

## RESEARCH

# Confirming and Identifying New Loci for Rice Blast Disease Resistance using *Magnaporthe oryzae* Field Isolates in the US

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## ABSTRACT

Quantitative trait loci (QTL) play important roles in controlling rice blast disease. In the present study, 10 field isolates of the races IA1, IB1, IB17, and IC1 of US rice blast fungus *Magnaporthe oryzae* collected in 1996 and 2009 were used to identify blast resistance QTL with a recombinant inbred line (RIL) population consisting of 227 F<sub>7</sub> individuals derived from the cross of rice (*Oryza sativa* L.) cultivars Lemont and Jasmine 85. Jasmine 85 is an *indica* cultivar that is moderately resistant, and Lemont is a tropical *japonica* cultivar susceptible to rice blast in greenhouse inoculation. Disease reactions of the parents and RILs were evaluated under greenhouse conditions. A total of six resistance QTL, *qBLR8*, *qBLR10-1*, *qBLR10-2*, *qBLR10-3*, *qBLR12-1*, and *qBLR12-2*, were identified on chromosomes 8, 10, and 12, respectively. Phenotypic variation, conditioned by these six resistance QTL, ranged from 5.37 to 39.18%. Among them, *qBLR12-1* and *qBLR12-2* provided the strongest resistance to the newest isolates of the most virulent race IA1 of *M. oryzae*. Three of these resistance QTL have been identified using different blast isolates in a previous study. *qBLR10-1*, *qBLR10-2*, and *qBLR10-3* have not been previously found in this cross. These confirmed and new resistance QTL will be useful for the development of rice cultivars with improved effective resistance to rice blast via a marker-assisted selection (MAS) approach.

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**Abbreviations:** LJRIL, ‘Lemont’ × ‘Jasmine 85’ recombinant inbred line; MAS, marker-assisted selection; QTL, quantitative trait loci; R, resistance; RIL, recombinant inbred line.

GENETIC RESISTANCE in rice to rice blast, caused by the fungus *M. oryzae*, includes qualitative (complete) resistance, which is often governed by major genes and quantitative resistance (incomplete) by minor genes. Both types of resistance (*R*) genes have been identified in rice germplasm worldwide. Major *R* genes are only effective to isolates (races) of *M. oryzae* that contain the corresponding avirulence (*AVR*) gene (Silué et al., 1992), and their deployments have been productive in preventing blast disease in numerous rice production areas. However, the *AVR* genes in *M. oryzae* have been known to be highly plastic with frequent deletions, insertions, and transposable elements (Zhou et al., 2007; Lee et al., 2009; Dai et al., 2010). Consequently, more virulent races of *M. oryzae* overcoming the deployed *R* genes have been found in commercial rice fields (Xing et al., 2012). The efficacy of major *R* genes has been limited by the corresponding *AVR* genes. In contrast, incomplete resistance, referred to as QTL, is generally considered to be nonrace specific with few exceptions (Bonman, 1992; Wang et al., 1994). Historical observations indeed suggest that resistance mediated by QTL is more durable. To date, more than 350 QTL have been identified in rice germplasm worldwide (Ballini et al., 2008); however, none of them were used for MAS. The major problem was that QTL mapping relies on phenotypic data collected from multiyear, replicated field-plot experiments,

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and these phenotypic data are often not reliable because of complex, uncontrollable variables in the field. Consequently, it has been difficult to validate and adapt QTL markers identified from field experiments for breeding using MAS approach.

Jasmine 85, an *indica* cultivar, was developed at the International Rice Research Institute, the Philippines, and was adapted to the southern United States. Jasmine 85 is moderately resistant to *M. oryzae* in the southern U.S. (Marchetti et al., 1998). Lemont, a tropical *japonica* cultivar, has partial resistance to *M. oryzae* under field conditions but is susceptible in greenhouse inoculations (Bollich et al., 1985; Jia and Liu, 2011). Infection types observed under greenhouse conditions were predicted to be reliable for identifying the corresponding QTL. Under this assumption, a mapping population derived from the cross of Lemont with Jasmine 85 has been used to map blast resistance QTL using numerical disease rating data instead of visual determination of disease reactions (Jia and Liu, 2011). Six resistance QTL, *qBLAST8.1*, *qBLAST8.2*, *qBLAST9.2*, *qBLAST9.3*, *qBLAST12.1*, and *qBLAST12.2*, in Jasmine 85 and three resistance QTL, *qBLAST3*, *qBLAST9.1*, and *qBLAST11*, in Lemont have been identified with several blast races under greenhouse conditions (Jia and Liu, 2011). These newly identified QTL will need to be validated using different blast isolates under the same environment before their application for MAS.

The objectives of this study were to (i) validate these identified resistance QTL using new isolates of *M. oryzae* under greenhouse conditions and (ii) identify new QTL and new genetic stocks for resistance to rice blast.

## MATERIALS AND METHODS

### Mapping Population and Plant Growth

A total of 227 F<sub>7</sub> RILs developed from a cross between Lemont and Jasmine 85 (LJRIL) by the single-seed descent method in a greenhouse at Dale Bumpers National Rice Research Center, Stuttgart, AR, US, was used for QTL mapping (Jia et al., 2015). The soil was provided by Hoskyn Enterprises Inc., Stuttgart, AR. The soil was from nonfarming areas and was free of agricultural chemicals (e.g., fertilizer, pesticide). The soil was sterilized by a high temperature steam generator that runs off of natural gas for 8 h. Eight to ten seeds yielding three to six plants per pot, one pot per replication, and two to three replications per RIL per blast isolate treatment were grown in a 4- by 4-cm pot in a 26- by 52-cm plastic container with sterilized soil under greenhouse conditions at 24 to 30°C and 16:8 h light/day. Similar seeding was done for the two parents, Lemont and Jasmine 85. After germination, 0.02 g of controlled-release fertilizer (Osmocote Plus, 15-9-12 [N-P-K]; Hummert International) was applied in each pot. The RILs were grown for 2 to 3 wk until they reached the three- to four-leaf stage and then were inoculated with blast fungus.

