Identification of the Quantitative Trait Loci in *Japonica* Rice Landrace Heikezijing Responsible for Broad-Spectrum Resistance to Rice Blast

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ABSTRACT

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Rice blast is one of the most devastating diseases affecting rice production worldwide. One *japonica* landrace, Heikezijing, from the Taihu Lake area in China, has been reported to be highly resistant to most of the rice blast isolates collected from China and Japan. To effectively dissect the inheritance of its resistance, a population of recombinant inbred lines (RILs) ($F_{2:8}$) was constructed from a cross between Heikezijing and Suyunuo, a blast-susceptible cultivar. Nineteen blast isolates from China and Japan were inoculated into 166 RILs and their

parents, and 22 quantitative trait loci (QTLs) conferring resistance to these isolates were identified and mapped onto rice chromosomes 1, 7, 9, 11, and 12. Most of the QTLs conferred race-specific resistance to blast. Some QTLs, such as *qtl11-5-5*, conferred resistance to two or more isolates. One blast-resistant gene cluster, including *qtl11-2-2*, *qtl11-3-1*, *qtl11-4-1*, *qtl11-5-5*, *qtl11-6-1*, *qtl11-7-5*, *qtl11-8-2*, *qtl11-9-2*, *qtl11-10-4*, and *qtl11-11-1*, was found on the long arm of chromosome 11 in the *japonica* landrace. These loci offered effective resistance toward as many as 17 isolates, including 16 isolates from seven Chinese race groups and 1 isolate from Japan. The results from this study suggest that the Heikezijing landrace involves a number of genes that are associated with broad-spectrum resistance to rice blast.

Rice blast, caused by the fungus *Magnaporthe grisea* (T. T. Hebert) M. E. Barr (anamorph, *Pyricularia grisea* (Cooke) Sacc), is one of the most devastating diseases affecting rice, the staple food for the largest portion of the world population (48). Many resistant rice cultivars often remain effective only a few years because of the genetic instability and pathogenic variability of *M. grisea* (31). However, the breeding of resistant rice remains the most promising choice in order to avoid an epidemic outbreak of rice blast (5,47). It has been a major goal to find and characterize effective resistance genes from rice, and to map and clone these genes for breeding resistant plants.

To date, >50 blast-resistant genes with qualitative traits have been reported. These genes are distributed on 11 of the 12 rice linkage groups, with the exception of chromosome 3, and many of them are clustered on chromosomes 6, 11, and 12 (54,64). Most of the resistance genes are dominant, except the recessive gene *pi21*. Thus far, 13 genes resistant to rice blast (*Pib*, *Pi-ta*, *Pi9*, *Pi2*, *Piz-t*, *Pi-d2*, *Pi36*, *Pi37*, *Pik-h*, *Pit*, *Pik-m*, *Pi5*, and *pi21*) have been isolated through a map-based cloning approach (2,6,10,14,15,21,24,28,41,44,53). Among these cloned genes, 11 genes belong to a large resistance (*R*) gene family encoding receptor proteins that contain nucleotide-binding sites (NBSs) and leucine-rich repeats (LRRs). In particular, the blast resistance mediated by

Pik-m and *Pi5* require the cooperation of two NBS-LRR genes (2,21). *Pi-d2*, however, encodes a receptor-like kinase protein with a predicted extracellular domain of a bulb-type mannose-specific binding lectin (B-lectin) and an intracellular serine-threonine kinase domain (10). Interestingly, a single amino acid difference in the Pita protein correlates with the gene-for-gene-specific characteristic of the *Pita/AVR-Pita* system (6). It has also been found that only a single amino acid change in *Pi-d2* is responsible for the difference between resistant and susceptible alleles (10). The only recessive gene conferring non-race-specific resistance, *pi21*, encodes a proline-rich protein with a putative heavy-metal-binding domain and putative protein-protein interaction motifs. The loss of function of *pi21* is likely due to the absence of the 18- and 48-bp PxxPxxP sequences (14).

Broad-spectrum disease *R* genes have been used for disease control in crops. Broad-spectrum disease resistance can be achieved in two ways: resistance to the majority of geographically different isolates of the same pathogen or resistance to two or more unrelated pathogens (41). To date, several broad-spectrum *R* genes have been cloned in plants (7,14,41,50,55). In rice, some broad-spectrum rice blast resistance genes belonging to the first category, such as *Pi1*, *Pi2*, *Pi9*, *Piz-t*, *Pi33*, and *Pigm(t)*, have been identified. These are all resistant to a number of rice blast isolates (4,11,35,41,61). Genes such as *Pi34* (58), *Pi35(t)* (38), and *pi21* (14) confer partial resistance to blast. Recently, Manosalva et al. (32) reported a broad-spectrum disease resistance gene from *indica* cv. Sanhuangzhan 2 that is effective against two distinct, important rice pathogens.

