Molecular Mapping of the New Blast Resistance Genes *Pi47* and *Pi48* in the Durably Resistant Local Rice Cultivar Xiangzi 3150

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ABSTRACT

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The *indica* rice cultivar Xiangzi 3150 (XZ3150) confers a high level of resistance to 95% of the isolates of *Magnaporthe oryzae* (the agent of rice blast disease) collected in Hunan Province, China. To identify the resistance (R) gene(s) controlling the high level of resistance in this cultivar, we developed 286 F₉ recombinant inbred lines (RILs) from a cross between XZ3150 and the highly susceptible cultivar CO39. Inoculation of the RILs and an F₂ population from a cross between the two cultivars with the avirulent isolate 193-1-1 in the growth chamber indicated the presence of two dominant R genes in XZ3150. A linkage map with 134

polymorphic simple sequence repeat and single feature polymorphism markers was constructed with the genotype data of the 286 RILs. Composite interval mapping (CIM) using the results of 193-1-1 inoculation showed that two major R genes, designated *Pi47* and *Pi48*, were located between RM206 and RM224 on chromosome 11, and between RM5364 and RM7102 on chromosome 12, respectively. Interestingly, the CIM analysis of the four resistant components of the RILs to the field blast population revealed that *Pi47* and *Pi48* were also the major genetic factors responsible for the field resistance in XZ3150. The DNA markers linked to the new R genes identified in this study should be useful for further fine mapping, gene cloning, and marker-aided breeding of blast-resistant rice cultivars.

Additional keywords: *Oryza sativa*.

Rice blast, caused by the fungus *Magnaporthe oryzae*, is the most severe disease of rice (*Oryza sativa* L.) in many countries. This disease causes about 10 to 20% yield loss in regular seasons and as high as 100% yield loss in years with blast epidemics (5). Although the breeding of resistant rice varieties is an economical, effective, and environmentally responsible way to control the disease, newly released varieties planted in large areas frequently lose their resistance within a few years because of shifts in strains of the *M. oryzae* population. Another problem is that most of the major rice blast resistance (R) genes thus far identified confer resistance to only a small set of blast isolates in a limited geographical region (1,18).

Genetic studies of resistance to rice blast began when Goto established the differential system for *M. oryzae* races in Japan in the early 1960s (22). After the first molecular linkage map was published in 1988 (19), efforts to map resistance genes increased, especially once the rice genome was sequenced in 2002 (7,30). To date, over 85 major rice blast R genes have been identified and mapped on rice chromosomes with molecular markers (1). Although these genes are located on all 12 chromosomes, many are clustered on chromosomes 6, 11, and 12 (1). Extensive efforts in the last 15 years have led to the cloning of 13 R genes via map-

based cloning strategies (18). Among the cloned genes, several confer high levels of resistance to diverse rice blast strains or races. For example, *Pi2* confers resistance to 455 isolates collected from different regions of the Philippines and to most of the 792 isolates from the 13 major rice regions of China (2,3,32). In a test with 43 isolates from 13 countries, a *Pi9*-carrying line was resistant to all isolates (17,24). These two R genes (*Pi2* and *Pi9*) have been used in the rice breeding programs in the last decade, and many new, highly resistant rice cultivars have been bred for rice production in China and several other Asian countries (18). Because the two genes are widely used in rice production, however, their resistance will gradually be lost with the emergence of virulent strains in the field. Although the new broad-spectrum resistance gene *Pi40* was recently identified from *O. australiensis*, its durability to rice blast needs more field evaluations in different regions (11). Thus, identification of new broad-spectrum resistance genes is urgently needed for stable rice production.

Xiangzi 3150 (XZ3150), a local *indica* cultivar in Hunan Province, China, conferred resistance to 95% of the blast isolates collected from Jilin, Zhejiang, Hunan, Fujian, Guangdong, and Hainan Provinces, China (23). Previous genetic analysis showed that the resistance in XZ3150 to *M. oryzae* isolates was controlled by two major R genes, but the gene locations were not determined (16). This cultivar has been used in rice blast resistance breeding programs as a donor line for over 20 years. To understand the genetic basis of the broad-spectrum and stable resistance in XZ3150, in the current study we developed a recombinant inbred line (RIL) population from a cross between XZ3150 and the highly susceptible variety CO39. The resistance of the RILs was

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evaluated in both the growth chamber and the field. Two R genes, designated *Pi47* and *Pi48*, were identified in XZ3150 and were mapped on chromosomes 11 and 12, respectively.