### Fungal Isolate (race) of *Magnaporthe oryzae*, Growth, Experiment Design, and Inoculation

Ten isolates (races) of *M. oryzae* from different rice growing areas of the southern United States, ARB82 (IA1), ARB4 (IA1), ARB9 (IA1), ARB132 (IA1), ARB94 (IB1), ARB157 (IB1), ARB151 (IB1), ARB154 (IB17), ARB86 (IC1), and ARB8 (IC1) (Xing et al., 2012), were used to inoculate all the LJRILs and parents, Lemont and Jasmine 85, in which race IA1 was recently found, IB1 was the most commonly found, and IC1 was one of the virulent races that infects the *Pita*-containing rice cultivar Katy. It has been observed that different isolates of the same race may be genetically different (Jia, unpublished data, 2015). Therefore, the isolate was used to represent respective blast race in this study.

The pots of RILs and parental lines were rearranged into multiple randomized replications per isolate treatment and placed in trays in a greenhouse. Desiccated filter paper pieces containing mycelia and spores were removed from a -20°C freezer and placed on oatmeal agar plates under black-white fluorescent light for 7 to 10 d at 23 to 25°C. Fungal spores were gently scrapped from the agar with 0.25% gelatin solution and were filtered through four layers of cheesecloth. Spore concentration was determined with a hemocytometer following the method described by Sambrook and Russell (2001). Ten to fifteen milliliters of spore suspensions ( $1-5 \times 10^6$  spores mL<sup>-1</sup>) were used to inoculate 200 to 300 LJRIL seedlings using the following procedure. First, spores were evenly sprayed onto seedlings in a plastic bag using a Bager-250 artist airbrush, and then the inoculated seedlings were sealed in the same bags overnight in a laboratory at 23 to 25°C to maintain at least 70% humidity. After 24 h, inoculated seedlings were removed from the bags and moved to a greenhouse with 24 to 30°C and 16-h light/day for an additional 6 d to develop disease symptoms. Disease reactions were determined when parents of the mapping population exhibited expected phenotypes. Disease reactions were rated using a scale of 0 to 5 where 0 to 2 is resistant and 3 to 5 is susceptible, as described by RoyChowdhury et al. (2011). Disease reaction scores were determined for each of the three to six plants per pot. For most of the RILs, the same disease reaction was obtained for all plants per RIL. Two replications were performed for all isolates except for ARB82, ARB94, and ARB86, for which three replications were performed. The RIL disease ratings used for QTL mapping per blast race were averaged and calculated across all plants of that RIL in all replications treated with that race.

### Data Analysis and Quantitative Trait Loci Mapping

A total of 199 polymorphic simple-sequence repeat markers were used for the construction of a linkage map (Jia and Liu, 2011). The QTL Cartographer 2.5 tool was used for QTL mapping with composite-interval mapping method (Wang et al., 2007; Jia and Liu, 2011; Yang et al., 2013). The logarithm of the odds threshold of  $\geq 2.5$  was used to declare the presence of a putative QTL to compare with the previously identified resistance QTL on rice chromosomes. The permutation was 1000 times, the other factors were default by the software. Additive effect and percentage of phenotypical variation were estimated.

## RESULTS

### Disease Reactions and New Blast Resistant Genetic Stocks

Lemont was susceptible and was rated as 4 to 5, and the disease reactions of Jasmine 85 varied from 0 to 4 indicating the presence of partial resistance to *M. oryzae*. Based on the disease reactions of 10 isolates (disease score < 3 for each isolate), 17 RILs were identified for blast resistance breeding (Table 1). Transgressive segregation of disease reactions of all RILs to all isolates, except to ARB 151, was observed. Frequencies and distributions of disease reactions of all individuals to each isolate of *M. oryzae* verified phenotypic effects of QTL resistance (Fig. 1).

### Quantitative Trait Loci Mapping

In the present study, a total of six resistance QTL, *qBLR8*, *qBLR10-1*, *qBLR10-2*, *qBLR10-3*, *qBLR12-1*, and *qBLR12-2*, were mapped on chromosomes 8, 10, and 12, respectively, with 10 isolates of *M. oryzae*. Among them, *qBLR10-2* and *qBLR10-3* are new QTL from Lemont; the others were mapped at the same region in previous studies (Jia and Liu, 2011; Loan et al., 2003), and their chromosomal location and function were validated.

The resistance QTL *qBLR8*, *qBLR12-1*, and *qBLR12-2* were from Jasmine 85 and *qBLR10-1*, *qBLR10-2*, and *qBLR10-3* were from Lemont (Table 2). The resistance QTL *qBLR8*, identified with the isolates (races), ARB86 (IC1), ARB132 (IA1), and ARB8 (IC1) of *M. oryzae*, was

**Table 1. Summary of blast disease reactions, quantitative trait loci (QTL), and closest-linked DNA markers of recommended recombinant inbred lines (RILs) for blast resistant donors, and parents.**

