Molecular Mapping of the Blast Resistance Genes *Pi2-1* and *Pi51(t)* in the Durably Resistant Rice ‘Tianjingyeshengdao’

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ABSTRACT

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‘Tianjingyeshengdao’ (TY) is a rice cultivar with durable resistance to populations of *Magnaporthe oryzae* (the causal agent of blast) in China. To understand the genetic basis of its resistance to blast, we developed a population of recombinant inbred lines from a cross between TY and the highly susceptible ‘CO39’ for gene mapping analysis. In total, 22 quantitative trait loci (QTLs) controlling rice blast resistance were identified on chromosomes 1, 3, 4, 5, 6, 9, 11, and 12 from the evaluation

of four disease parameters in both greenhouse and blast nursery conditions. Among these QTLs, 19 were contributed by TY and three by CO39. Two QTL clusters on chromosome 6 and 12 were named *Pi2-1* and *Pi51(t)*, respectively. *Pi2-1* was detected under both growth chamber and natural blast nursery conditions, and explained 31.24 to 59.73% of the phenotypic variation. *Pi51(t)* was only detected in the natural blast nursery and explained 3.67 to 10.37% of the phenotypic variation. Our results demonstrate that the durable resistance in TY is controlled by two major and seven minor genes. Identification of the markers linked to both *Pi2-1* and *Pi51(t)* in this study should be useful for marker-aided selection in rice breeding programs as well as for molecular cloning of the identified resistance genes.

Rice, one of the most important food crops worldwide, is continuously challenged by the devastating fungal disease rice blast (causal agent *Magnaporthe oryzae*) (21,39). Blast disease occurs in almost all rice production regions and commonly reduces yield by 10 to 30% (36). In the last three decades, rice breeders incorporated many resistance (*R*) genes identified from diverse resources into rice cultivars. Use of these *R* genes has been effective in reducing yield losses in many rice-growing areas. However, most of the rice cultivars are short lived due to the high variation in *M. oryzae* populations in a conducive environment (24). Therefore, discovery and use of novel *R* genes and development of broad-spectrum resistant varieties by pyramiding different types of *R* genes have become urgent goals in breeding for rice blast resistance.

Rice blast resistance can be classified into two main types: complete resistance and partial resistance. Complete resistance is race specific and controlled by a single dominant or recessive *R* gene that can be recognized by a cognate avirulence (*Avr*) gene in the pathogen (32). During the last two decades, more than 85 major *R* genes have been mapped on all rice chromosomes, except for chromosome 3 (2,21). Interestingly, a large group of *R* genes are clustered in several genomic regions such as chromosome 6, 11, and 12. Currently, 18 *R* genes have been cloned: *Pi37*(20), *Pit* (10), *Pi-sh* (37), *Pib* (41), *Pi9* (32), *Pi2* (51), *Piz-t* (51), *Pid2* (5), *Pid3* (33), *Pi25* (4), *Pi36* (22), *Pi5* (18), *Pikm* (1), *Pi54* (34), *Pia* (29), *Pik-p* (46), *Pita* (3), and *Pik* (50). All of

these genes belong to the nucleotide-binding site (NBS) leucine-rich repeat (LRR) gene family, except for *Pid2*, which encodes a serine/threonine-kinase membrane-spanning protein (5). Although cloning of these *R* genes has advanced our understanding of the molecular basis of blast resistance in rice, few of them have been tested for their effectiveness against a diverse blast population of a relative long time period in the rice-growing countries. For example, both *Pi2* and *Pi9* confer a high level of resistance to many blast isolates collected in different countries (13,32,51). Because these two genes are being widely used in rice production, new virulent isolates that can overcome the resistance have been found in some regions in the last few years. Thus, it is important to search for new *R* genes in local cultivars to replace these genes in rice production.

