDPC ratings used here. The relatively high importance of *qBLASTads-2* to field resistance is apparently more than just a reflection of the hypersensitive reaction induced by the major gene *Pi-tq5* to the prevalent races. Two other QTLs, *qBLASTads-6* and *qBLASTads-12-1*, also mapped similarly with major genes conferring resistance to these three major races of blast, but they only accounted for one-fourth to one-half as much of the observed phenotypic variation as did *qBLASTads-2* (Tables 1, 2, 3). The differential levels of susceptibility associated with *qBLASTads-2*, *qBLASTads-6* and *qBLASTads-12-1*, as detected here in the presence of virulent races, suggests that these regions contain genetic factors conferring a hypersensitive response to some races and partial resistance to other races.

The locus accounting for the second largest portion of variability for all three traits, *qBLASTads-3* (Tables 1, 2, 3), was not previously associated with a major resistance gene. Teqing is completely resistant to more than the five races employed by Tabien et al. (2000); however, the screening of the RIL population with additional races may associate this or other newly identified QTLs with major-gene resistance. The next two most-important QTLs in terms of the percent of variance individually explained are *qBLASTads-12-1* and *qBLASTads-6*. These QTLs correspond to previously determined locations of *Pi-tq6* and *Pi-tq1*, which also confer resistance to the three predominant blast races, IC-17, IB-49 and IE-1. A second resistance QTL, *qBLASTa-12-2*, was located on chromosome 12 but was detected only through the analysis of AUDPC resistance data, and did not reveal itself until the larger *qBLASTads-12-1* was "fixed" in the genetic model.

Impact of QTL/gene combinations

The data indicate that Teqing's broad-spectrum resistance to U.S. races of rice blast pathogen is due both to major genes capable of inducing hypersensitive reactions and to minor genes with a smaller and less phenotypically distinctive impact, similar to that reported previously for the resistance in Moroberekan (Wang et al. 1994). The resistance of Lemont, which has proven to be durably resistant in the U.S., has also been attributed to a combination of at least one major resistance gene (Tabien et al. 1996, 2000) and one minor gene (*qBLASTads-4*, Tables 1, 2, 3).

Because new resistance alleles are often incorporated one, or a few at a time, into improved breeding lines, knowledge on the relative impacts of various combinations of resistance loci on field resistance can be used by breeders to prioritize their efforts. All possible pairwise and triplet combinations were evaluated using two-way and three-way analyses. The use of *F*-values to rank the importance of various genes and gene combinations can be biased against genes that are less closely linked with markers, causing the markers to poorly represent the genes in the stepwise regression. The three most-distantly tagged QTLs were *qBLASTads-2*, *qBLASTa-1* and *qBLASTads-9*, whose closest markers were estimated to be 9, 8 and 6 cM away from the LOD peaks. Thus, the fact that *qBLASTads-2* was present in all locus pairs having a high R^2 in the analysis of SES data (Table 4) was particularly noteworthy. Triplets including this QTL also

Table 4 Pairwise combinations of loci resulting in the highest 20% of R^2 values in two-way analyses of AUDPC, %DLA and SES data