LJRIL <sup>†</sup> no.	GSOR <sup>§</sup>	QTL identified	Closest markers	Size (bases)	Disease reaction to isolates and races of <i>Magnaporthe oryzae</i> <sup>†</sup>									
					ARB82 (IA1)	ARB4 (IA1)	ARB9 (IA1)	ARB132 (IA1)	ARB157 (IB1)	ARB94 (IB1)	ARB151 (IB1)	ARB86 (IC1)	ARB8 (IC1)	ARB154 (IB17)
14	101614	<i>qBLR8</i>	RM72	152–198	1.3	1	1	3	1	0.7	0	2.7	3	1.5
		<i>qBLR12-1</i>	RM6998	210–230										
		<i>qBLR12-2</i>	OSM89	290–300										
15	10615	<i>qBLR8</i>	RM72	152–198	1.7	0	1	1	2	1.3	1	1	3	1
		<i>qBLR10-3</i>	RM258	139–147										
21	101621	<i>qBLR8</i>	RM72	152–198	1.3	1	0	1	0.5	0.3	1	2.7	0	1
		<i>qBLR12-1</i>	RM6998	210–230										
		<i>qBLR12-2</i>	OSM89	290–300										
28	101628	<i>qBLR8</i>	RM72	152–198	1.3	1	2	1	1	1.3	1	1	1	0
		<i>qBLR10-1</i>	RM228	103–155										
		<i>qBLR10-2</i>	RM147	92–97										
		<i>qBLR10-3</i>	RM258	139–147										
38	101638	<i>qBLR10-1</i>	RM228	103–155	0.7	0	0	0	0.5	1.3	0	2.3	0	0.5
		<i>qBLR10-3</i>	RM258	139–147										
		<i>qBLR12-1</i>	RM6998	210–230										
		<i>qBLR12-2</i>	OSM89	290–300										
45	101645	<i>qBLR8</i>	RM72	152–198	1	1	2	nd <sup>¶</sup>	1	1.7	1	1.7	3	1
		<i>qBLR12-1</i>	RM6998	210–230										
		<i>qBLR12-2</i>	OSM89	290–300										
62	101662	<i>qBLR12-2</i>	OSM89	290–300	0.7	0	0	1	2	0.7	0	2.7	3	0.5
76	101676	<i>qBLR10-1</i>	RM228	103–155	1	1	2	nd	0.5	0.7	1	1.7	3	0.5
		<i>qBLR10-2</i>	RM147	92–97										
		<i>qBLR12-1</i>	RM6998	210–230										
		<i>qBLR12-2</i>	OSM89	290–300										
78	101678	<i>qBLR12-1</i>	RM6998	210–230	0.7	0	4	0	0.5	1.7	3	1.3	2	0
		<i>qBLR12-2</i>	OSM89	290–300										
89	101689	<i>qBLR12-1</i>	RM6998	210–230	1.7	1	1	nd	1.5	0.7	1	2	0	2
		<i>qBLR12-2</i>	OSM89	290–300										
110	101710 <sup>#</sup>	<i>qBLR10-1</i>	RM228	103–155	1.3	3	2	1	3	1	1	2.3	1	2
		<i>qBLR10-2</i>	RM147	92–97										
		<i>qBLR12-1</i>	RM6998	210–230										
127	101727	<i>qBLR8</i>	RM72	152–198	1.3	2	2	0	0	1.3	1	1.3	2	0
		<i>qBLR10-1</i>	RM228	103–155										
		<i>qBLR10-2</i>	RM147	92–97										
		<i>qBLR12-2</i>	OSM89	290–300										

(cont'd.)

Table 1. Continued.

LJRIL <sup>‡</sup> no.	GSOR <sup>§</sup>	QTL identified	Closest markers	Size (bases)	Disease reaction to isolates and races of <i>Magnaporthe oryzae</i> <sup>†</sup>										
					ARB82 (IA1)	ARB4 (IA1)	ARB9 (IA1)	ARB132 (IA1)	ARB157 (IB1)	ARB94 (IB1)	ARB151 (IB1)	ARB86 (IC1)	ARB8 (IC1)	ARB154 (IB17)	
193	101793	<i>qBLR10-1</i>	RM228	103–155	2	1	1	1	0.5	1	1	1.3	3	0.5	
		<i>qBLR10-2</i>	RM147	92–97											
		<i>qBLR12-2</i>	OSM89	290–300											
215	101815	<i>qBLR8</i>	RM72	152–198	1.7	1	0	0	1	1	1	1.3	1	0.5	
		<i>qBLR12-1</i>	RM6998	210–230											
		<i>qBLR12-2</i>	OSM89	290–300											
227	101827	<i>qBLR12-1</i>	RM6998	210–230	1.7	1	1	0	1.5	1	1	1.3	3	3	
		<i>qBLR12-2</i>	OSM89	290–300											
238	101838	<i>qBLR10-1</i>	RM228	103–155	1	3	3	0	1	2.3	1	1.3	3	2.5	
		<i>qBLR10-2</i>	RM147	92–97											
		<i>qBLR12-1</i>	RM6998	210–230											
		<i>qBLR12-2</i>	OSM89	290–300											
245	101845	<i>qBLR12-1</i>	RM6998	210–230	1.7	1	3	1	0.5	1.3	1	2.7	0	1.5	
		<i>qBLR12-2</i>	OSM89	290–300											
Lemont	102173	<i>qBLR10-1</i>	RM228	103–155	4.7	5	5	4	4	4	5	4.7	5	5	
		<i>qBLR10-2</i>	RM147	92–97											
		<i>qBLR10-3</i>	RM258	139–147											
Jasmine 85	102174	<i>qBLR8</i>	RM72	152–198	1.7	1	1	1	2	0.7	0	2.3	4	1	
		<i>qBLR12-1</i>	RM6998	210–230											
		<i>qBLR12-2</i>	OSM89	290–300											

<sup>†</sup> The average of disease reaction was shown where 0 to 2 indicates resistance and 3 to 5 indicates susceptibility (RoyChowdhury et al., 2011).

<sup>‡</sup> LJRIL, 'Lemont' × 'Jasmine 85' recombinant inbred lines

<sup>§</sup> GSOR, genetic stock *Oryza* collection number (for seed request and information on agronomic traits, <http://www.ars.usda.gov/Main/Docs.htm?docid=24954>).

<sup>¶</sup> nd, no data.

<sup>#</sup> Indicates seed for this line is currently unavailable.

