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Genetic control of rice blast resistance in the durably resistant cultivar Gumei 2 against multiple isolates

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Abstract To further our understanding of the genetic control of blast resistance in rice cultivar Gumei 2 and, consequently, to facilitate the utilization of this durably blast-resistant cultivar, we studied 304 recombinant inbred lines of indica rice cross Zhong 156/Gumei 2 and a linkage map comprising 181 markers. An analysis of segregation for resistance against five isolates of rice blast suggested that one gene cluster and three additional major genes that are independently inherited are responsible for the complete resistance of Gumei 2. The gene cluster was located to chromosome 6 and includes two genes mapped previously, *Pi25*(t), against Chinese rice blast isolate 92-183 (race ZC15) and *Pi26*(t) against Philippine rice blast isolate Ca89 (lineage 4), and a gene for resistance against Philippine rice blast isolate 92330-5 (lineage 17). Of the two genes conferring resistance against the Philippine isolates V86013 (lineage 15) and C923-39 (lineage 46), we identified one as *Pi26*(t) and mapped the other onto the distal end of chromosome 2 where *Pib* is located. We used three components of partial blast resistance, percentage diseased leaf area (DLA), lesion number and lesion size, all measured in the greenhouse, to measure the degree of susceptibility to isolates Ca89 and C923-39 and subsequently identified nine and eight quantitative trait loci (QTLs), respectively. Epistasis was determined to play an important role in partial resistance against Ca89. Using DLA measured on lines susceptible in a blast nursery, we detected six QTLs. While different QTLs were detected for partial resistance to Ca89 and C923-39, respectively, most were involved in the partial resistance in the field. Our results suggest that the blast resistance in Gumei 2 is controlled by multiple major genes and minor genes with epistatic effects.

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Introduction

Rice blast resistance, caused by *Magnaporthe grisea*, has been studied extensively, but this diverse and rapidly changing pathogen has never been permanently brought under control. New varieties frequently lose their resistance within a few years—sometimes within 1 year—of their release to farmers (Kiyosawa 1982; Reddy and Bonman 1987). Nevertheless, breeding for resistant varieties remains the most promising choice for managing the blast problem, and gene pyramiding seems promising to provide broad spectrum and durable resistance (McClung et al. 1997; Tabien et al. 2002).

Mapping studies on blast resistance genes (R genes) have been carried out extensively since the 1990s as a result of the increasing availability of DNA molecular markers. To date, at least 25 R genes have been identified and mapped using molecular markers. These genes are distributed on 10 of the 12 rice linkage groups, with the exception of chromosomes 3 and 10, and many of them are clustered on chromosomes 6, 11 and 12 (Kinoshita 1995; Pan et al. 1996, 1998; Chen et al. 1999; Zhuang et al. 2002). Wang et al. (1994) located *Pi7*(t) on chromosome 11 using recombinant inbred lines (RILs) derived from a cross between Moroberekan, a durable resistant cultivar from Ivory Coast, and susceptible control CO39. This group was also the first to identify a number of quantitative trait loci (QTLs) for partial blast

resistance, which they measured using three components of partial blast resistance, percentage diseased leaf area (DLA), lesion number (LN) and lesion size (LS). At the present time, at least seven R genes have been identified in Moroberekan (Wang et al. 1994; Naqvi et al. 1995; Inukai et al. 1996; Naqvi and Chattoo 1996; Chen et al. 1997a, 1999), indicating that the genetic control of blast resistance is complicated and involves both major and minor genes. Blast resistance in some of the traditional Chinese resistant cultivars has been shown to be mainly controlled by multiple minor genes with complementary or additive effects (He et al. 1989a), although major genes do contribute under certain circumstances (He et al. 1989b). In general, durable resistance to blast has been considered to be complicated and influenced by environment (Bonman 1992).

A long-term nationwide evaluation of blast resistance of 38,000 rice accessions by 33 Chinese rice research organizations resulted in the selection of 140 resistant accessions. These were tested further for durable resistance from 1990 to 1994 in six blast nurseries covering five rice-growing regions from southern to northern China. Rice cultivar Gumei 2 was tested in 34 blast nurseries for 12 years and found to be the only semi-dwarf cultivar possessing durable blast resistance (Peng et al. 1996). The objective of the investigation reported here was to determine the genetic control of resistance to blast in Gumei 2.