MATERIALS AND METHODS

Development of the XZ3150/CO39 RILs. A cross was made between XZ3150 and the susceptible variety CO39, and the F_1 plants were selfed to produce F_2 seeds in Hunan, China. The single seed descend method was used from F_2 to F_9 generations. A total of 286 F_9 RILs were obtained for blast inoculation and genotyping experiments.

Evaluation of blast disease severity in the growth chamber. *M. oryzae* 193-1-1 was isolated from the blast nursery in Taojiang County and belongs to the Chinese blast race ZC11 (23). It is one of the predominant strains in Taojiang and other regions of Hunan Province, and has stable virulence and sporulation capacity in the laboratory (*unpublished data*). The isolate was maintained on dry filter paper at -20°C . Actively growing mycelia of 193-1-1 from agar medium with tomato juice and oats were transferred to fresh medium and cultured for 5 days at 27°C under darkness, and then moved to an incubator with continuous fluorescent light at 27°C for another 5 days.

The F_2 plant and F_9 RIL inoculations were conducted in 2002 and 2009, respectively, in Changsha, China. Germinated seeds were sown in plastic trays ($30 \times 25 \times 5$ cm) in the greenhouse. Each tray contained 17 RILs, with 15 to 20 plants per RIL. CO39 and XZ3150 were included in each tray as the susceptible and resistant control, respectively. Seedlings at the three-to-four-leaf stage (≈ 18 to 20 days after sowing) were sprayed with 50 ml of a conidial suspension of isolate 193-1-1 ($\approx 1 \times 10^5$ spores/ml in 0.1% Tween 20) using a hand sprayer connected to an air compressor. The inoculated plants were sealed in plastic bags in the growth chamber at 26 to 28°C . The plants were kept in the dark for 24 h. The inoculated plants were subsequently kept in the same room with normal illumination (12 h light and 12 h dark) and high humidity (70 to 80%) for an additional 6 days, at which time the susceptible check CO39 exhibited a highly susceptible reaction. The trays were maintained in plastic bags containing about 3 cm of water to keep the soil moist during the evaluation period. For each replication, 286 RILs and the susceptible and resistant controls were arranged in a completely randomized block design. Each line was inoculated three times. The inoculation for the entire RIL population was conducted during April and June 2009.

Disease severity on individual plants was evaluated with a 0 to 9 blast scoring system (9). F_2 plants with a score of 0 to 3 were considered resistant, and those with a score of 4 to 9 were considered susceptible. For each F_9 RIL, the disease severity score was recorded as the average severity of 15 to 20 plants in each replication. The average disease severity score of each RIL in the growth chamber (DS_G) was averaged from the three replications. Among the 286 RILs tested, only 251 lines with consistent resistant reaction in the three replications were used for the composite interval mapping (CIM).

Evaluation of rice blast disease severity in the field. A field experiment was conducted during April and August 2009, in the blast nursery of Taojiang County ($28^{\circ}22'\text{N}$ and $112^{\circ}03'\text{E}$), Hunan Province, China. The blast nursery contained 11 major blast races, i.e., ZC9, ZC11, ZE3, ZB29, ZG1, ZB25, ZB31, ZB13, ZC7, ZA9, and ZF1 (*unpublished data*). The field contained clay soil. The blast infection was severe because there was only about 6 h of sunshine each day during the growing season and there are mountains to the north and south of the field. The humidity in the field was about 70 to 80% at night.

About 20 F_9 seeds of the 286 RILs were sown in single-row plots, each row was 50 cm long. There were three replications for each line, and the plots were arranged in a completely randomized

block design. The seedlings of all lines were separated by the susceptible spreader varieties Xiangzaizao7 and Xiangwanxian11, which were planted in the middle of each block to enhance blast infection. The highly resistant XZ3150 and the highly susceptible CO39 were also seeded alternately in every 20 rows. Rice leaves infected with *M. oryzae* were obtained from other parts of the blast nursery. The leaves were cut into small sections (≈ 2 to 3 cm) and were sprinkled over each plant of the susceptible spreader varieties to induce rice blast at the two-leaf stage.