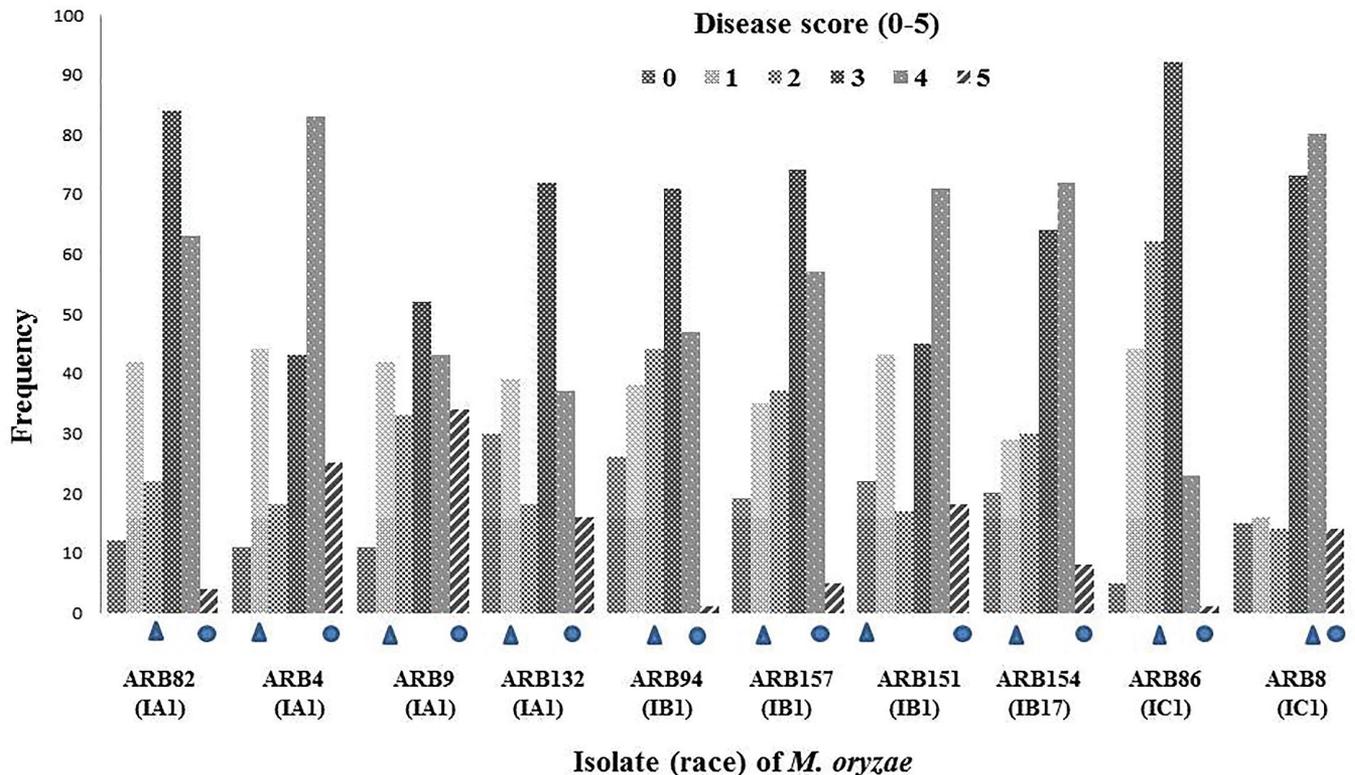


Figure 1. Frequencies and distributions of disease reactions of all individuals to each isolate of *Magnaporthe oryzae*. Disease reaction was determined using the indicated 0-to-5 scale. The triangle and circle indicate the disease score of Jasmine85 and Lemont to each isolate, respectively.

**Table 2. Summary of quantitative trait loci (QTL) responsible for blast resistance, chromosomal and physical locations, and phenotypic variation percentage.**

QTL	Chr <sup>†</sup>	Marker interval	R source <sup>‡</sup>	Nearest marker locus	Isolate (race) of <i>Magnaporthe oryzae</i> <sup>§</sup>	LOD <sup>¶</sup> value	Phenotypic variation	AE <sup>#</sup>
<i>qBLR8</i>	8	RM310-RM404	<i>J</i>	RM72	ARB86 (IC1)	8.0	13.01	0.35
					ARB132 (IA1)	6.3	9.83	0.48
					ARB8 (IC1)	4.9	8.76	0.39
<i>qBLR10-1</i>	10	RM228-RM484	<i>L</i>	RM228	ARB86 (IC1)	4.2	6.93	-0.28
<i>qBLR10-2</i>	10	RM147-RM590	<i>L</i>	RM147	ARB86 (IC1)	4.6	8.11	-0.30
<i>qBLR10-3</i>	10	RM271-RM228	<i>L</i>	RM258	ARB154 (IB17)	4.3	5.37	-0.35
<i>qBLR12-1</i>	12	RM247-RM6998	<i>J</i>	RM6998	ARB94 (IB1)	9.4	17.97	0.71
					ARB151 (IB1)	9.8	18.29	0.71
					ARB157 (IB1)	7.0	13.04	0.48
<i>qBLR12-2</i>	12	OSM89-RM277	<i>J</i>	OSM89	ARB82 (IA1)	22.9	39.18	0.78
					ARB132 (IA1)	9.8	20.89	0.73
					ARB94 (IB1)	16.0	28.19	0.71
					ARB151 (IB1)	11.4	21.05	0.75
					ARB157 (IB1)	13.5	22.89	0.64
					ARB154 (IB17)	19.2	34.19	0.87

<sup>†</sup> Chr, name of chromosome.

<sup>‡</sup> R source, source of resistance where *J* indicates Jasmine 85 and *L* indicates Lemont, respectively.

<sup>§</sup> The reference for these blast races (isolates) is in Xing et al. (2012).

<sup>¶</sup> LOD, logarithm of the odds.

<sup>#</sup> AE, additive effect; a positive AE indicates the Jasmine 85 allele decreases the disease score (confers enhanced resistance) and a negative AE indicates the Lemont allele decreases the disease score.

located between the markers RM310 and RM404 on chromosome 8. The QTL *qBLR8* is located between 5.38 and 16.5 Mb, explaining 13.01, 9.83, and 8.76% of phenotypic variation to these three isolates, respectively.

The resistance QTL *qBLR10-1* and *qBLR10-2* on chromosome 10 were identified with the isolate ARB86 (IC1). The *qBLR10-1* was located between markers RM228 and RM484, explaining 6.93% phenotypic variation. The *qBLR10-2* was identified between the markers RM147 and RM590, explaining 8.11% phenotypic variation. The QTL *qBLR10-3* identified with the isolate ARB154 (IB17) was mapped between the markers RM271 and RM228 on chromosome 10, explaining 5.37% phenotypic variation.