In a previous study, we found that a *japonica* rice landrace, Heikezijing from the Taihu Lake region in China, exhibited a high level of resistance to nine blast isolates (51). Here, we identified resistance quantitative trait loci (QTLs) to 19 isolates in Heikezijing with a simple-sequence repeat (SSR) molecular linkage map.

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MATERIALS AND METHODS

Plant materials. Two rice (*Oryza sativa* L.) accessions were used in this study. The *japonica* landrace, Heikezijing, is highly resistant to the blast, and Suyunuo is susceptibility to most blast isolates (22,51). The population of recombinant inbred lines (RILs) (F_{2:8}) from the cross of Heikezijing × Suyunuo consisted of 166 lines.

Pathogens. Eighteen Chinese blast isolates from all seven races (ZA, ZB, ZC, ZD, ZE, ZF, and ZG), based on the Chinese Rice Blast Identification System (25), provided by the Plant Protection Research Institute, Jiangsu Academy of Agricultural Sciences, and one Japanese isolate, Hoku 1, provided by the Institute of Crop Research, Chinese Academy of Agricultural Sciences, were used in this study (Table 1).

Inoculation. Rice seeds were grown in plastic trays of 60 by 30 by 5 cm as described by Wang et al. (51). In each tray, 30 lines and 2 parents (P₁ and P₂) as control were sown, with 10 seeds per line or parents for each inoculation. Three-week-old rice seedlings were put into inoculation chambers and inoculated by spraying with conidial suspensions (5×10^4 conidia/ml) with several drops of Tween-20 as described by Wang et al. (51). The inoculated plants were kept in chambers at 26°C with 95% relative humidity and darkness for 24 h, and then transferred to the greenhouse for incubation with 100% relative humidity by intermittently spraying with water. Each line was inoculated in two independent experiments, with two replications of each experiment. The lesion types on rice leaves were scored after 1 week of inoculation, according to standard procedures (31). Lesion scores of 0 to 5 were based on lesion types with appropriate reference of the disease area of the highest score of each plant, and the average lesion score of 10 plants was recorded as the final score of each line. RILs with scores of 0 to 2 were considered to be resistant and those with scores of 3 to 5 were considered to be susceptible. For QTL mapping, the average score of each RIL was used as a quantitative variable (43).

Construction of molecular linkage map. To construct the molecular linkage map, 0.2 to 0.5 g of leaves from RILs (F_{2:7}) and parents were collected specifically for DNA extraction by the sodium dodecyl sulfate (SDS) method (40). According to the International Rice Microsatellite Initiative (IRMI; <http://www.gramene.org>), 1,436 SSR markers were used in this study, and 128 of them with polymorphisms between the two parents were used for map construction. Polymerase chain reaction (PCR) amplifications were carried out in 10-μl reaction mixtures containing 0.25 μm primer, Taq polymerase (Tiangen, Beijing) at 0.01 U/μl, 1× buffer mix, and 250 μm dNTP. The PCR conditions was as follows: predenaturing at 94°C for 5 min; followed by 35 cycles of denaturing at 94°C for 40 s, annealing at 55°C for 40 s, and extension at 72°C for 45 s; and a final extension at 72°C for 10 min. The PCR products were separated on 8% nondenaturant polyacrylamide gels followed by a silver staining method according to the manufacturer's recommendations (Promega Corp., Madison, WI). A genetic map of molecular markers was computed using the multipoint functions performed by MapMaker/EXP v. 3.0 (20). The logarithm of the odds (LOD) score threshold was set to 3.0. Ordering of markers was achieved using the "order", "try", and "ripple" commands, which calculate likelihood ratios for the different possible multipoint orders. Conversion of recombination fractions into centimorgans (cM) was obtained with Kosambi's mapping function (18). The final map was drawn using MapDraw 2.1 (27).

Data analysis. Composite interval mapping (CIM) (59) was performed for QTL analysis and CIM was computed using Windows QTL Cartographer 2.5 (52). A stringent LOD threshold of 3.0 was used to declare major QTLs in this study while a LOD score value of 2.5 was used to detect minor QTLs, as suggested by Lander (19). Mapmaker/QTL (20) and QTLnetwork 2.0 (56)

were also used to confirm putative loci affecting blast resistance based on point and interval analysis and mixed-model-based CIM (MCIM). In this study, QTLs were named *qtlA-B-C*, in which A means the chromosome number, B the QTL, and C the number of isolates corresponding to the QTL.

RESULTS

Resistance spectrum of Heikezijing to blast. Nineteen blast isolates from China and Japan were inoculated onto Heikezijing, Suyunuo, and 166 RILs derived from the cross Heikezijing × Suyunuo. The results show that Heikezijing is resistant to all isolates, with lesion scores of 0 to 1.3, and that Suyunuo is susceptible to all isolates, with lesion scores of 4 to 5 (Table 1). The RIL population showed different effects from the blast isolates, and the distribution frequency of lesion scores differed depending on the blast isolate that was inoculated (Supplementary Fig. 1).