Materials and methods

Plant materials and inoculation

In the early growing season of 1990, Zhong 156, as female parent, was crossed to Gumei 2 at the China National Rice Research Institute (CNRRI), Hangzhou, China. From a single F_1 plant, seeds were collected and advanced by single seed descent. In 1995, 304 RILs at the F_8 generation were obtained.

The two parents (10 plants each for two replications in two independent experiments) were inoculated with 40 Philippine isolates at IRRI (International Rice Research Institute, Manila, Philippines) by means of a standard inoculum (1×10^5 conidia/ml). No isolates were able to successfully infect Gumei 2, but eight isolates were compatible to Zhong 156, and four of these, representing four different lineage groups, were chosen to test the RILs for blast resistance in the greenhouse. These isolates were Ca89 (lineage 4), V86013 (lineage 15), 92330-5 (lineage 17) and C923-39 (lineage 46) (Chen 1993). The RILs were inoculated in a complete randomized block design with three replications of ten plants per line. For each of the four isolates, lesion types 0–3 were scored as resistant and lesion types 4 and 5 as susceptible (Bonman et al. 1986). Partial resistance was only measured for lines susceptible to Ca89 and C923-39, respectively. DLA was estimated visually following the Standard Evaluation System (SES) for rice [International Rice Research Institute (IRRI) 1996], while LN and LS were measured using 30 plants in the three replications.

In the blast nursery, RILs, parents and susceptible control CO39 were planted as described by Wu et al. (2004b). Between 30 and 40 seeds were sown in a row of $10\times3\times1$ cm. The experiment was a complete randomized block design with three replications. DLA was estimated once a week beginning 14 days after sowing; data collected at the fourth week were used for analysis.

Molecular marker analysis

Roughly 10 g of fresh leaf tissue from 20 plants per line were bulked for DNA extraction following the sodium dodecyl sulfate (SDS) method of Lu and Zheng (1993). Southern blotting was performed according to standard methodology (Sambrook et al. 1989). Six restriction enzymes (*Bam*HI, *Dra*I, *Eco*RI, *Eco*RV, *Hin*dIII, *Xba*I) were used for the parental survey of polymorphism. Probe hybridization and signal detection were carried out using ECL Direct Nucleic Acid Labelling and Detection Systems (Amersham Pharmacia Biotech, Chalfont, UK). Restriction fragment length polymorphism (RFLP) probes were from Cornell University (USA) and the Rice Genome Research Program (RGP) of Japan, except that candidate genes were provided by Kansas State University (see http://www.ksu.edu/ksudgc/).

The simple sequence repeat (SSR) primers were amplified according to Chen et al. (1997b). The PCR products were detected on 1.5% agarose gels or on 4% denaturing polyacrylamide gels using silver staining following the manufacturer's recommendation (Promega, Madison, Wis.). Resistance gene analogue (RGA) primers were amplified based on the method described by Chen et al. (1998) with a minor modification: an initial denaturation at 94°C for 4 min, followed by 40 cycles of 1 min at 94°C, 1 min at 42°C and 1.5 min at 72°C and terminated by a final extension of 8 min at 72°C.

Data analysis

MAPMAKER ver. 3.0b (Lander et al. 1987) was employed for the construction of a framework map. A LOD of 3.0 was used to determine linkage groups, and the order of linked markers was determined at LOD of 2.0 within a linkage group. Map distances presented in centiMorgans (cM) between markers were derived from the Kosambi function (Kosambi 1944). The chromosomal locations of major genes were inferred using MAPMAKER 3.0b.

The QTLs conditioning partial resistance against Ca89 and C923-39 were identified by employing the Multiple Interval Mapping (MIM) approach of Windows QTL CARTOGRAPHER ver. 2.0 (Wang et al. 2004).