The resistance QTL *qBLR12-1* and *qBLR12-2* were mapped on chromosome 12 with the isolates ARB94 (IB1), ARB151 (IB1), and ARB157 (IB1) of *M. oryzae*. The QTL *qBLR12-1* was located between the markers RM247 and RM6998, explaining 17.97, 18.29, and 13.04% of phenotypic variation to isolates ARB94, ARB151, and ARB157, respectively. The major resistance QTL *qBLR12-2* was mapped between the markers OSM89 and RM277 with six blast isolates, ARB82 (IA1), ARB132 (IA1), ARB94 (IB1), ARB151 (IB1), ARB157 (IB1), and ARB154 (IB17), explaining 39.18, 20.89, 28.19, 21.05, 22.89, and 34.19% of phenotypic variation, respectively (Table 2; Fig. 2).

## DISCUSSION

In the present study, we used newly characterized isolates from the commonly found races IA1, IB1, IB17, and IC1 of *M. oryzae* to validate three previously identified QTL, *qBLR8*, *qBLR12-1*, and *qBLR12-2* on chromosomes 8 and 12, respectively, and to identify three new resistance QTL, *qBLR10-1*, *qBLR10-2*, and *qBLR10-3* on chromosome 10 in LJRILs under greenhouse conditions. All disease assays were performed in a greenhouse to eliminate confounding environmental factors. These resistance QTL and resistance RILs are reliable sources for introducing new blast resistance using MAS.

Rice varieties with *Pi-ta* have been effective for blast control for over two decades. The presence of multiple blast *R* genes including *Pi-ta* and resistance QTL surrounding the centromere of chromosome 12 in rice cultivars may explain the durability and effectiveness of blast resistance of these resistant cultivars (Jia, 2009; Jia et al., 2012). *qBLR12-1* was mapped at regions harboring *Pi-6(t)*, *Pi-4(t)*, and *Pi-ta* (Yu, 1991; Mackill and Bonman, 1992; Bryan et al., 2000). *qBLR12-2* was mapped at regions harboring *Pi-ta* and near *Pi-31(t)*, *Pi-12(t)*, *Pi-tq6*, and *qRBr-12.1* (Zheng et al., 1996; Tabien et al., 2000; Sallaud et al., 2003; Ashkani et al., 2013). It was demonstrated that there is a large linkage block among elite rice cultivars carrying *Pi-ta* (Jia, 2009). It is possible that *qBLR12-2* may have been bred into blast-resistant cultivars carrying *Pi-ta* because of the linkage block at the *Pi-ta* locus (Jia, 2009). Examining the effects of *qBLR12-2* in rice

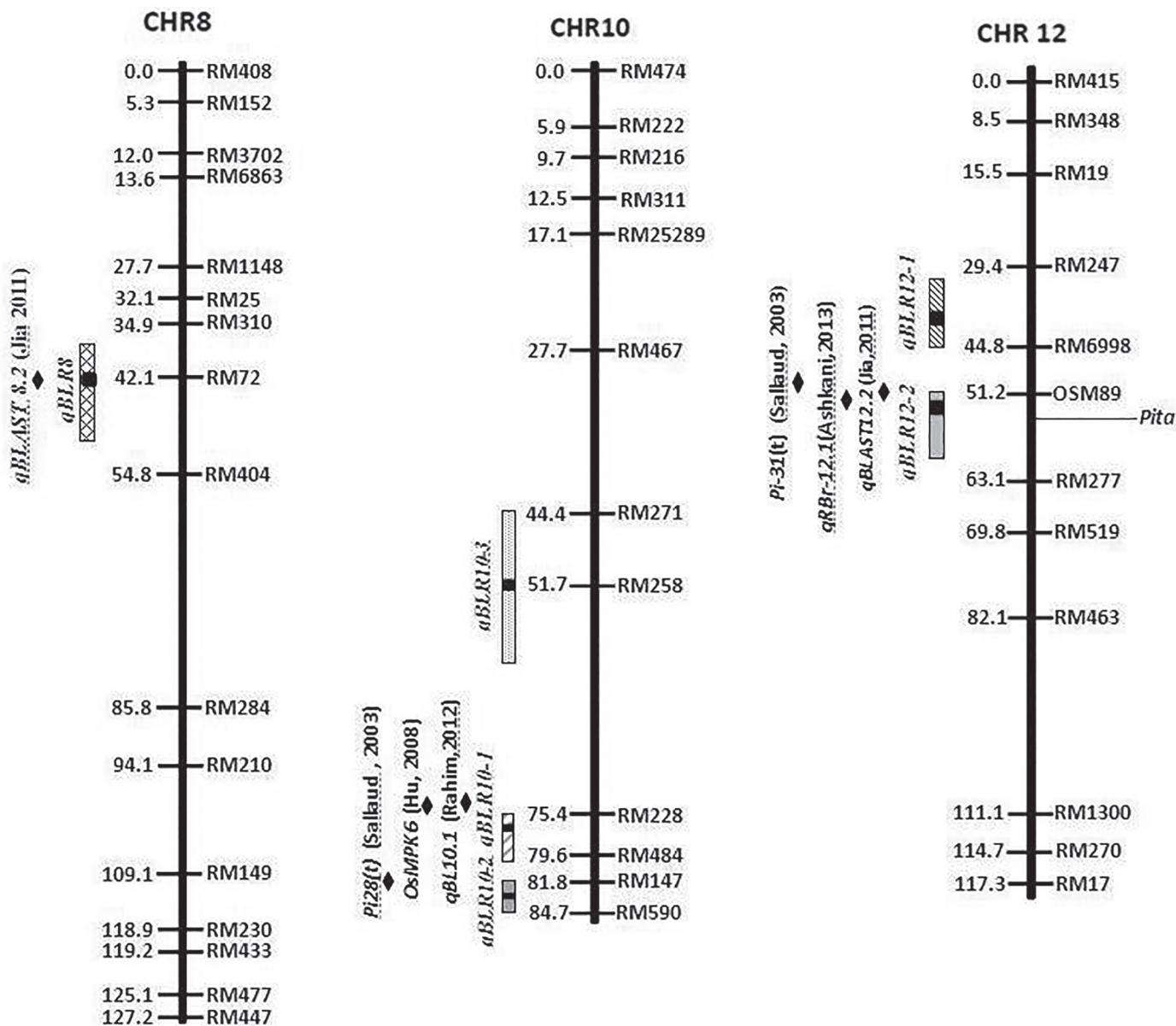


Figure 2. Chromosomal position of rice blast resistance quantitative trait loci (QTL) identified in this study from ‘Lemont’ × ‘Jasmine 85’ recombinant inbred lines and marked with a rectangle; previously identified resistance QTL or genes in the same regions were marked beside related QTL and marked with a diamond.

cultivars carrying *Pi-ta* under field conditions would provide more insight of this prediction.