Loci in paired combination			Markers representing loci in the stepwise regression		*F*-value[a]	R^2
AUDPC	Total 45 pairwise comparison[b]					
qBLASTads-6	/	*qBLASTads-12-1*	C236	RZ397	15.8	0.1872
qBLASTads-2	/	*qBLASTads-12-1*	RG520	RZ397	13.8	0.1865
qBLASTads-2	/	*qBLASTads-6*	RG520	C236	13.5	0.1695
qBLASTads-2	/	*qBLASTa-7*	RG520	RG678	13.3	0.1658
qBLASTads-2	/	*qBLASTads-12-2*	RG520	RG574	12.2	0.1612
qBLASTads-2	/	*qBLASTads-9*	RG520	G103b	10.5	0.1563
qBLASTads-6	/	*qBLASTa-7*	C236	RG678	14.7	0.155
qBLASTads-9	/	*qBLASTa-12-2*	G103b	RG574	9.9	0.1486
qBLASTads-6	/	*qBLASTa-12-2*	C236	RG574	3.2	0.1469
%DLA	Total 36 pairwise comparison[b]					
qBLASTads-6	/	*qBLASTads-12-1*	C236	RZ397	14.4	0.1745
qBLASTads-6	/	*qBLASTads-9*	C236	G103b	13.4	0.1697
qBLASTads-6	/	*qBLASTa-7*	C236	RG678	15.8	0.1659
qBLASTads-2	/	*qBLASTads-6*	RG520	C236	12.9	0.1645
qBLASTads-2	/	*qBLASTads-12-1*	RG520	RZ397	11.5	0.1619
qBLASTads-9	/	*qBLASTads-12-1*	G103b	RZ397	9.6	0.1458
qBLASTads-6	/	*Pi-lm2*	C236	RZ536x	12.9	0.1449
SES	Total 36 pairwise comparison[b]					
qBLASTads-2	/	*qBLASTads-12-1*	RG520	RZ397	24.0	0.2842
qBLASTads-2	/	*qBLASTads-6*	RG520	C236	17.4	0.2086
qBLASTads-2	/	*qBLASTa-7*	RG520	RG678	16.1	0.1936
qBLASTads-2	/	*qBLASTads-4*	RG520	RZ69x	15.6	0.1915
qBLASTads-2	/	*qBLASTads-9*	RG520	G103b	12.7	0.1833
qBLASTads-2	/	*Pi-lm2*	RG520	RZ536x	14.4	0.1821
qBLASTads-2	/	*qBLASTads-3*	RG520	RZ474	14.6	0.1794

[a] All reported *F*-values were significant at $P \leq 0.0001$

[b] Analysis of AUDPC included the nine QTLs associated with AUDPC (listed in Table 1) and the major gene previously mapped to chromosome 11, *Pi-lm2*. Care must be taken in the interpretation of this analysis, however, since linkage between *qBLASTads-12-1* and *qBLASTa-12-2* would bias their associations upwards. Loci pairs considered in the analyses of %DLA and SES did not include *qBLASTa-12-2* because this probe exhibited enhanced association due to its linkage with *qBLASTads-12-1*

generally exhibited a high R^2 (Table 5). This estimated importance of *qBLASTads-2* is consistent with the fact that this QTL explained the largest portion of phenotypic variation within the SES data (Table 3). Although *qBLASTads-2* was also the QTL that individually explained the largest portion of %DLA, *qBLASTads-6* was of greater significance in the two-way analysis of %DLA data. Three QTLs previously associated with major resistance genes (*qBLAST-ads-2*, *qBLAST-ads-6* and *qBLAST-ads-12-1*) were of nearly equal importance in the three-way analysis of %DLA, as well as in the two- and three-way analyses of AUDPC. While the specific pairs differed in ranking of importance between the three traits, two pairwise combinations appeared near the top of each of the lists (Table 4); *qBLASTads-2/qBLASTads-6* (range of $R^2 = 0.16 – 0.21$, $P \leq 0.0001$) and *qBLASTads-2/qBLASTads-12-1* (range of $R^2 = 0.20 – 0.25$, $P \leq 0.0001$).

Although no QTLs were identified on chromosome 11, analysis of data for RZ536x which was linked to *Pi-lm2* (Tabien et al. 2000) indicated that a genetic factor in this region contributed significantly to field resistance when combined with either *qBLASTads-2* or *qBLASTads-6*. In combination, *Pi-lm2*, appeared in the top 20% of digenic R^2 values for %DLA and for SES, but not for AUDPC (Table 4). Though not in the top 20%, some combinations including *Pi-lm2* did have significant *F*-values ($P \leq 0.0001$) and a relatively high R^2; the *qBLASTads-2*/*Pi-lm2* combination had $R^2 = 0.1143$, $P \leq 0.0001$ and the *qBLASTads-6*/*Pi-lm2* combination had $R^2 = 0.1136$, $P \leq 0.0001$. The importance of the *Pi-lm2* locus appears to be dependent on the genetic background. A similar observation was reported in soybean (Lark et al. 1995) where a QTL was found to be conditional on another allele located at an unlinked locus.