Partial resistance is usually considered to be non-race specific and durable, and is controlled by quantitative trait loci (QTLs). Since Wang et al. (40) mapped the first set of blast QTLs using a recombinant inbred line (RIL) population derived from a cross between the durably resistant upland rice ‘Moroberekan’ and the susceptible ‘CO39’, 362 QTLs have been identified on all 12 chromosomes in different mapping populations under greenhouse and field conditions. A meta-analysis involving the exclusion of the redundant or overlapping QTLs identified 49 metaQTL responsible for partial resistance (2). Several of these QTLs have been finely mapped, and these include *Pi21* (8), *Pb1* (11), *Pi34* (48), *Pif* (47), *Pikur1* (35), *Pikur2* (9), *Pi-se1* (42), *Pi35* (27), *Pikahei-1(t)* (26), *qBR6.1* (23), and *qBR9.1* (23). Among them, the QTL genes *Pi21* and *Pb1* have been cloned. *Pi21* is a recessive gene and encodes a novel proline-rich protein with a putative heavy-metal-binding domain and protein–protein interaction motifs, suggesting that *pi21*-mediated partial resistance might represent a novel defense mechanism (8). The panicle blast QTL

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gene *Pb1* also encodes an NBS-LRR protein, raising the possibility that similar resistance mechanisms are mediated by major and minor blast *R* genes (11).

‘Tianjingyeshengdao’ (TY) is a local *indica* cultivar that confers high levels of resistance to 92.7% of the blast isolates collected from Jilin, Zhejiang, Hunan, Fujian, Guangdong, and Hainan Provinces in China (30). A previous study characterized the resistance in TY to *M. oryzae* isolates collected in different blast nurseries but the gene location was not determined (12). To understand the genetic basis of the broad-spectrum and durable resistance of TY, in the current study we used 363 RILs derived from a cross between TY and the susceptible CO39 to identify QTLs responsible for blast resistance under both growth chamber and blast nursery conditions. Our results show that two major genes, designated *Pi2-1* and *Pi51(t)*, and seven minor QTLs are responsible for the high level of blast resistance in TY. *Pi2-1* is a new allelic member of the broad-spectrum blast resistance locus

TABLE 1. Response of rice ‘Tianjingyeshengdao’ (TY) and ‘CO39’ to 49 blast isolates^a

Isolate	Origin	TY	CO39
RB1	China (Guangdong)	R	R
RB3	China (Guangdong)	R	R
RB4	China (Guangdong)	R	S
RB5	China (Guangdong)	R	S
RB6	China (Guangdong)	R	S
RB7	China (Guangdong)	R	MS
RB9	China (Guangdong)	R	S
RB10	China (Guangdong)	R	R
RB11	Japan	R	R
RB12	China	R	S
RB13	China	R	S
RB14	China	R	MS
RB15	China	R	S
RB16	China (Fujian)	R	S
RB17	China (Fujian)	R	MS
RB18	China (Fujian)	R	MS
RB19	China (Fujian)	R	S
RB20	China (Fujian)	R	S
RB21	China	R	MS
CHL438	China (Hunan)	R	S
CHL440	China (Hunan)	R	S
CHL471	China (Hunan)	R	S
CHL473	China (Hunan)	R	S
CHL506	China (Fujian)	R	S
CHL528	China (Fujian)	R	S
CHL645	China (Guizhou)	MS	MR
CHL1743	China (Guangdong)	R	S
CHL1752	China (Guangdong)	R	S
CHL1962	China (Guangdong)	R	MR
C1-3-1	China (Sichuan)	R	MS
C8-61-3	China (Sichuan)	R	MS
X2007A-7	China (Hunan)	R	S
ROR1	Korea	R	MR
236-2	China (Yanxi)	R	S
195-2-2	China (Taojiang)	R	MS
236-1	China (Yanxi)	R	S
CHNOS60-2-3	China	R	S
110-2	China (Qidong)	R	S
318-2	China (Xinning)	R	S
87-4	China (Hanshou)	R	S
193-1-1	China (Taojiang)	R	S
220-1-1	China (Yanxi)	R	S
107-2	China (Qidong)	R	S
95097AZC13	China	R	S
ES6	Spain	R	S
KJ201	Korea	R	S
ROR1	Korea	R	S
IC-17	Philippines	R	S
P06-6	Philippines	R	S