MIM simultaneously fits multiple putative QTLs in the model for mapping QTLs, and the QTL effects and variance partition are additive. The QTLs are determined using model selection criteria that could supersede the requirement of a true threshold. In this study, the Bayesian Information Criterion (BIC) default value $c(n) = \ln(n)$ was used to search QTLs and QTL epistasis at a walk speed of 1 cM. Given the detection of QTLs with significant additive effects, QTL positions were optimized and fitted into the model. The QTL epistasis was then searched using the same criteria. Given significant epistasis detected, the QTL positions were optimized again and fitted into the model. The QTL effects and variance partition were calculated based on the final model. The additive-by-additive epistatic effect was defined as the difference between the effects of parental genotype combinations and recombinant genotype combinations.

Results and discussion

Genetic control of blast resistance

Gumei 2 was completely resistance to all four isolates. Zhong 156 was susceptible to all four isolates with typical lesion type 4, but LN and DLA were relatively low in contrast to the susceptible control CO39. Resistance to isolate Ca89 was mediated by a single gene, while resistance to each of the other three isolates was governed by two R genes (Table 1). Since only Gumei 2 was resistant, the resistance alleles had to have originated from Gumei 2.

Of the 304 RILs, 301 showed a consistent, identical response pattern to isolates V86013 and C923-39, while the remaining three lines (G116, G128 and G282) showed inconsistent reactions to either V86013 or C923-39 (Table 1). This result indicated that the same set of two R genes probably controlled blast resistance to these two isolates. The 73 lines susceptible to both V86013 and C923-39 were all susceptible to Ca89, implying that the gene conditioning resistance to Ca89 was likely one of the two R genes against V86013 and C923-39. Of the 78 lines susceptible to 92330-5, 76 lines were also susceptible to Ca89, while the remaining two lines were resistant to Ca89, indicating that one of the two R genes against 92330-5 was tightly linked to the gene against Ca89. On the other hand, 38 of the 73 lines susceptible to both V86013 and 923-39 were resistant to 92330-5, and 43 of the 78 lines susceptible to 92330-5 were resistant to V86013 and 923-39. Thus, the second gene for resistance to V86013 and 923-39 was independently inherited from the second gene for resistance to 92330-5.

The gene conferring resistance against Ca89 has already been mapped and tentatively designated as *Pi26*(t). A different gene conferring neck blast resistance to a Chinese blast isolate 92-183 (race ZC15) has also been mapped using the same population and tentatively designated as *Pi25*(t) (Wu et al. 2004a). Mapping located *Pi25*(t) and *Pi26*(t) to closely linked intervals on chromosome 6. A total of 287 rice lines showed consistent resistance or susceptibility to neck blast isolate 92-183 over a 3-year period. Of these,183 and 93 lines had a consistent resistance or susceptible reaction, respectively. Of the 93 lines susceptible to Chinese blast isolate 92-183, 87 and 6 were respectively susceptible and resistance to Philippine blast isolate Ca89, 39 and 54, respectively, for Philippine blast isolate V86013, 39 and 53, respectively, for Philippine blast isolate C923-39 and 62 and 30, respectively, for Philippine blast isolate 92330-5. This results indicated that the second resistance gene against Chinese isolate 92-183 was independently inherited from genes controlling resistance to the Philippine isolates. In addition to the resistance gene cluster in the *Pi25*(t)/*Pi26*(t) region, at least three more major genes were responsible for the blast resistance of Gumei 2.

Mapping of major genes

Wu et al. (2004a) used a linkage map consisting of 177 marker loci for mapping *Pi25*(t) and *Pi26*(t). We added 14 additional SSR markers in the region harbouring *Pi25*(t)/*Pi26*(t) for the present survey of the parents.