The disease severity for individual plants in the field (DS_F) was evaluated using a 0 to 9 blast scoring system (9). Lesion number (LN), percentage of diseased leaf area (DLA%), and lesion size (LS) were determined 40 days after seeding. For each F_9 RIL, the score of all resistance parameters was taken from five plants randomly selected in each plot. The final score of each RIL was the average score of three replications. Leaf area (LA) was determined using a portable area meter (ADC AM300, BioScientific Ltd., Herts, UK). DLA% data were estimated according to Notteghem et al. (21). LS was calculated using the formula $\text{LS} = \text{LA} \times \text{DLA\%/LN}$.

DNA extraction, parental polymorphism survey, and genotyping with molecular markers. The molecular marker analysis was conducted in Changsha, China. Genomic DNA was extracted from the young leaves of each F_9 RIL according to Saghai Maroof et al. (25). A total of 848 simple sequence repeat (SSR) and 66 single feature polymorphism (SFP) markers were used to survey the polymorphism between the two parents. The markers are evenly distributed on 12 rice chromosomes according to the results in Chen et al. (4), Temnykh et al. (26), McCouch et al. (20), the International Rice Genome Sequencing Project (8), and Jeremy et al. (10). The SSR marker assay was conducted as described by Wu and Tanksley (29). Polymerase chain reactions (PCRs) were conducted in a 10- μl reaction mix containing 37.5 ng of genomic DNA, $1 \times$ PCR buffer, 0.025 units of *Taq* DNA polymerase (TaKaRa Dalian Co., Dalian, China), 0.2 mmol dNTPs, and 0.8 pmol forward and reverse primers. PCR amplification was performed with a PTC-200 thermal cycler (MJ Research Inc., Waltham, MA). The PCR reactions involved an initial denaturation at 94°C for 5 min, followed by 35 cycles of denaturation for 30 s at 94°C , annealing for 30 s at 55°C , and extension for 1 min at 72°C and then a final extension for 7 min at 72°C . DNA bands were visualized via silver staining on an 8% polyacrylamide gel.

Map construction and CIM analysis. A genetic linkage map was constructed from the genotypic data of the 286 RILs using Mapmaker version 3.0 software (14). The map distance was calculated based on the Kosambi function. Quantitative trait loci (QTL) Cartographer version 2.5 (31) was used to identify R genes conferring blast resistance on the basis of CIM analysis. The percentage of total phenotypic variation explained by each locus and their additive effects were estimated by the same software. Tests were performed at every 2-cM interval on the linkage map, and cofactors were selected by forward/backward stepwise regression (model 6) in QTL Cartographer (31). The significance threshold for CIM was determined by 300 permutation tests. In this study, the threshold value of LOD was 4.0. The phenotypic variation explained by a QTL (r^2) conditioned by the CIM cofactors included in the model was calculated at the most likely QTL position.

RESULTS

Reevaluation of the resistance spectrum of XZ3150. Because the resistance of XZ3150 to rice blast had not been evaluated for about 15 years (23), we inoculated the cultivar with 303 isolates, representing 30 races of *M. oryzae*; these isolates had been recently collected from different regions of Hunan Province. Inoculation experiments showed that XZ3150 was resistant to 95.4% of

the 303 isolates and was highly resistant to several predominant races in Hunan Province, including ZG1, ZB13, and ZC11 (Table 1).

Polymorphism survey of SSR and SFP markers. Among the 914 markers (848 SSRs and 66 SFPs) tested, 202 SSR and 20 SFP markers showed polymorphism between the two parents. A total of 134 codominant and reliable polymorphic markers evenly distributed on the 12 chromosomes were selected for the construction of a linkage map with the genotype data of the 286 RILs. Based on the Kosambi function, the linkage map consisting of 12 chromosomes was 2,085.8 cM long with an average distance of 15.56 cM between adjacent markers.