^a Lesion score: R indicates resistant, MR indicates moderately resistant, MS indicates moderately susceptible, and S indicates susceptible. Resistance evaluations are based on the 0 to 9 scale of the standard evaluation system and three replications.

Pi2/9 on chromosome 6, and *Pi51(t)* is a novel gene on chromosome 12 located between the simple sequence repeat (SSR) markers RM5364 and RM27990.

MATERIALS AND METHODS

RIL population development. In total, 363 F₈ RILs were obtained from a cross of the resistant *indica* TY and the susceptible CO39 using the single-seed descent method. All of the lines were used for both blast disease evaluations and genotyping experiments.

Disease evaluations in the growth chamber and blast nursery. For growth chamber inoculation experiments, ≈20 seeds of each RIL were germinated in a petri dish and sowed in potting soil in plastic trays. Three replicates of all RILs and the two parents were included in the inoculations. Blast isolates 318-2 and 110-2, collected from Hunan Province, China, were used for evaluating blast resistance in growth chambers following the method described by Correa-Victoria and Zeigler (6). Briefly, 18- to 20-day-old seedlings with three or four fully expanded leaves were sprayed with a spore suspension (1 × 10⁵ conidia/ml in 0.1% Tween-20). The inoculated plants were kept in the dark, with high humidity (>90%). After 24 h, they were moved to a growth chamber (12 h of light, 12 h of darkness, ≈80 to 90% humidity) for an additional 6 days. Ten randomly selected plants of each line were then scored with the 0 to 9 blast scoring system (14). Plants with scores between 0 and 3 were classified as resistant and those with scores between 4 and 9 were classified as susceptible.

The blast nursery evaluations were performed in April and August 2010 in Taojiang Country (28°22'N and 112°03'E), Yiyang, China, as previously described by Huang et al. (13). This nursery contains 11 major blast races including ZC9, ZC11, ZE3, ZB29, ZG1, ZB25, ZB31, ZB13, ZC7, ZA9, and ZF19 (13, 19,31). The RILs and two parents were arranged in a randomized complete block design with four replications. Two highly susceptible varieties, ‘Xianggaizao7’ and ‘Xiangwanxian11’, were planted in every 10 rows as inducers of blast infection. At ≈40 days after sowing, disease severity (DS) was scored using the 0 to 9 scoring system (14). Also measured were the percentage of leaf area that was diseased (DLA), lesion number (LN), lesion size (LS), and leaf area (LA) of each line. Three randomly selected plants of each line were scored for all the parameters. DLA was estimated following the method of Notteghem et al. (28). LS was calculated as (LA × DLA%)/LN. LA was measured with a portable area meter (ADC AM300; BioScientific Ltd., Herts, UK).

DNA isolation and genotyping. Total DNA was isolated from leaf tissues using a modified cetyltrimethylammonium bromide method (25). In total, 1,286 SSR and 66 single-feature polymorphism (SFP) markers were used to survey the polymorphism between TY and CO39. The SSR marker assay was conducted as described by Wu and Tanksley (44). Polymerase chain reaction (PCR) amplification was performed using a PTC-200 thermal cycler (MJ Research Inc., Waltham, MA) with a 10-μl reaction volume containing 37.5 ng of DNA, 1× PCR buffer, 0.025 units of Taq DNA polymerase (TaKaRa Dalian Co., Dalian, China), 0.2 mmol of dNTPs, and 0.8 pmol of forward and reverse primers. The PCR program was as follows: 1 cycle at 94°C for 5 min; followed by 35 cycles at 94°C for 30 s, 55°C for 30 s, and 72°C for 60 s; and a final extension at 72°C for 7 min. DNA bands were visualized via silver staining on an 8% polyacrylamide gel.