Jasmine 85, carrying *qBLR8*, *qBLR12-1*, and *qBLR12-2*, has shown considerable resistance to *M. oryzae* in the southern United States. (Jia and Liu, 2011). *qBLR8* colocalized with QTL identified by different labs using different mapping populations under different environmental conditions (Zhu et al., 1993; Wang et al., 1994; Chen et al., 2003; Kongprakhon et al., 2010; Jia and Liu, 2011), suggesting that *qBLR8* may harbor a large gene family. Fine mapping and cloning of this QTL may reveal the genetic basis of this QTL. Lemont did not exhibit resistance to most races of *M. oryzae* under greenhouse conditions in this study (Table 1); however, Lemont did express partial resistance under field conditions and in a previous greenhouse study (Bollich et

al., 1985; Jia and Liu, 2011, Loan et al., 2003). Previous studies identified blast resistance QTL *qBLAST11*, *qBLAST3*, *qBLAST9.1*, and *qBLASTads-4* from Lemont (Tabien et al., 2002; Jia and Liu, 2011), and Loan et al. (2003) found an association with blast resistance from Lemont using Chi-square analysis. The *R* gene *Pi-lm2* has been reported at similar region on chromosome 11 from Lemont (Tabien et al., 2000). Lemont did not exhibit resistance to all 10 races; differential rating results for each RIL allowed us to identify three new resistance QTL, *qBLR10-1*, *qBLR10-2* and *qBLR10-3*, from Lemont. It is possible to identify QTL from lines that perform poorly for a particular trait that can greatly enhance the trait in a different background. Imai et al. (2013) validated that QTL from a low-yielding *O. rufipogon* Griff. that greatly enhance grain yield in *O. sativa*.

Numerous studies have identified blast QTL from susceptible lines under greenhouse conditions (Xu et al., 2004; Lopez-Gerena, 2006). The resistance QTL *qBLR10-1* and *qBLR10-2* colocalized with *qBL10.1* (Rahim et al., 2012) and *OsMPK6* (Hu et al., 2008), suggesting that they may be involved in similar blast-resistant responses. It is also possible that differences in resistance QTL detected in the greenhouse was a result of confounding environmental effects, including the presence of different blast races in the field. Further analysis of these resistance QTL and candidate genes will clarify this prediction. Additionally, *qBLR10-2* was mapped near *Pi28(t)* (Sallaud et al., 2003). Both *qBLR10-1* and *qBLR10-2* were identified using the race IC1 (ARB86), while the race IB17 (ARB154) identified *qBLR10-3*.

Three resistance QTL were newly identified from Lemont in this study. The fact that they were not identified in previous study, which used different field isolates (races) of blast (Jia and Liu, 2011), suggests the measured effectiveness of resistance QTL may depend on which blast isolates are used. Even though Lemont was susceptible to all test isolates in the greenhouse individually, Lemont showed partial resistance in a field plot study (Tabien et al., 2000). This suggested that weak resistance QTL may function in nature. These three newly identified QTL can be validated with different blast isolates (races) from the southern United States to verify their reliability before their application in MAS. However, the four resistance QTL validated in Lemont, *qBLAST11*, *qBLAST3*, *qBLAST9.1*, and *qBLASTads-4*, can be used as a source of partial resistance in combination with major blast *R* genes to effectively manage rice blast disease.

*Pi-ta* has been effective, in the southern United State at managing blast disease since its release in the 1990s. It is known that IA1 infects all eight international differentials (Atkins et al., 1967). The LJRIL carrying *qBLR12-2* was resistant to IA1 (ARB82 and ARB132), IB1 (ARB94, ARB151, and ARB157), IB17 (ARB154), IB49 (ZN61), and ID (ZN42) of *M. oryzae* (this study, Jia and Liu, 2011). Furthermore, Jasmine 85 does not contain *Pi-ta* (Wang et al., 2010); therefore, *qBLR12-2* from Jasmine 85 would be another useful blast *R* gene to use in the southern United States. Another QTL, *qBLR8*, was identified using IA1 (ARB132), IC1 (ARB8 and ARB86), and IC17 (ZN60) of *M. oryzae*, and most of isolates in IC1 were virulent to the resistant cultivar Katy carrying *Pi-ta*. Marker-assisted selection can be used to combine *qBLR8* with *Pi-ta*, and RILs found here that carry *qBLR8* (LJRIL 14, 15, 21, 28, 45, 127, and 215) could be useful donors of the *qBLR8* resistance allele.

Several studies suggest that resistance QTL were isolate (race) specific (Sallaud et al., 2003; Talukder et al., 2004; Lopez-Gerena, 2006). However, in this study, *qBLR12-2* was effective in preventing infections by at least six blast isolates, that is, *qBLR8* for at least three isolates. The resistance

QTL *qPi93-3* from the rice cultivar 93-11 was responsible for preventing infections by the isolates CHE86, ARB52, and ARB94 of *M. oryzae* (Yang et al., 2013). Moreover, the race identity has been traditionally determined with eight international differentials (Ling and Ou, 1969). Before the present study, a total of 26 isolates belonging to eight groups was determined to be the race IB1 (Xing et al., 2012). The three genetically different isolates of IB1, ARB94, ARB151, and ARB157, identified *qBLR12-1* and *qBLR12-1* with different percentages of contribution to the resistant phenotypes. In contrast, major blast *R* genes, such as *Pi-ta* and *Pi-9*, confer resistance to multiple isolates (races) that contain corresponding avirulence genes, respectively (Qu et al., 2006; Jia, 2009). It would be of significant importance to identify the corresponding pathogenicity factors of race-specific QTL to understand the molecular basis of QTL-mediated blast resistance.