In combinations of three loci, the three QTLs that mapped similarly with major genes on chromosomes 2, 6 and 12 were again common among the combinations having high R^2 values (Table 5). Two QTLs not associated with major genes, *qBLASTa-7* and *qBLASTads-9*, are also common in the lists of combinations having a high R^2 from both two- and three-way analyses of all three traits. Indeed, a combination of these QTLs with the major-gene-associated QTLs *qBLASTads-2* and *qBLASTads-12-1* resulted in R^2 values nearly as high or higher than the combination of all three QTL-identified major genes (*qBLASTads-2*/*qBLASTads-6*/*qBLASTads-12-1*), indicating that loci not providing major-gene resistance do play a significant role in field resistance. The durability of resistance stemming from such gene combinations can only be determined after multiple year, environment, and race studies.

It is interesting to note that, especially in the analysis of triplet combinations, the inclusion of *Pi-lm2*, the major gene locus not identified in QTL analysis, again appeared in triplets with a high R^2, especially when those triplets included *qBLASTads-2* and/or *qBLASTads-6*. The resistance allele for *Pi-lm2* originated from Lemont and may indeed contribute to the durable resistance of this variety, as may *qBLASTads-4*, the QTL whose resistance allele was also derived from Lemont. The wide use of both Lemont and ‘Gulfmont’, a line that is identical with Lemont for both pedigree and blast response (Marchetti

Table 5 Triplet combinations of loci resulting in the highest 20% of R^2 values in three-way analyses of AUDPC, %DLA and SES data