Linkage map construction and QTL mapping analysis. The genetic linkage map was constructed using the program Mapmaker 3.0 based on the genotyping data of the 363 F₇ RILs (17). The Kosambi function was used for calculation of map distance. Windows QTL Cartographer 2.5 was used for QTL identification using the composite interval mapping (CIM) strategy, and for the

estimation of percentage of total phenotypic variation explained by each target locus and their additive effects (49). Tests were performed at every 0.5-centimorgan (cM) interval on the linkage map, and the forward/backward stepwise regression (Model 6) was used for the selection of cofactors. The significance threshold of CIM was determined by 300 permutation tests, and 2.0 was the threshold value of the logarithm of likelihood ratio (LOD) in this study. The approximate percentage of the phenotypic variation explained by a QTL was estimated with CIM.

RESULTS

Evaluation of the resistance spectrum of TY. To confirm the resistance spectrum of TY, we inoculated the cultivar with 49 blast isolates collected from five countries, including China, Spain, Korea, Philippines, and Japan. The inoculations showed that TY was highly resistant to 48 isolates (97.96%) and was only moderately susceptible to isolate CHL645 (Table 1). In contrast, the control CO39 was highly susceptible to most of the isolates and was resistant to only 14.29% of tested isolates (Table 1).

Genetic polymorphism and linkage map construction. In total, 1,352 markers (1,286 SSRs and 66 SFPs) were selected to analyze the polymorphism between TY and CO39. The survey showed that 187 of the 1,352 markers (13.83%) were polymorphic between the two parents. Among them, 138 co-dominant markers were selected for genotyping of the 363 RILs, and the segregation data were used for the linkage map construction. Finally, a linkage map with a coverage of 1,832 cM in 12 groups was constructed, with an average interval of 13.28 cM between adjacent markers.

Identification of QTL to individual blast isolates in growth chambers. To identify the resistance QTLs in TY to single isolates, we inoculated the 363 F₈ RILs in growth chambers with isolates 318-2 and 110-2. DS in the growth chamber (DS_G) was measured using the 0 to 9 scoring system (14). Using the CIM method, we identified a total of eight QTLs for these two isolates (Table 2; Fig. 1). Four QTLs conferring resistance to isolate 318-2 are individually located on chromosomes 4, 5, 6, and 12 (Table 2;

Figs. 1 and 2). The QTL with the largest contribution, named *qDS_G6.1*, was mapped in the interval between markers AP4791 and AP4007 and was located near the centromere on chromosome 6 (Figs. 1 and 2), which was the same location for the blast resistance gene cluster *Pi2/9* locus (32,51). This locus explained 50.64% of the phenotypic variation, with the highest LOD value of 41.25, which suggested that *qDS_G6.1* is a major *R* gene. The other two QTLs from TY, *qDS_G5.1* and *qDS_G12.1*, were mapped in the interval between RM334 and RM1054 on chromosome 5 and between RM27990 and RM511 on chromosome 12, respectively. These two loci explained 2.79% (LOD = 2.6) and 5.44% (LOD = 3.25) of the phenotypic variation, respectively. The fourth QTL, *qDS_G4.1*, which was located between markers RM471 and SFP4-3 on chromosome 4, originated from CO39 and explained 1.69% of the phenotypic variation (LOD = 2.11).