In conclusion, we validated three previously identified resistance QTL to *M. oryzae*, three new resistance QTL, and 17 improved genetic stocks with user-friendly molecular markers possessing considerable resistance to *M. oryzae* (Table 1). Agronomic traits of these 17 rice genetic stocks are also available at <http://www.ars.usda.gov/Main/Docs.htm?docid=24954>. These findings are useful for fine mapping of these QTL and improving resistance to *M. oryzae* using MAS.

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## References

- Ashkani, S., M.Y. Rafii, H.A. Rahim, and M.A. Latif. 2013. Genetic dissection of rice blast resistance by QTL mapping approach using an  $F_3$  population. *Mol. Biol. Rep.* 40:2503–2515. doi:10.1007/s11033-012-2331-3
- Atkins, A.T., A.L. Robert, C.R. Adair, K. Goto, T. Kozaka, R. Yanagida, M. Yamada, and S. Matsumoto. 1967. An international set of rice varieties for differentiating races of *Pyricularia oryzae*. *Phytopathology* 57:297–301.
- Ballini, E., J.B. Morel, G. Droc, A. Price, B. Courtois, J.L. Notteghem, and D. Tharreau. 2008. A genome-wide meta-analysis of rice blast resistance genes and quantitative trait loci provides new insights into partial and complete resistance. *Mol. Plant Microbe Interact.* 21:859–868. doi:10.1094/MPMI-21-7-0859
- Bollich, C.N., B.D. Webb, M.A. Marchetti, and J.E. Scott. 1985. Registration of 'Lemont' rice. *Crop Sci.* 25:883–885. doi:10.2135/cropsci1985.0011183X002500050038x
- Bonman, J.M. 1992. Durable resistance to rice blast disease—environmental influences. *Euphytica* 63:115–123. doi:10.1007/BF00023917