Loci in triplet combinations			Markers representing loci in stepwise regressions			*F*-value[a]	R^2
AUDPC	Total 120 triples analysed[b]						
qBLASTads-2	/ *qBLASTa-7*	/ *qBLASTads-12-1*	RG520	RG678	RZ397	10.9	0.3054
qBLASTads-2	/ *qBLASTads-6*	/ *qBLASTads-12-1*	RG520	C236	RZ397	10.9	0.3015
qBLASTads-2	/ *qBLASTads-12-1*	/ *qBLASTa-12-2*	RG520	RZ397	RG574	9.7	0.2915
qBLASTads-2	/ *qBLASTads-9*	/ *qBLASTads-12-1*	RG520	G103b	RZ397	8.0	0.2750
qBLASTa-1	/ *qBLASTads-2*	/ *qBLASTads-9*	RZ14	RG520	G103b	8.2	0.2677
qBLASTads-6	/ *qBLASTads-9*	/ *qBLASTads-12-1*	C236	G103b	RZ397	8.2	0.2612
qBLASTads-2	/ *qBLASTads-6*	/ *qBLASTa-7*	RG520	C236	RG678	9.3	0.2556
qBLASTads-2	/ *qBLASTa-7*	/ *qBLASTads-9*	RG520	RG678	G103b	7.9	0.2526
qBLASTads-2	/ *qBLASTads-4*	/ *qBLASTads-12-1*	RG520	RZ69x	RZ397	8.3	0.2517
qBLASTads-2	/ *qBLASTads-6*	/ *qBLASTads-9*	RG520	C236	G103b	7.6	0.2480
qBLASTads-2	/ *qBLASTads-4*	/ *qBLASTa-7*	RG520	RZ69x	RG678	8.9	0.2479
qBLASTads-2	/ *Pi-lm2*	/ *qBLASTads-12-1*	RG520	RZ536x	RZ397	7.8	0.2411
qBLASTads-2	/ *qBLASTa-7*	/ *qBLASTa-12-2*	RG520	RG678	RG574	8.4	0.2409
qBLASTa-1	/ *qBLASTads-2*	/ *qBLASTads-12-1*	RZ14	RG520	RZ397	7.5	0.2402
qBLASTads-2	/ *qBLASTads-6*	/ *qBLASTa-12-2*	RG520	C236	RG574	8.1	0.2379
qBLASTa-1	/ *qBLASTads-6*	/ *qBLASTads-12-1*	RZ14	C236	RZ397	8.3	0.2351
qBLASTads-4	/ *qBLASTads-6*	/ *qBLASTads-12-1*	RZ69x	C236	RZ397	8.1	0.2241
qBLASTads-2	/ *qBLASTads-4*	/ *qBLASTa-12-2*	RG520	RZ69x	RG574	7.4	0.2232
qBLASTads-6	/ *Pi-lm2*	/ *qBLASTads-12-1*	C236	RZ536x	RZ397	8.0	0.2217
qBLASTa-1	/ *qBLASTads-2*	/ *qBLASTa-12-2*	RZ14	RG520	RG574	7.4	0.2212
qBLASTads-2	/ *qBLASTads-4*	/ *qBLASTads-6*	RG520	RZ69x	C236	7.6	0.2201
qBLASTads-6	/ *qBLASTads-12-1*	/ *qBLASTa-12-2*	C236	RZ397	RG574	7.5	0.2197
qBLASTa-1	/ *qBLASTads-2*	/ *qBLASTads-6*	RZ14	RG520	C236	7.2	0.2178
qBLASTads-6	/ *qBLASTa-7*	/ *qBLASTads-12-1*	C236	RG678	RZ397	7.9	0.2176
%DLA	Total 84 triples[b]						
qBLASTads-2	/ *qBLASTads-6*	/ *qBLASTads-12-1*	RG520	C236	RZ397	9.4	0.2746
qBLASTads-2	/ *qBLASTa-7*	/ *qBLASTads-12-1*	RG520	RG678	RZ397	9.1	0.2705
qBLASTads-6	/ *qBLASTads-9*	/ *qBLASTads-12-1*	C236	G103b	RZ397	8.3	0.2668
qBLASTads-2	/ *qBLASTads-6*	/ *qBLASTads-9*	RG520	C236	G103b	7.9	0.2576
qBLASTads-2	/ *qBLASTads-6*	/ *qBLASTa-7*	RG520	C236	RG678	8.8	0.2475
qBLASTads-2	/ *qBLASTa-7*	/ *qBLASTads-9*	RG520	RG678	G103b	7.6	0.2471
qBLASTads-2	/ *qBLASTads-4*	/ *qBLASTads-12-1*	RG520	RZ69x	RZ397	7.8	0.2441
qBLASTads-2	/ *qBLASTads-9*	/ *qBLASTads-12-1*	RG520	G103b	RZ397	6.6	0.2427
qBLASTads-6	/ *qBLASTa-7*	/ *qBLASTads-9*	C236	RG678	G103b	8.6	0.2408
qBLASTads-2	/ *qBLASTads-4*	/ *qBLASTa-7*	RG520	RZ69x	RG678	8.1	0.2327
qBLASTads-6	/ *Pi-lm2*	/ *qBLASTads-12-1*	C236	RZ536x	RZ397	8.2	0.2286
qBLASTads-4	/ *qBLASTads-6*	/ *qBLASTads-12-1*	RZ69x	C236	RZ397	8.0	0.2239
qBLASTads-4	/ *qBLASTads-6*	/ *qBLASTa-7*	RZ69x	C236	RG678	9.0	0.2218
qBLASTads-2	/ *qBLASTads-4*	/ *qBLASTads-6*	RG520	RZ69x	C236	7.6	0.2205
qBLASTads-2	/ *qBLASTads-9*	/ *Pi-lm2*	RG520	G103b	RZ536x	6.2	0.2179
qBLASTads-6	/ *qBLASTads-9*	/ *Pi-lm2*	C236	G103b	RZ536x	7.0	0.2128
qBLASTads-4	/ *qBLASTads-9*	/ *qBLASTads-12-1*	RZ69x	G103b	RZ397	6.0	0.2086
SES	Total 84 triples[b]						
qBLASTads-2	/ *qBLASTads-4*	/ *qBLASTads-12-1*	RG520	RZ69x	RZ397	12.8	0.3418
qBLASTads-2	/ *qBLASTads-6*	/ *qBLASTads-12-1*	RG520	C236	RZ397	13.0	0.3402
qBLASTads-2	/ *qBLASTa-7*	/ *qBLASTads-12-1*	RG520	RG678	RZ397	12.7	0.3391
qBLASTads-2	/ *Pi-lm2*	/ *qBLASTads-12-1*	RG520	RZ536x	RZ397	11.9	0.3270
qBLASTads-2	/ *qBLASTads-9*	/ *qBLASTads-12-1*	RG520	G103b	RZ397	9.8	0.3173
qBLASTads-2	/ *qBLASTads-3*	/ *qBLASTads-12-1*	RG520	RZ474	RZ397	11.3	0.3118
qBLASTa-1	/ *qBLASTads-2*	/ *qBLASTads-12-1*	RZ14	RG520	RZ397	10.7	0.3100
qBLASTads-2	/ *qBLASTads-4*	/ *qBLASTa-7*	RG520	RZ69x	RG678	10.9	0.2877
qBLASTads-2	/ *qBLASTads-3*	/ *qBLASTads-4*	RG520	RZ474	RZ69x	9.4	0.2577
qBLASTads-2	/ *qBLASTads-6*	/ *qBLASTa-7*	RG520	C236	RG678	9.3	0.2558
qBLASTads-2	/ *qBLASTads-6*	/ *qBLASTads-9*	RG520	C236	G103b	7.5	0.2461
qBLASTads-2	/ *qBLASTads-4*	/ *qBLASTads-6*	RG520	RZ69x	C236	8.8	0.2459
qBLASTads-2	/ *qBLASTads-6*	/ *Pi-lm2*	RG520	C236	RZ536x	8.3	0.2419
qBLASTads-2	/ *qBLASTads-4*	/ *Pi-lm2*	RG520	RZ69x	RZ536x	8.2	0.2394
qBLASTads-2	/ *qBLASTads-4*	/ *qBLASTads-9*	RG520	RZ69x	G103b	7.1	0.2362
qBLASTads-2	/ *qBLASTa-7*	/ *qBLASTads-9*	RG520	RG678	G103b	7.2	0.2357
qBLASTads-2	/ *qBLASTads-9*	/ *Pi-lm2*	RG520	G103b	RZ536x	6.6	0.2270