The other four QTLs conferring resistance to isolate 110-2 are located on chromosomes 3, 6, 11, and 12 (Table 2; Fig. 1). Whereas *qDS_G6.1* was the major locus conferring resistance to isolate 318-2, QTL *qDS_G6.2* was also the major locus in TY conferring resistance to isolate 110-2 (Table 2), and it explained 54.59% of phenotypic variation (LOD = 49.43). The phenotypic contributions of other three QTLs (i.e., *qDS_G3.2*, *qDS_G11.2*, and *qDS_G12.2*) were 2.69% (LOD = 2.2), 2.49% (LOD = 2.53), and 2.28% (LOD = 3.29), respectively. All three of the minor QTLs were from TY.

Identification of loci in TY conferring resistance to the blast population in the blast nursery. The following four parameters were investigated for all RILs and two parents in the blast nursery: DS in the field (DS_F), DLA%, LN, and LS. A continuous distribution of the DS_F frequency was observed (Fig. 3). The CIM mapping showed that, in total, 14 QTLs for the four parameters were identified in response to the field population of *M. oryzae* (Table 2). Among them, two QTL clusters had large effects on all four parameters. One QTL cluster (*qDS_F6*, *qLN_F6*, *qLS_F6*, and *qDLA_F6*) was located on chromosome 6 in the interval between AP4791 and AP4007, and the other (*qDS_F12*, *qLN_F12*, *qLS_F12*, and *qDLA_F12*) was located on chromosome 12 in the interval between RM5364 and RM27990. Interestingly, these two major loci were

TABLE 2. Quantitative trait loci (QTLs) for blast resistance detected in the ‘Tianjingyeshengdao’ (TY)/‘CO39’ F₈ recombinant inbred line population

Trait ^a	QTL ^b	Chromosome	Makers bordering QTL	Genetic distance (cM) ^c	LOD ^d	Variance explained (%) ^e	DPE ^f
DS _G 318-2	<i>qDS_G4.1</i>	4	RM471-SFP4-3	0 (SFP4-3)	2.11	1.69	C
DS _G 318-2	<i>qDS_G5.1</i>	5	RM334-RM1054	5 (RM334)	2.6	2.79	T
DS _G 318-2	<i>qDS_G6.1</i>	6	AP4791-AP4007	1.5 (AP4791)	41.25	50.64	T
DS _G 318-2	<i>qDS_G12.1</i>	12	RM27990-RM511	10 (RM27990)	3.25	5.44	T
DS _G 110-2	<i>qDS_G3.2</i>	3	RM5626-RM3525	5.7 (RM3525)	2.2	2.69	T
DS _G 110-2	<i>qDS_G6.2</i>	6	AP4791-AP4007	4 (AP4791)	49.43	54.59	T
DS _G 110-2	<i>qDS_G11.2</i>	11	RM5599-RM3133	5.9 (RM3133)	2.53	2.49	T
DS _G 110-2	<i>qDS_G12.2</i>	12	RM5364-RM27990	0 (RM27990)	3.29	2.28	T
DS _F	<i>qDS_F1</i>	1	RM5536-RM8136	0 (RM5536)	2.07	0.99	T
DS _F	<i>qDS_F5</i>	5	RM267-RM5140	6.5 (RM267)	2.59	2.29	T
DS _F	<i>qDS_F6</i>	6	AP4791-AP4007	4 (AP4791)	72.08	59.73	T
DS _F	<i>qDS_F9</i>	9	RM7212-RM3769	9.5 (RM3769)	2.85	2.99	T
DS _F	<i>qDS_F12</i>	12	RM1337-RM27990	0.3 (RM27990)	17.98	9.7	T
DLA%	<i>qDLA4</i>	4	SFP4-3-RM3866	0.1 (RM3866)	2.1	1.56	C
DLA%	<i>qDLA5</i>	5	RM164-RM5948	0.4 (RM5948)	2.05	1.46	T
DLA%	<i>qDLA6</i>	6	AP4791-AP4007	0.5 (AP4791)	36.76	32.93	T
DLA%	<i>qDLA12</i>	12	RM1337-RM27990	0.3 (RM27990)	13.81	10.37	T
LN	<i>qLN6</i>	6	AP4791-AP4007	1.5 (AP4791)	40.74	38.31	T
LN	<i>qLN12</i>	12	RM1337-RM27990	0.3 (RM27990)	10.42	7.35	T
LS	<i>qLS4</i>	4	RM3866-RM2636	0 (RM3866)	2.37	2.35	C
LS	<i>qLS6</i>	6	AP4791-AP4007	1 (AP4791)	28.17	31.24	T
LS	<i>qLS12</i>	12	RM5364-RM1337	0.01 (RM1337)	4.14	3.67	T