Table 1 Genetic analysis of blast resistance of 304 RILs from the indica rice cross Zhong 156/Gumei 2 to different Philippine isolates

Group	Phenotype[a]				Number of lines
	Ca89	V86013	C923-39	9233-5	
I	S	S	S	S	35
II	S	S	S	R	38
III	S	R	R	S	41
IV	S	S	–	R	1
V	S	–	R	–	1
VI	S	R	–	R	1
VII	S	R	R	R	31
VIII	R	R	R	R	153
IX	–	R	R	R	1
X	R	R	R	S	2
R:S	155:148	229:74	229:73	225:78	
Chi-test	$P(1:1) = 0.69$	$P(3:1) = 0.82$	$P(3:1) = 0.74$	$P(3:1) = 0.77$	

[a]R, Resistance (lesion types 0–3); S, susceptible (lesion types 4 and 5); –, inconsistent reaction

Four polymorphic markers were assayed in the population, and the map was updated to include 181 marker loci. The locations of *Pi25*(t) and *Pi26*(t) were revised (Fig. 1). *Pi25*(t) stayed in the same location in interval A7-RG456, while *Pi26*(t) was modified to a location in the region flanked by markers K17 and R2123 at a distance 9.1 cM from K17 and 2.0 cM from R2123.

To avoid the masking effects of one gene on the other in the presence of two R genes against isolates 92330-5, V86013 and C923-39, we used susceptible lines to locate the R genes. The 78 lines susceptible to 92330-5 were used for mapping genes controlling resistance to this isolate. One of the two R genes against 92330-5 was located on chromosome 6 at a distance 8.5 cM from marker K17 and 2.7 cM from marker R2123. As suggested by the segregation analysis and supported by the linkage analysis, the resistance gene against Ca89 was tightly linked to one of the genes for resistance to 92330-5. Because we were unable to determine if these were two independent loci, no new gene designations were given. Markers in other regions, including those unlinked, showed no associations to blast resistance against 92330-5 and 92-183. Therefore, the second resistant gene to 92330-5 and the second one to 92-183 could not be mapped.

As all the 73 lines susceptible to both V86013 and C923-39 were also susceptible to Ca89, the 73 lines were not used for mapping the gene on chromosome 6 but were used to tag the second resistance gene. This second gene was mapped on chromosome 2, with all susceptible lines having the allele from the susceptible parent Zhong 156 at marker locus RM208. Since the R gene *Pib* and marker RM208 were located at the same position (186.4 cM) on chromosome 2 in the Cornell 2001 SSR map, the gene symbol *Pib* was used for the second resistance gene against V86013 and C923-39.

Mapping of partial resistance genes

DLA, LN and LS are the three major components of partial resistance. In the present study, partial blast resistance was tested for DLA in the blast nursery at IRRI and all three components were tested for isolates