Identification and mapping of two R genes in XZ3150. Among the 378 F₂ plants inoculated with isolate 193-1-1 in the growth chamber, 351 plants were highly resistant (as resistant as XZ3150) and 27 plants were highly susceptible (as susceptible as

CO39). The segregation of resistant and susceptible plants fit a 15:1 ratio (Table 2, $\chi^2 = 0.51 < \chi^2_{0.05} = 3.84$), indicating that XZ3150 carried two dominant R genes conferring resistance to 193-1-1.

A total of 251 RILs were inoculated with isolate 193-1-1 in the growth chamber. Among them, 182 RILs were highly resistant and 69 RILs were highly susceptible to the local blast population based on the 0 to 9 blast scoring system. The numbers of resistant and susceptible lines fit a 3:1 ratio (Table 2, $\chi^2 = 0.83 < \chi^2_{0.05} = 3.84$), suggesting that two independent R loci in XZ3150 confer resistance to 193-1-1.

To map these two R genes, we performed CIM analysis using the marker genotype and resistance phenotype data of the 251 RILs. This analysis detected two genomic region with large LOD scores, one on chromosome 11 and one on chromosome 12 (Fig. 1). The locus on chromosome 11 was designated *Pi47*, and the

TABLE 1. Evaluation of the resistance spectrum of Xiangzi 3150

Race group	Race	No. of isolates	Isolate origin in Hunan Province, China ^a	No. of incompatible isolates	Resistance frequency (%)
ZA	ZA ₁	5	Taojiang	5	100
	ZA ₃	2	Xinning	2	100
	ZA ₉	3	Xinning (1), Suining (1), Taojiang (1)	3	100
	ZA ₁₁	2	Xinning (1), Yanxi (1)	1	50
	ZA ₁₃	2	Taojiang	2	100
	ZA ₁₅	1	Taojiang	1	100
	ZA ₂₇	1	Suining	1	100
	ZA ₄₃	1	Yanxi	1	100
	ZA ₅₇	2	Taojiang	2	100
	ZA ₅₉	1	Yanxi	1	100
ZB	ZB ₁	11	Suining (6), Yanxi (4), Guidong (1)	11	100
	ZB ₃	4	Yanxi (3), Guidong (1)	4	100
	ZB ₅	1	Taojiang	1	100
	ZB ₇	4	Daweishan (2), Taojiang (2)	4	100
	ZB ₉	6	Taojiang (2), Yanxi (4)	6	100
	ZB ₁₁	3	Taojiang	3	100
	ZB ₁₃	35	Taojiang (13), Xinning (10), Guidong (12)	35	100
	ZB ₁₅	24	Yanxi (12), Qidong (6), Chaling (6)	21	87.5
	ZB ₁₇	1	Taojiang	1	100
	ZB ₂₁	1	Daweishan	1	100
	ZB ₂₃	1	Daweishan	1	100
	ZB ₂₅	3	Taojiang (1), Yanxi (1), Daweishan (1)	2	66.67
	ZB ₂₇	3	Taojiang (1), Guidong (1), Daweishan (1)	3	100
	ZB ₂₉	31	Taojiang (11), Daweishan (20)	30	96.77
	ZB ₃₁	13	Taojiang (8), Daweishan (5)	12	92.31
	ZC ₁	1	Daweishan	1	100
	ZC ₃	3	Yanxi (1), Qidong (1), Taojiang (1)	3	100
ZC	ZC ₅	1	Taojiang	1	100
	ZC ₇	1	Xinning	1	100
	ZC ₉	2	Taojiang	1	50
	ZC ₁₁	7	Yuanjiang (3), Taojiang (2), Taoyuan (1), Qidong (1)	7	100
	ZC ₁₃	10	Qidong (3), Daweishan (7)	9	90
	ZC ₁₅	10	Jianghua (3), Guidong (2), Yizhang (2), Suining (2), Taoyuan (1)	9	90
	ZD ₁	8	Daweishan (4), Yanxi (2), Taojiang (2)	8	100
	ZD ₃	4	Daweishan (1), Suining (3)	3	75
ZD	ZD ₅	2	Daweishan	2	100
	ZD ₇	4	Daweishan (3), Taoyuan (1)	4	100
	ZE ₁	11	Yanxi (3), Daweishan (5), Jianghua (3)	11	100
ZE	ZE ₃	12	Qidong (3), Taojiang (1), Junshan (2), Jianghua (5), Daweishan (1)	10	83.33
	ZF ₁	13	Daweishan (8), Jianghua (3), Taojiang (2)	12	92.31
ZG	ZG ₁	53	Taojiang (16), Daweishan (15), Yanxi (7), Jianghua (5), Xinning (3), Dongshan (3), Xinhua (1), Dongjiao (1), Suining (1), Yizhang (1)	53	100