^a DS_G, disease severity in the growth chamber to isolates 318-2 (DS_G318-2) and 110-2 (DS_G110-2); DS_F, disease severity in the nursery; LN, lesion number; DLA%, percentage of leaf area that is diseased; LS, lesion size.

^b QTLs are named by abbreviations plus chromosome number.

^c Genetic distance of nearest marker to QTL in centimorgans (cM).

^d Logarithm of likelihood ratio.

^e Percentage of phenotypic variation explained by each QTL.

^f Direction of phenotypic effect: T and C indicate that the TY and CO39 alleles, respectively, increase the values.

Fig. 1. Linkage map constructed from the ‘Tianjinyeshengdao’ TY/CO39’ recombinant inbred line population and the location of blast resistance genes and quantitative trait loci. DS_G, disease severity in the growth chamber to isolates 318-2 (DS_{G318-2}) and 110-2 (DS_{G110-2}); DS_F, disease severity in the blast nursery; DLA%, percentage of diseased leaf area; LN, lesion number; and LS, lesion size.

Fig. 2. Map position of the *Pi2-1* and *Pi51(t)* genes that confer resistance to *Magnaporthe oryzae* isolates 318-2 and 110-2 in the growth chamber and to the *M. oryzae* population in the blast nursery. Four disease parameters were used: DS_G, disease severity in the growth chamber to isolates 318-2 (DS_{G318-2}) and 110-2 (DS_{G110-2}); DS_F, disease severity in the blast nursery; DLA%, percentage of diseased leaf area; LN, lesion number; and LS, lesion size.

also detected in plants that were separately inoculated with the two isolates in the growth chamber (Fig. 3). The contribution of the QTL on chromosome 6 was 59.73% for DS_F (LOD = 72.08), 38.31% for LN (LOD = 40.74), 32.93% for DLA% (LOD = 36.76), and 31.24% for LS (LOD = 28.17). The QTL on chromosome 12 contributed 9.7% phenotypic variance to DS_F (LOD = 17.98), 7.35% to LN (LOD = 10.42), 10.37% to DLA% (LOD = 13.81), and 3.67% to LS (LOD = 4.14) (Table 2; Fig. 2). Except the eight QTLs in the two clusters, six minor QTLs were also detected: two on chromosome 4, two on chromosome 5, and one each on chromosomes 1 and 9. The contribution of these minor QTLs to phenotypic variation was 0.99 to 2.99%, and the LOD scores were 2.05 to 2.85. Similar to the result obtained in the growth chamber, the QTL on chromosome 4 that was detected in the nursery originated from CO39.

Based on the results from the growth chamber and the blast nursery, we identified two new major QTLs that are located on chromosomes 6 and 12 in TY (Figs. 2 and 3). We designated the QTL located on chromosome 6 between the markers AP4791 and AP4007 as *Pi2-1* as new allelic gene to the *Pi2* gene, and the QTL located on chromosome 12 between the markers RM5364 and RM27990 as *Pi51(t)*.

Resistance spectrum evaluation of the *Pi2-1* and *Pi51(t)* genes. To further confirm the resistance spectrum of *Pi2-1* and *Pi51(t)*, for each gene we selected six RILs that carried the markers linked to only one of the loci. These 12 RILs were inoculated with 11 isolates and then evaluated for disease. The inoculations revealed that *Pi2-1* was resistant to all the isolates whereas *Pi51(t)* was resistant to only approximately half of the isolates (Table 3).