[a] All reported *F*-values were significant at $P \leq 0.0001$

[b] Analysis of AUDPC included nine QTLs associated with AUDPC (listed in Table 1) and the major gene previously mapped to chromosome 11, *Pi-lm2*. Care must be taken in the interpretation of this analysis because linkage between *qBLASTads-12-1* and *qBLASTa-12-2* would bias their associations upwards. Loci pairs considered in the analyses of %DLA and SES did not include *qBLASTa-12-2* because this probe exhibited enhanced association due to its linkage with *qBLASTads-12-1*

1994), as breeding parents in rice breeding programs both in the U.S. and worldwide suggests that the *Pi-lm2* and *qBLASTads-4* resistance alleles are most likely present in many breeding populations. Triplet analysis indicated that the most-significant single resistance locus for breeders to combine with the two resistance alleles already present in Lemont, is *qBLASTads-2*. This triplet was included in the top 20% for three-way analyses of SES ($R^2 = 0.2394$, Table 5), and provided R^2 values of 0.1791 ($P = 0.0001$) for AUDPC, and 0.1876 ($P = 0.0001$) for the %DLA, each of which are significant increases over the *qBLASTads-4*/*Pi-lm2* pairwise R^2 values of 0.0373 for SES, 0.0619 for AUDPC, and 0.0760 for %DLA.

Correlation between presently identified QTLs and previously reported disease resistance loci

Figure 1 shows the estimated location of the blast resistance QTLs identified from this study and how they compare with the estimated locations of genes and QTLs previously reported to affect rice blast resistance (Yu et al. 1991; McCouch et al. 1994; Wang et al. 1994; Hittalmani et al. 1995; Inukai et al. 1996; Miyamoto et al. 1996; Naqvi and Chattoo 1996; Pan et al. 1996; Rybka et al. 1997; Chen et al. 1999; Tabien et al. 2000). Of the nine presently identified putative blast QTLs, three (*qBLASTa-1*, *qBLASTads-2* and *qBLAST-ads-12-1*) correlated with blast resistance loci previously reported from other populations. The LOD peak for *qBLASTads-2* was very near the RG520 locus causing it to coincide with the *Pi-tq1* gene from Teqing, which itself may be allelic with the *Pi-b* as discussed by Tabien et al. (2000). Identification of the same locus in different populations suggests that the locus may be polymorphic in many different rice populations. For example, a single locus for flowering time was found to be polymorphic in three populations (McCouch and Doerge 1995).