^a Numbers in parentheses indicate the number of isolates from the region.

TABLE 2. Segregation of the resistant and susceptible plants or lines in F₂ generation or F₉ recombinant inbred lines after inoculation with *Magnaporthe oryzae* isolate 193-1-1 in the growth chamber

Generation	No. of plants or lines		Ratio of resistant to susceptible plants or lines		χ^2 for 15:1 or 3:1
	Resistant	Susceptible	Observed	Expected	
F ₂	351	27	13:1	15:1	0.51 ^a
F ₉	182	69	2.6:1	3:1	0.83 ^a

^a Not significant at the 0.05 level.

locus on chromosome 12 was designated *Pi48*. The *Pi47* region had a LOD score of 14.2, explained 27.2% of the total phenotypic variation (Table 3), and was mapped between RM206 and RM224 (Fig. 1). *Pi48* had a LOD score of 16.7, explained 22.5% of the total phenotypic variation, and was located in a 4.2-cM region between the SSR markers RM5364 and RM7102 and in a 0.2-cM region near the marker RM7102 (Table 3). The additive effects of the two R genes (resulting in improving the resistance) were derived from the XZ3150 alleles.

Major genetic factors for the blast resistance of XZ3150 in the field. To identify the QTL in XZ3150 for the *M. oryzae* population in the field, we evaluated the DS_F, LN, DLA%, and LS of the 286 RILs and analyzed the data using the CIM method. The analysis showed that the two regions in chromosome 11 and 12, i.e., *Pi47* and *Pi48*, were responsible for all four blast-resistant phenotypes in the field (Fig. 1). The peaks of the two regions closely overlapped with the regions of *Pi47* and *Pi48*, which were identified in the inoculation with the isolate 193-1-1 in the growth chamber experiment (Fig. 1). The LOD score for the four resistant phenotypes at the *Pi47* region ranged from 8 to 15 while those at the *Pi48* region ranged from 7 to 18. When the RILs carrying the two major genes were removed from the CIM analy-

sis, no QTL with LOD scores over 3.0 were identified (data not shown), suggesting that the two R genes were the major contributors to XZ3150 blast resistance in the field.

To test the correlation between the number of R genes and disease resistance level among the RILs, we classified the RILs into three groups: those carrying both genes, those carry one gene, and those carry neither gene. Under both growth chamber and field conditions, the RILs with markers RM224 (linked to *Pi47*) and RM7102 (linked to *Pi48*) had significantly lower disease severity levels than the lines with the CO39 alleles at the loci. Disease severity level was negatively correlated with the number of R genes (Fig. 2). As the number of R genes in the RILs was increased, the DS_G, DS_F, LN, DLA%, and LS were decreased. The correlation coefficients between the number of R genes and DS_G, DS_F, LN, DLA%, and LS were -0.909, -0.917, -0.894, -0.902, and -0.947, respectively.

Validation of the markers linked to the *Pi47* and *Pi48* genes. To validate the above mapping results, we selected three lines carrying the flanking markers of *Pi47* but not those of *Pi48*, or carrying the flanking markers of *Pi48* but not those of *Pi47*, for backcrossing with the susceptible parent CO39. The segregation ratio for resistant to susceptible plants in the derived F₂ popu-

Fig. 1. Locations of the two resistance genes in Xiangzi 3150 to *Magnaporthe oryzae* isolate 193-1-1 in the growth chamber and to the *M. oryzae* population in the field. DS_G, disease severity in growth chamber; DS_F, disease severity in the field; LN, lesion number; DLA%, percentage of diseased leaf area; and LS, lesion size.