Fig. 3. Frequency distribution of disease reactions of the 'Tianjingyeshengdao' TY/CO39² F₈ recombinant inbred lines in the blast nursery.

DISCUSSION

Major resistance genes are being widely used in rice breeding programs because of their effectiveness against blast isolates and their ease of transfer to susceptible varieties. However, frequent loss of major gene resistance due to virulence shifts in the field populations has motivated rice breeders to search for durably resistant cultivars. Although many durably resistant cultivars have been identified in the last two decades, the molecular mechanism underlying the durable resistance is still unclear (13,40). In this study, we used molecular markers to analyze the durable resistance in Chinese TY. TY has been used in rice breeding in China for more than 13 years as a blast resistance donor because its resistance against blast in the field is substantial and stable (12,30). We confirmed that TY is highly resistant to 48 blast isolates from five countries. We developed a set of 363 F₈ RILs from a cross between TY and CO39 for identification of blast QTLs in the growth chamber and in a natural rice blast nursery. We identified 22 QTLs that were responsible for the resistance to two isolates in the growth chambers and to the field population in the blast nursery. These QTLs are distributed on chromosomes 1, 3, 4, 5, 6, 9, 11, and 12 (Table 2; Figs. 2 and 3). Among the 22 QTLs, 19 are from TY and 3 are from CO39. Two major *R* genes located on chromosomes 6 and 12 were detected in both the growth chamber and the blast nursery. However, the minor QTLs were detected only after inoculations with specific isolates or under different infection conditions, indicating that these QTLs may be isolate or environment specific. A previous study reported that eight QTLs were effective against one isolate, seven QTLs were effective against two isolates, and only three QTLs were predicted to be effective against all three isolates (38). Therefore, further characterization of these QTLs will focus on their resistance specificity in different isolates or different field blast populations once near-isogenic lines of these minor QTLs are identified.

The QTL that conferred the highest blast resistance was named *Pi2-1* and was detected in both the growth chamber experiment and the blast nursery experiment. In contrast, the second major QTL, which we named *Pi51(t)*, explained ≈10% to the variation in disease severity in the nursery but was not detected in the growth chamber experiment, suggesting that *Pi51(t)* is a QTL for the field blast population. It is noteworthy to mention that *Pi2-1* is mapped between the markers AP4791 and AP4007 on chromosome 6, where the broad-spectrum blast resistance cluster *Pi2/9* locus is located. At the *Pi2/9* locus, at least 10 alleles have been mapped, including *Pi9* (32), *Pi2* (51), *Piz-t* (51), *Piz* (45), *Pigm*

TABLE 3. Reactions of the *Pi2-1* and *Pi51(t)*-containing recombinant inbred lines (RILs) to *Magnaporthe oryzae* isolates