- Bryan, G.T., K.S. Wu, L. Farrall, Y. Jia, H.P. Hershey, S.A. McAdams, K.N. Faulk, G.K. Donaldson, R. Tarchini, and B. Valent. 2000. A single amino acid difference distinguishes resistant and susceptible alleles of the rice blast resistance gene *Pi-ta*. *Plant Cell* 12:2033–2045. doi:10.1105/tpc.12.11.2033
- Chen, H., S. Wang, Y. Xing, C. Xu, P. Hayes, and Q. Zhang. 2003. Comparative analyses of genomic locations and race specificities of loci for quantitative resistance to *Pyricularia grisea* in rice and barley. *Proc. Natl. Acad. Sci. U.S.A.* 100:2544–2549. doi:10.1073/pnas.0437898100
- Dai, Y., Y. Jia, J. Correll, X. Wang, and Y. Wang. 2010. Diversification and evolution of the avirulence gene *AVR-Pita1* in field isolates of *Magnaporthe oryzae*. *Fungal Genet. Biol.* 47:973–980. doi:10.1016/j.fgb.2010.08.003
- Hu, K., D. Qiu, X. Shen, X. Li, and S. Wang. 2008. Isolation and manipulation of quantitative trait loci for disease resistance in rice using a candidate gene approach. *Mol. Plant* 1:786–793. doi:10.1093/mp/ssn039
- Imai, I., J.A. Kimball, B. Conway, K.M. Yeater, S.R. McCouch, and A. McClung. 2013. Validation of yield-enhancing quantitative trait loci from a low-yielding wild ancestor of rice. *Mol. Breed.* 32:101–120. doi:10.1007/s11032-013-9855-7
- Jia, Y. 2009. Artificial introgression of a large chromosome fragment around the rice blast resistance gene *Pi-ta* in backcross progeny and several elite rice cultivars. *Heredity* 103:355–356. doi:10.1038/hdy.2009.117
- Jia, Y., M.H. Jia, X. Wang, and G. Liu. 2012. *Indica* and *japonica* crosses resulting in linkage block and recombination suppression on rice chromosome 12. *PLoS ONE* 7:e43066. doi:10.1371/journal.pone.0043066
- Jia, Y., and G. Liu. 2011. Mapping quantitative trait loci for resistance to rice blast. *Phytopathology* 101:176–181. doi:10.1094/PHYTO-06-10-0151
- Jia, Y., G. Liu, M.H. Jia, and A. McClung. 2015. Registration of a rice gene mapping population of Lemont × Jasmine 85 Recombinant Inbred lines. *J. Plant Reg.* 9:128–132. doi:10.3198/jpr2013.03.0014crmp
- Kongprakhon, P., A. Cuesta-Marcos, P.M. Hayes, V. Hongtrakul, P. Sirit-hunya, T. Toojinda, and N. Sangduen. 2010. Four QTL in rice associated with broad spectrum resistance to blast isolates from rice and barley. *J. Phytopathol.* 158:125–131. doi:10.1111/j.1439-0434.2009.01587.x
- Lee, F.N., R.D. Cartwright, Y. Jia, and J.C. Correll. 2009. Field resistance expressed when the *Pi-ta* gene is compromised by *Magnaporthe oryzae*. In: G.L. Wang and B. Valent, editors, *Advances in genetics, genomics and control of rice blast disease*. Springer Science and Business Media LLC, New York. p. 281–289.
- Ling, K.C., and S.H. Ou. 1969. Standardization of the international race numbers of *Pyricularia oryzae*. *Phytopathology* 59:339–342.
- Loan, L.C., P.V. Du, and Z.K. Li. 2003. Identification of genes conferring resistance to some Philippine and Vietnamese races of blast. *Omonrice*. 11:49–62.
- Lopez-Gerena, J. 2006. Mapping QTL controlling durable resistance to rice blast in the cultivar Oryzica Llanos 5. Ph.D. diss., Kansas State University, Manhattan, KS.
- Mackill, D.J., and J.M. Bonman. 1992. Inheritance of blast resistance in near-isogenic lines of rice. *Phytopathology* 82:746–749. doi:10.1094/Phyto-82-746
- Marchetti, M.A., C.N. Bollich, B.D. Webb, B.R. Jackson, A.M. McClung, J.E. Scott, and H.H. Hung. 1998. Registration of 'Jasmine 85'. *Rice. Crop Sci.* 38:896. doi:10.2135/cropsci1998.0011183X003800030072x
- Qu, S.H., G.F. Liu, B. Zhou, M. Bellizzi, L.R. Zeng, L.Y. Dai, B. Han, and G.L. Wang. 2006. The broad-spectrum blast resistance gene *Pi9* encodes a nucleotide-binding site-leucine-rich repeat protein and is a member of a multigene family in rice. *Genetics* 172:1901–1914. doi:10.1534/genetics.105.044891
- Rahim, H.A., M.A.R. Bhuiyan, L.S. Lim, K.K. Sabu, A. Saad, M. Azhar, and R. Wickneswari. 2012. Identification of quantitative trait loci for blast resistance in BC2F3 and BC2F5 advanced backcross families of rice. *Genet. Mol. Res.* 11:3277–3289. doi:10.4238/2012.September.12.11
- RoyChowdhury, M., Y. Jia, A. Jackson, M.H. Jia, R. Fjellstrom, and R. Cartwright. 2011. Analysis of rice blast resistance gene *Pi-z* using pathogenicity assays and DNA markers. *Euphytica* 184:35–46. doi:10.1007/s10681-011-0481-3
- Sallaud, C., M. Lorieux, E. Roumen, D. Tharreau, R. Berruyer, P. Svestas-rani, O. Garsmeur, A. Ghesquiere, and J.L. Notteghem. 2003. Identification of five new blast resistance genes in the highly blast-resistant rice cultivar IR64 using a QTL mapping strategy. *Theor. Appl. Genet.* 106:794–803.
- Sambrook, J., and D.W. Russell. 2001. Estimation of cell number. In: D.W. Russell, editor, *Molecular cloning: A laboratory manual*, 3rd ed. J. Sambrook and Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York. p. A8.6–A8.7
- Silué, D., J.L. Nottenghem, and D. Tharreau. 1992. Evidence for a gene for-gene relationship in the *Oryza sativa*–*Magnaporthe grisea* pathosystem. *Phytopathology* 82:577–580. doi:10.1094/Phyto-82-577
- Tabien, R.E., Z. Li, A.H. Paterson, M.A. Marchetti, J.W. Stansel, and S.R.M. Pinson. 2002. Mapping QTLs for field resistance to the rice blast pathogen and evaluating their individual and combined utility in improved varieties. *Theor. Appl. Genet.* 105:313–324. doi:10.1007/s00122-002-0940-2
- Tabien, R.E., Z. Li, A.H. Paterson, M.A. Marchetti, J.W. Stansel, and S.R.M. Pinson. 2000. Mapping of four rice blast resistance genes from 'Lemont' and 'Teqing' and evaluation of their combinatorial effect for field resistance. *Theor. Appl. Genet.* 101:1215–1225. doi:10.1007/s001220051600
- Talukder, Z.I., D. Tharreau, and A.H. Price. 2004. Quantitative trait loci analysis suggests that partial resistance to rice blast is mostly determined by race-specific interactions. *New Phytol.* 162:197–209. doi:10.1111/j.1469-8137.2004.01010.x
- Wang, G.L., D.J. Mackill, J.M. Bonman, S.R. McCouch, M.C. Champoux, and R.J. Nelson. 1994. RFLP mapping of genes conferring complete and partial resistance to blast in a durably resistant rice cultivar. *Genetics* 136:1421–1434.
- Wang, S., C.J. Basten, and Z.B. Zeng. 2007. Windows QTL cartographer 2.5. Department of Statistics, North Carolina State University, Raleigh, North Carolina, U.S.A. (<http://statgen.ncsu.edu/qtlcart/WQTLCart.htm>)
- Wang, X., R. Fjellstrom, Y. Jia, W.G. Yan, M.H. Jia, B.E. Scheffler, D. Wu, Q. Shu, and A. McClung. 2010. Characterization of *Pi-ta* blast resistance gene in an international rice core collection. *Plant Breed.* 129:491–501.
- Xing, J., Y. Jia, J.C. Correll, F.N. Lee, R. Cartwright, M. Cao, and L. Yuan. 2012. Analysis of genetic and molecular identity among field isolates of the rice blast fungus with an international differential system, Rep-PCR, and DNA sequencing. *Plant Dis.* 97:491–495. doi:10.1094/PDIS-04-12-0344-RE
- Xu, J., J. Wang, Z. Ling, and L. Zhu. 2004. Analysis of rice blast resistance genes by QTL mapping. *Chin. Sci. Bull.* 49:337–342. doi:10.1007/BF02900315
- Yang, H., M.H. Jia, Y. Jia, J. Xing, R.C. Venu, M. Bellizzi, L. Yuan, Z. Wang, C. Sun, and G. Wang. 2013. Molecular mapping of four blast resistance genes using recombinant inbred lines of 93-11 and Nipponbare. *J. Plant Biol.* 56:91–97. doi:10.1007/s12374-012-0462-7
- Yu, Z.H. 1991. Molecular mapping of rice (*Oryza sativa* L.) gene via linkage to restriction fragment length polymorphism (RFLP) markers. Ph.D. diss. Cornell University, Ithaca, New York.
- Zheng, K.L., J.Y. Zhuang, J. Lu, H.R. Qian, and H.X. Lin. 1996. Identification of DNA markers tightly linked to blast resistance genes in rice. In: G.S. Khush, editor, *Rice genetics III*. IRRRI, Manila, the Philippines. p. 565–569.
- Zhou, E., Y. Jia, J. Correll, and F.N. Lee. 2007. Instability of the *Magnaporthe oryzae* avirulence gene *AVR-Pita* alters virulence. *Fungal Genet. Biol.* 44:1024–1103. doi:10.1016/j.fgb.2007.02.003
- Zhu, L., Y. Chen, Y. Xu, J. Xu, H. Cai, and Z. Ling. 1993. Construction of a molecular map of rice and gene mapping using a double-haploid population of a cross between *Indica* and *Japonica* varieties. *Rice Genet. Newsl.* 10:132–135.