TABLE 3. The chromosomal locations and additive effects of the *Pi47* and *Pi48* genes

Gene	Chromosome	Marker interval	Distance ^a (cM)	LOD score	r ^{2b}	Additive ^c
<i>Pi47</i>	11	RM206-RM224	5.5	14.15	0.2716	-1.0658
<i>Pi48</i>	12	RM5364-RM7102	0.2	16.76	0.2253	-0.9675

^a Refers to the distance of the gene to the nearest marker.

^b Refers to the percentage of phenotypic variation explained by a single gene.

^c Refers to the additive effect of the XZ3150 alleles in increasing disease severity for individual plants in growth chamber or in field.

lations fit a 3:1 ratio, suggesting that there is a single resistant gene in these lines (data not shown). Molecular analysis confirmed that the flanking markers of *Pi47* were linked to the resistance in the *Pi47*-carrying RILs and that the flanking markers of *Pi48* were linked to the resistance in the *Pi48*-carrying RILs.

The SSR markers RM224 and RM7102, which are the nearest markers linked to *Pi47* and *Pi48*, respectively, were used to select candidate RILs for resistance spectrum tests. Six RILs each for *Pi47* and *Pi48* were selected for resistance spectrum evaluation against 14 XZ3150 incompatible isolates (Table 4). The inoculations revealed that *Pi48* conferred resistant to more isolates than *Pi47*, suggesting that *Pi48* may have a broader resistance spectrum than *Pi47*.

DISCUSSION

Developing rice cultivars with broad-spectrum and durable resistance to rice blast has been a daunting challenge for rice breeders. One of the difficulties is the lack of R genes that are effective against many *M. oryzae* strains in different geographic regions. Among the 85 identified blast R genes, most confer

resistance to only a few strains and have not been widely used in breeding programs. Although several durably blast-resistant cultivars have been reported, the genetic mechanism of the resistance in these cultivars has not been elucidated. Wang et al. (27) pioneered the use of RIL population to dissect the durable resistance in the upland rice cultivar Moroberekan. In that study, two major genes, *Pi5* and *Pi7*, and over 10 QTL were identified. The authors concluded that durable resistance in Moroberekan is due to the combination of both major genes and QTL. One of the major genes, *Pi5*, was recently cloned via a map-based cloning strategy (12). Interestingly, the *Pi5*-mediated broad-spectrum resistance requires two nucleotide binding site-leucine-rich repeat genes, suggesting a novel defense strategy against blast infection in these rice plants. Similarly, Wu et al. (28) identified *Pi25*, *Pi26*, and eight QTL from the durably resistant cultivar Gumei 2, and Lee et al. (13) identified both *Pi42* and *Pi43* from the known blast-resistant cultivar Zhe733 using the KBNT $lpa1-1 \times$ Zhe733 RILs. In this study, we developed a set of 286 F₉ RILs to identify the R genes in XZ3150 against a single isolate in the growth chamber and multiple isolates in the field. Both *Pi47* on chromosome 11 and *Pi48* on chromosome 12 confer the resistance to

Fig. 2. The correlation between the disease resistance level and number of resistance genes in the recombinant inbred lines under both growth chamber and field conditions.

TABLE 4. Reactions to several *Magnaporthe oryzae* isolates of recombinant inbred lines (RILs) determined from flanking marker data to contain *Pi47* or *Pi48* gene for rice blast resistance

Line	Marker genotype ^a		Isolate													
	RM 224	RM 7102	CHL 645	CHL 446	CHL 1743	CHL 440	e2007 046A2	RB 6	CHL 471	CHL 506	CHL 473	0901-2	ES 6-1	RB 14	0905 -1	0904-3
RIL005	C	X	R	R	S	R	R	R	R	R	R	R	R	R	S	R
RIL053	C	X	R	R	R	R	R	R	R	R	R	R	R	R	R	R
RIL079	C	X	R	R	S	R	R	R	R	R	R	R	R	R	R	R
RIL158	C	X	R	R	S	R	R	R	S	R	R	R	R	R	R	R
RIL171	C	X	R	R	S	R	R	R	R	R	R	R	R	R	R	R
RIL266	C	X	S	R	S	R	R	R	R	R	R	R	R	R	R	R
RIL018	X	C	S	S	R	R	R	R	R	R	R	R	R	R	R	R
RIL040	X	C	S	S	R	S	S	R	S	S	R	S	R	—	R	R
RIL070	X	C	R	S	R	R	R	R	R	R	R	R	R	R	S	R
RIL147	X	C	R	S	R	S	R	R	R	S	R	S	R	R	R	R
RIL230	X	C	S	S	R	S	S	R	R	R	R	R	R	R	R	R
RIL279	X	C	S	S	S	S	S	R	R	R	R	R	R	R	—	R