QTL ^a	Line	Marker genotype ^b		Blast ^c										
		AP4791	RM27990	CHL477	RB5	X2007A-1	87-4	X2007A-2	CHL471	CHL446	CHL473	P06-6	IC-17	CHL438
<i>qtl6</i>	RIL33	T	C	HR(0)	R(2)	R(2)	R(2)	MR(3)	MR(3)	MR(2)	R(2)	R(2)	MR(3)	R(2)
<i>qtl6</i>	RIL38	T	C	HR(0)	HR(0)	R(1)	R(1)	R(1)	HR(0)	R(2)	R(2)	R(1)	R(2)	R(2)
<i>qtl6</i>	RIL42	T	C	R(2)	R(1)	MR(3)	MR(3)	R(1)	R(1)	...	R(2)	HR(0)	R(1)	R(2)
<i>qtl6</i>	RIL51	T	C	R(1)	R(1)	MS(4)	R(2)	MR(3)	MR(3)	MR(3)	R(2)	R(1)	MR(3)	R(2)
<i>qtl6</i>	RIL70	T	C	R(1)	HR(0)	R(1)	R(2)	R(1)	MR(3)	MR(2)	R(1)	R(2)	MR(3)	R(1)
<i>qtl6</i>	RIL104	T	C	HR(0)	HR(0)	MR(3)	MR(3)	R(1)	R(1)	HR(0)	HR(0)	R(2)	R(1)	R(1)
<i>qtl12</i>	RIL74	C	T	R(2)	R(1)	R(2)	R(2)	MR(3)	MR(3)	R(2)	R(2)	R(2)	MR(3)	R(2)
<i>qtl12</i>	RIL101	C	T	R(1)	MR(3)	S(6)	S(5)	MS(4)	MS(4)	S(5)	R(2)	R(1)	MR(3)	MR(3)
<i>qtl12</i>	RIL118	C	T	HR(0)	HR(0)	R(1)	R(2)	MR(3)	MR(3)	MR(3)	R(2)	R(2)	R(1)	R(2)
<i>qtl12</i>	RIL220	C	T	R(2)	HR(0)	S(5)	MR(3)	MR(3)	MR(3)	R(2)	R(2)	R(1)	R(2)	MR(3)
<i>qtl12</i>	RIL221	C	T	HR(0)	R(1)	S(5)	S(6)	MS(4)	R(1)	HR(0)	HR(0)	R(1)	MS(4)	MR(3)
<i>qtl12</i>	RIL369	C	T	R(1)	R(2)	MS(4)	S(5)	S(5)	MS(4)	R(2)	R(1)	R(1)	MS(4)	R(2)
	T	...	...	HR(0)	HR(0)	R(1)	R(1)	R(1)	R(1)	HR(0)	HR(0)	HR(0)	R(2)	R(1)
	C	...	...	S(6)	S(5)	S(6)	S(5)	S(6)	S(6)	S(7)	S(5)	S(6)	MS(4)	MS(4)

^a Quantitative trait locus.

^b Genotype of the nearest marker linked to the resistance gene. AP4791 and RM27990 are the nearest markers linked to *Pi2-1* and *Pi51(t)*, respectively. T and C denote the 'Tianjingyeshengdao' and 'CO39' genotypes, respectively, at the linked markers.

^c Blast was rated on a scale of 0 to 9 (from low to high disease), and scores are indicated in parentheses. HR indicates highly resistant, MS indicates medium resistant, and S indicates susceptible.

(7), *Pi26* (43), *Pi40* (15), *Pi50* (52), *qBR6* (23), and *Pi-Jef* (unpublished data). Our allelism test indicated that *Pi2-1* is one of the alleles at the *Pi2/9* locus (unpublished data). Unlike *Pi2-1*, *Pi51(t)* is mapped in the interval between RM5364 and RM27990, where no known resistance gene is located. The *Pi48* gene linked to the SSR marker RM7102 is 2.3 cM away from the *Pi51(t)* gene (13). The cloned NBS-LRR gene *Pita* is also located 4.7 cM away of the *Pi51(t)* (3). In addition, *Pi51(t)* is different from *qBLAST12.1* and *qBLAST12.2* that are located between RM247 and RM277 (16). Therefore, it is likely that *Pi51(t)* is a novel blast resistance gene on chromosome 12.

Durable resistance to rice blast has been a major goal in rice breeding programs in the last few decades. With recent identification of major genes and QTLs in durably resistant cultivars, molecular-aided selection of durable resistance has become feasible in rice breeding. In the current study, we found that the resistance of TY is controlled by two major genes and seven minor QTLs. Our laboratory is now fine-mapping the two major genes and confirming the mapping location of the QTLs in back-cross populations with a range of single isolates and in different blast nursery sites. Once markers tightly linked to major genes and QTLs are developed, we will transfer these genes into high-yielding but highly susceptible cultivars using marker-aided methods.

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