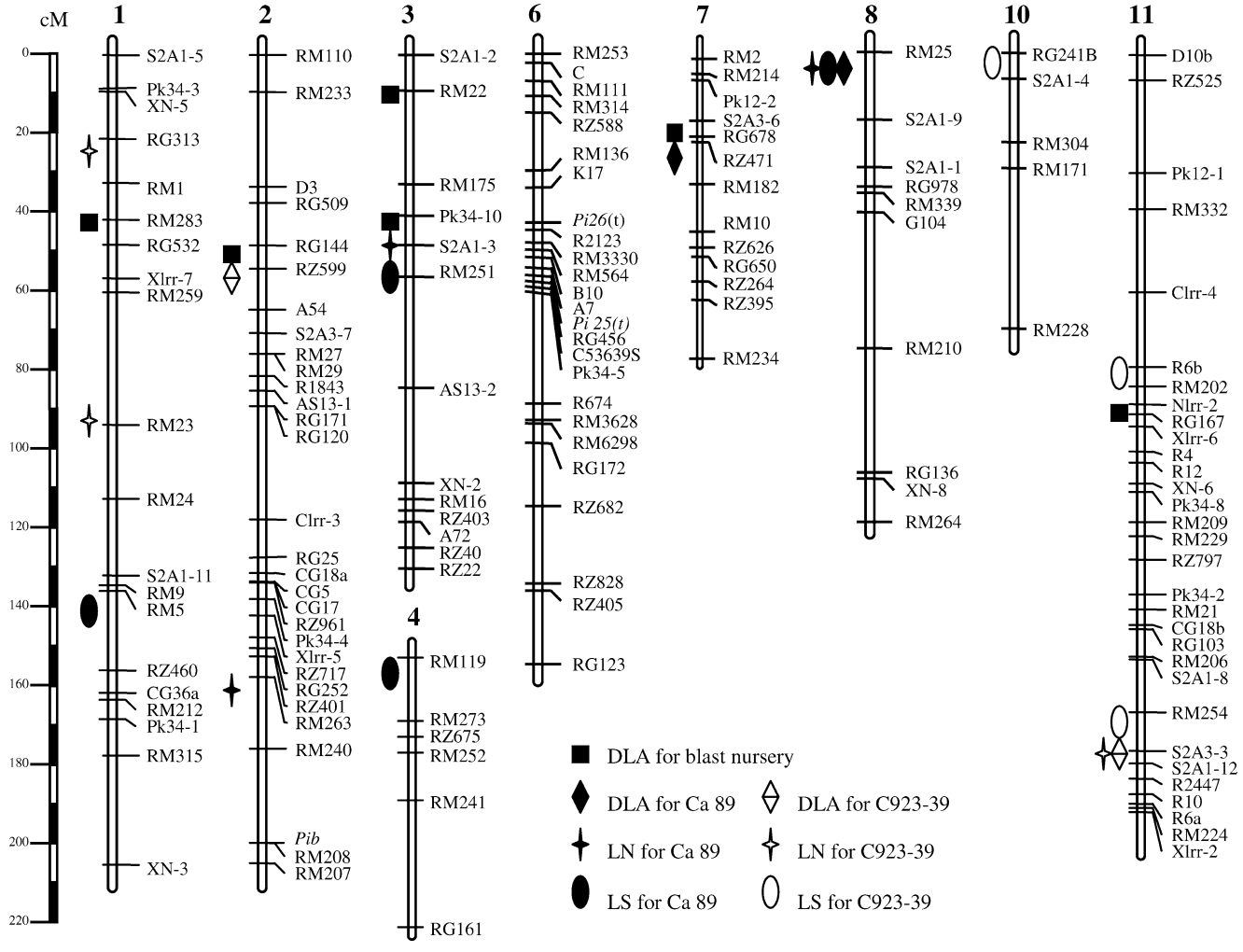

Fig. 1 Genetic map constructed using 304 RILs derived from Gumei 2/Zhong 156. The map contains 181 markers from all 12 linkage groups based on the assignment of Causse et al. (1994), but only linkage groups in which major genes or QTLs were detected are shown

Ca89 and C923-39 in the greenhouse, using the monocyclic test. The analysis of variance indicated highly significant differences among the lines tested and for each parameter measured ($P = 4.21\times10^{-4}$–1.37×10^{-23}).

Based on the data collected from the 148 RILs susceptible to Ca89, nine QTLs and two digenic interactions were detected for partial resistance against Ca89 (Table 2). Two QTLs were detected for DLA, and these interacted with each other, with the epistatic effect between the two DLA QTLs being larger than the effect of each individual QTL alone. Three QTLs and one digenic interaction were detected for LN. Similar to the epistasis detected for DLA, the epistatic effect for LN was larger than the individual effect of any of the three LN QTLs. Four QTLs were detected for LS, while no significant epistasis was found for this trait. The contribution of the LS QTLs to the phenotypic variance ranged from 2.8% to 11.5%. As expected, LN was the trait for which the detected QTLs and epistasis had the largest general contribution to the phenotypic variance.

Using the 74 RILs susceptible to C923-39, we detected eight QTLs for partial resistance against C923-39, including two for DLA, three for LN and three for LS (Table 2). No epistasis was detected for DLA and LS, while one epistatic effect was found for LN. The two DLA QTLs explained 10.7% and 9.3% of the phenotypic variance, respectively, and the three LS QTLs explained a range of LS variance from 6.4% to 8.0%. Two of the three QTLs detected for LN were located on chromosome 1, and these showed major effects, accounting for 25.9% and 17.1% of the phenotypic variance, respectively. In addition, the two QTLs interacted with each other and had an additional contribution of 12.5% to the LN variance. The third QTL was located on chromosome 11 and had a smaller contribution of 6.6%.