Based on single-marker analyses, most of the markers on chromosome 12 were associated with one or more of the resistance traits measured here. The possibility of multiple linked resistance loci was carefully evaluated by comparing LODs of one- and two-QTL models in interval analysis and by stepwise regression. The %DLA and SES data each support a single locus, *qBLASTads-12-1*, while the AUDPC data indicate the presence of a second QTL, *qBLASTa-12-2*, located near RG574. Chromosome 12 reportedly has several loci for blast resistance. Pending allelism tests, there were nine major genes and 13 QTLs previously mapped to this chromosome, and the majority of these were reported to be close to RG869 (McCouch et al. 1994; Wang et al. 1994; Zheng et al. 1995) which is near the location we estimated for *qBLASTads-12-1*. This region of chromosome 12 could be a resistance gene block or cluster similar to those reported in lettuce (Witsenboer et al. 1995) and maize (Sudupak et al. 1993), and for resistance to bacterial blight in rice (Song et al. 1997).

The major genes affecting blast resistance are clustered on chromosomes 4, 6, 11 and 12. The common genetic location between the major genes and QTLs identified from different genetic populations using different blast races/isolates suggests the importance of these specific segments in studying broad-spectrum resistance, i.e., resistance to multiple races or even resistance to multiple pathogens. Several major genes for resistance to rice bacterial blight (causal organism *Xanthomonas oryzae* pv *oryzae*) are clustered near reported blast resistance loci on chromosomes 4 and 11 (Abenes et al. 1993; Causse et al. 1994; Yoshimura et al. 1995; Lin et al. 1996; Zhang et al. 1996; Li et al. 1999). The use of degenerate oligonucleotide primers to map resistance gene analogues in rice have also shown clustering around the known major resistance genes on chromosomes 4, 11 and 12 (Leister et al. 1999; Mago et al. 1999), while the sequence of the *Pib* gene indicates that this major blast resistance gene located on chromosome 2 also belongs to the nucleotide-binding and leucine-rich repeat class of plant resistance genes (Wang et al. 1999). Figure 1 shows that QTLs affecting blast resistance may also be similarly located with QTLs affecting resistance to sheath blight disease (Li et al. 1995) and/or bacterial blight (Li et al. 1999), suggesting the possibility of a disease resistance mechanism of very broad spectrum.

Implications in field screening for blast resistance

The data support the hypothesis that combinations of major genes and QTLs are desirable for improved blast field resistance. The SES rating has been the most-popular method for screening blast resistance and was employed in the development of the durably resistant cultivar IR36 (Ahn 1994). AUDPC is a more-laborious screening method that has been found to be very useful in screening durable resistance to other diseases in other crops (Shaner and Finney 1977). Our results indicate that the SES and %DLA scores, as they are typically rated (once at 30 to 40 DAS), were equally effective at locating resistance QTLs. AUDPC is calculated from sequential %DLA ratings and allows one to distinguish between late infection and slow disease development. In this study, it proved both more laborious and more sensitive. AUDPC data revealed two smaller QTLs not detectable by single ratings of either %DLA or SES. One of these, *qBLASTa-1*, coincided with a QTL reported from Moroberekan.

In field screening for blast resistance, major genes can mask the effects of minor genes. Because partial resistance from minor genes has been associated with increased durability of resistance, it has been suggested that, to identify and select resistance that will withstand the development/selection of virulence in populations of *P. grisea*, breeders should remove completely resistant plants from their breeding populations and select only those that have high levels of partial resistance (Ahn 1994). However, this method may result in removal of

linked/pleiotropic genetic factors conferring partial resistance. Furthermore, this will not allow for evaluation of the effect of major genes in combination with QTLs, such as we found to be significant for *Pi-lm2*, and as were previously reported for blast resistance in Moroberekan (Wang et al. 1994).

Our data support the strategy of pyramiding QTLs and major genes in selected combinations to provide both broad spectrum and durable resistance to the blast fungus. The selection of alleles to be combined should consider both the race specificity of major genes and epistatic interactions between genes and QTLs. The employment of DNA markers to select for desired genetic combinations can be particularly useful when, as in the case of major-gene conferred complete blast resistance, the presence of some genes makes the detection of other genes/QTLs impossible to detect through phenotypic analysis alone.

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