^a The genotype of the nearest marker linked to the resistance gene. RM224 and RM7102 are the nearest markers linked to *Pi47* and *Pi48*, respectively. C and X denote the CO39 and XZ3150 genotype at the linked marker, respectively.

isolate 193-1-1 and to the *M. oryzae* population in the field. When the RILs carrying both R genes were removed from the data set for the CIM analysis, no significant QTL (LOD $\geq$ 3.0) was identified, suggesting that these two R genes are largely responsible for the broad-spectrum resistance in XZ3150. How these two R genes control the high level of resistance in XZ3150 and the molecular mechanism of their defense to rice blast infection will be revealed once both genes are cloned and characterized.

At least 17 R genes are mapped on chromosome 11, i.e., *Pi-a*, *Pi-f*, *Pi-k*, *Pik-h*, *Pik-m*, *Pi-1*, *Pi-7*, *Pi-12(t)*, *Pi-18*, *Pi-34*, *Pi-38*, *Pi-43(t)*, *Pi-44(t)*, *pbl*, *Pi-CO39(t)*, *Pi-y(t)*, and *Pi-zy(t)* (1). Among them, *Pi-43(t)*, *Pi-k*, and *Pik-h* are linked to the marker RM224 (http://www.ricedata.cn/gene/gene_pi.htm). *Pi-k* is located between the SSR markers of RM1233 and RM224 on the long arm of chromosome 11, with a distance of 1.6 and 0.2 cM to the linked marker, respectively (http://www.ricedata.cn/gene/gene_pi.htm). *Pik-h* was mapped to a 2-cM interval between RM224 and Y6855RA in the same region (http://www.ricedata.cn/gene/gene_pi.htm). *Pi43(t)* from the rice variety Zhe733 is also tightly linked to RM1233 and RM224 (13). The *Pi47* gene found in this study is located between RM206 and RM224. At least 16 R genes are located on rice chromosome 12 (1). Among them, only two are linked to the SSR marker RM7102 according to previous studies (6,15). *Pi-ta²* was allelic to *Pi-ta* (6) on chromosome 12, which is located outside of the marker interval of RM5364-RM7102. *Pi-GD-3(t)* is linked to the SSR marker of RM179 on chromosome 12 (15), outside of the interval of the RM5364-RM7102 region. The *Pi48* gene identified in our study is novel because no gene has been previously reported in the same region on chromosome 12. The exact physical location of the *Pi47* and *Pi48* genes and their relationship with known R genes in the same region will be revealed by fine-mapping of both genes. As shown in Table 4, the putative *Pi47*- or *Pi48*-carrying lines had different resistance reactions to the same isolates. This is because we selected them based on their linked marker genotypes. When near-isogenic lines of *Pi47* and *Pi48* in the CO39 background are obtained, more inoculations will be conducted to confirm the resistance spectrum of the two genes.

XZ3150 has maintained durable resistance to rice blast for over 20 years in Hunan Province and is being used in many rice breeding programs (23). Its poor agronomical traits (it produces low yields and often lodges), however, are difficult to eliminate by conventional backcross method in the progenies when it is crossed with an elite rice line. This problem has frustrated rice breeders who want to use XZ3150 in their breeding programs. The identification of the DNA markers linked to *Pi47* and *Pi48* in this study should allow rice breeders to efficiently transfer the resistance genes in XZ3150 into new, elite rice cultivars using marker-aided selection. Once new markers tightly linked to *Pi47* and *Pi48* are identified, PCR-based markers will be developed for rice blast resistance breeding.

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