The use of all the three traits inferring partial resistance against Ca89 and C923-39 provided an opportunity to compare the locations of the QTLs detected for different traits for the same isolate and for different isolates. We found that QTLs detected for different traits for the same isolate were frequently located in similar genomic regions: in the vicinity of RM25 on chromosome 8 and the intervals flanked by RM254 and S2A1-12 on chromosome 11, QTLs were detected for each of the three traits for Ca89 and C923-39, respectively; in the region marked by S2A1-3 and RM251 on chromosome 3, QTLs were detected for LN and LS for Ca89. In contrast, no QTLs were detected for the same or different traits for different isolates.

For DLA in the blast nursery, 301 lines showed consistent phenotypes, of which 234 lines were resistant and 67 were susceptible. DLA phenotypes of the 67 susceptible RILs were used to detect QTLs conditioning partial resistance in the blast nursery. Six QTLs were detected on five chromosomes (Table 2), but no significant digenic interactions were found. The contribution of these QTLs to the phenotypic variance ranged from 3.3% to 28.6%.

Table 2 Partial blast resistance QTLs and epistasis detected in the Zhong 156/Gumei 2 RIL population

Isolate	Trait[a]	QTL	Left marker	Distance from left marker (cM)	R^2 (%)	Effect[b]
Ca89	DLA	*qDLA7*	RZ471	2.2	3.8	−0.33
		qDLA8	RM25	2.9	7.7	0.43
		qDLA7×qDLA8			10.8	−0.54
	LN	*qLN2*	RM263	6.0	8.4	0.58
		qLN3	S2A1-3	2.6	5.0	0.44
		qLN8	RM25	0.1	7.4	0.52
		qLN2×qLN8			13.1	0.74
	LS	*qLS1*	RM5	11.6	11.5	−0.40
		qLS3	RM251	5.3	6.8	0.34
		qLS4	RM119	0.1	2.8	0.20
		qLS8	RM25	6.7	6.1	0.28
C923-39	DLA	*qDLA2*	RZ599	0.0	10.7	0.85
		qDLA11	S2A1-12	0.0	9.3	0.78
	LN	*qLN1-1*	RG313	2.9	25.9	−13.97
		qLN1-2	RM23	6.9	17.1	−12.64
		qLN11	S2A1-12	0.1	6.6	3.60
		qLN1-1×qLN1-2			12.5	11.01
	LS	*qLS10*	RG241B	2.8	6.4	−0.06
		qLS11-1	R6b	4.8	6.5	0.06
		qLS11-2	RM254	10.6	8.0	0.07
Blast nursery	DLA	*qDLA1*	RM283	0.1	12.5	−2.36
		qDLA2	RG144	5.8	3.3	1.41
		qDLA3-1	RM22	2.4	28.6	3.16
		qDLA3-2	Pk34-10	1.7	8.1	−1.96
		qDLA7	RG678	0.1	5.7	−1.68
		qDLA11	Nlrr-2	0.0	7.8	−1.98

[a]DLA, Percentage diseased leaf area; LN, lesion number; LS, lesion size

[b]The epistatic effect was measured as the difference between the effects of parental genotype combinations and the recombinant genotype combinations. A positive value indicates that the effect of the parental type is greater than that of the recombinant type. A negative value indicates that the effect of the parental type is lower than that of the recombinant type. The additive effect was measured as the genetic effect when a Zhong 156 allele was replaced by a Gumei 2 allele

A point worth noting is that QTLs responsible for DLA variation in the blast nursery were generally detected in genomic regions where QTLs conditioning partial resistance against either Ca89 or C923-39 were detected. Two QTLs, *qDLA2* and *qDLA7*, had similar locations to DLA QTLs for C923-39 and Ca89, respectively. In regions harbouring *qDLA3-2* and *qDLA11*, QTLs for related traits were found for Ca89 and C923-39, respectively. Of the remaining two QTLs, *qDLA3-1* is linked to *qDLA3-2*, and *qDLA1* was located adjacent to *qLN1-1* for C923-39. These results clearly show that the QTLs detected in the inoculation experiment played a major role in the genetic control of partial blast resistance in the blast nursery.

Effect of R genes in the blast nursery

Based on DNA markers most tightly linked to the three major blast R genes, *Pi26*(t), *Pi25*(t) and *Pib*, we were able to determine the resistance alleles present in the 301 RILs showing consistent DLA performance in the blast nursery. Of the 78 lines that possessed resistant alleles at all three loci, 77 lines were resistant and only one line was susceptible. Similarly, the 68 lines carrying resistant alleles at both the *Pi26*(t) and *Pi25*(t) loci and the susceptible allele at the *Pib* locus were all resistant. Of the 20 lines containing a resistance allele at either the *Pi26*(t) or *Pi25*(t) locus, four lines were susceptible and 16 lines were resistant. These results suggest that the existence of resistance alleles at both the *Pi26*(t) and *Pi25*(t) loci were sufficient to control the blast disease in the blast nursery, while the presence of a resistance allele at only one of the *Pi26*(t) and *Pi25*(t) loci may not be sufficient to confer resistance.

In the remaining groups—60 lines carrying a resistant allele at the *Pib* locus only and 75 lines carrying no resistant alleles at the three Pi loci—the susceptible and resistance lines segregated in a 1:1 ratio. These results suggest that *Pib* was relatively ineffective in the IRRI blast nursery, while one other gene that was not mapped in this study is involved in conferring resistance.

Conclusions

Our results show that multiple major genes are responsible for conferring blast resistance in Gumei 2. In addition to a gene cluster containing *Pi26*(t) and *Pi25*(t) on chromosome 6, major genes at three other regions were found to be present in Gumei 2. Based on the results from resistance segregation analysis, we determined that *Pi26*(t) and *Pi25*(t) are non-allelic but closely linked. The resistance performance of the RILs in the blast nursery also implied that *Pi25*(t) may act differently from *Pi26*(t). Nevertheless, these loci have been located to chromosome 6 in the region with a cluster of R genes, including *Piz*^{5}, *Pi2*(t), *Pi3*, *Pi8*, *Pi9*(t) and *Pi13*(t) (Yu et al. 1991; Mackill and Bonnman 1992; Causse et al. 1994; Pan et al. 1996, 1998; Liu et al. 2002). Another gene mapped in this study was located on the distal end of chromosome 2 where the cloned R gene, *Pib* (Wang et al. 1999), was located. The relationship between the genes carried by Gumei 2 and those found in other cultivars needs to be further studied.

Partial blast resistance to Ca89, C923-39 and the pathogen population was studied using susceptible lines. On the basis of the results, we conclude that partial resistance to Ca89 and C923-39 observed in the present study was likely controlled by different QTLs, while most of the QTLs detected using single isolates in the greenhouse were involved in the partial resistance in the field. Unlike the four major genes for which the resistant alleles are from Gumei 2, the sources of partial resistance were either from Zhong 156 or Gumei 2 both in the greenhouse and under field conditions. This phenomenon is similar to those observed in other studies on various disease and plant species (Wang et al. 1994; Ramalingam et al. 2003; Wu et al. 2004b).

Gumei 2 is considered to have unusual characteristics that are quite valuable in rice breeding. Unlike many other blast resistance donors, it is a semi-dwarf plant type and has a relatively short growth period. Studies on the inheritance of blast resistance in Gumei 2 should enhance the utilization of this germplasm. The yield traits of the Zhong 156/Gumei 2 RILs were evaluated in 1996 and 1998 and in two seasons in 2001 (Cao et al. 2003a; Zheng et al. 2003), and three lines carrying Gumei 2 alleles at marker loci K17 through R674 [i.e. carrying resistance alleles at *Pi25*(t) and *Pi26*(t)] that showed promising yield potential were identified. Although these lines are not desirable for commercial usage due to poor grain quality, they have shown a wide-spectrum of blast resistance. In other marker assisted selection practice utilizing bacterial blight (BB) resistance gene pyramids introduced from IRRI (Huang et al. 1997), a number of new restorer lines for three-line hybrid rice have been developed by the CNRRI (Cao et al. 2003b), and seven new hybrids have been released commercially. By crossing BB-resistant lines to blast resistance gene pyramids selected from a Zhong 156/Gumei 2 RIL population, populations are currently being used to select superior lines carrying multiple blast R genes and other desirable traits.

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