Identification of resistance loci in Heikezijing. In total, 128 SSR markers with polymorphisms between the two parents were used for constructing the molecular linkage map. This linkage map had a total distance of 1,867.1 cM, with an average distance of 14.6 cM between two markers, and covered the whole rice genome according to the information from the IRMI. The average lesion score of each RIL based on the 0 to 5 disease scale was used as the quantitative variable for QTL analysis. When the LOD threshold was set to 3.0, 15 major QTLs corresponding to resistance to 19 blast isolates were detected by the CIM method (Table 2). When the LOD threshold was set to 2.5, five minor Heikezijing QTLs and two minor Suyunuo QTLs were detected (Table 2).

Among the 20 QTLs detected in Heikezijing, 10 QTLs on chromosome 11 corresponded to all tested blast isolates except JS02-95 and JS2004-180, with LOD scores of 3.21 to 38.15 and phenotypic variance of 10.65 to 80.82%. The five QTLs on chromosome 9 were effective against 13 isolates, with LOD scores of 2.65 to 14.57 and phenotypic variance of 7.43 to 68.61%. The four QTLs on chromosome 1 were effective against four isolates, with LOD scores of 2.54 to 2.82, and the one QTL on chromosome 12 was effective against only one isolate, with a LOD score of 6.98 (Table 2). The two minor QTLs from Suyunuo mapped to chromosomes 7 and 11, corresponding to JS02-95 and JS2007-22-2, with 5.04 and 9.91% of the phenotypic variances, respectively.

TABLE 1. Responses of Heikezijing and Suyunuo to 19 blast isolates

Isolate (race)	Origin	Phenotype (lesion score) ^a	
		Heikezijing	Suyunuo
GD10-279a(ZB5)	China (Guangdong)	R (0)	S (5)
JS2004-180(ZG1)	China (Jiangsu)	R (0)	S (4.6)
JS02-68(ZC1)	China (Jiangsu)	R (0)	S (5)
JS05-74(ZG1)	China (Jiangsu)	R (0)	S (5)
JS02-43(ZD7)	China (Jiangsu)	R (0)	S (5)
JS90-78(ZF1)	China (Jiangsu)	MR (1.3)	S (5)
JS2004-185(ZE3)	China (Jiangsu)	R (0)	S (5)
JS2004-183(ZD5)	China (Jiangsu)	R (0)	S (5)
JS2004-141-1(ZD5)	China (Jiangsu)	R (0)	S (5)
JS02-95(ZE3)	China (Jiangsu)	R (0)	S (5)
JS03-44(ZA13)	China (Jiangsu)	R (0)	S (4.75)
JS2004-3(ZB5)	China (Jiangsu)	MR (1.2)	S (5)
JS2007-11-1(ZG1)	China (Jiangsu)	R (0)	S (5)
JS2006-25(ZE3)	China (Jiangsu)	R (0)	S (5)
JS2007-22-2(ZB29)	China (Jiangsu)	R (0)	S (4)
JS2007-2-3(ZC13)	China (Jiangsu)	R (0)	S (4.1)
JS2007-51-1(ZD5)	China (Jiangsu)	R (0)	S (5)
JS2007-2-2(ZF1)	China (Jiangsu)	R (0)	S (4.2)
Hoku1(ZG1)	Japan	R (0)	S (5)

^a R represents resistant, MR represents moderately resistant, S represents susceptible, and MS represents moderately susceptible.

Thirteen QTLs in Heikezijing, including *qtl1-1-1*, *qtl1-2-1*, *qtl1-3-1*, *qtl1-4-1*, *qtl9-2-1*, *qtl9-3-1*, *qtl9-4-1*, *qtl9-5-1*, *qtl11-3-1*, *qtl11-4-1*, *qtl11-6-1*, *qtl11-11-1*, and *qtl12-1-1*, were effective against only one blast isolate and, thus, are more likely to confer isolate-specific resistance. The other seven QTLs may be non-isolate-specific because each QTL confers resistance to more than one isolate, with different peak LOD scores for each isolate (Table 2). For instance, *qtl9-1-9* could be detected when inoculated with nine different isolates; *qtl11-5-5* and *qtl11-7-5* were detected when inoculated with five different isolates; *qtl11-10-4* could be detected when inoculated with four different isolates; and *qtl11-2-2*, *qtl11-8-2*, and *qtl11-9-2* were detected when inoculated with two isolates (Table 2). In addition, eight QTLs detected in Heikezijing contribute to >30% of the phenotypic variance, exhibiting a major gene effect. For example, *qtl11-5-5* on chromosome 11 could explain 74.34 and 80.82% of the phenotypic variance when inoculated with Hoku 1 and JS90-78, respectively, and *qtl9-4-1* could explain 68.62% of the phenotypic variance to the blast isolate JS02-95 (Table 2).

Comparison of QTL detection with the expected resistant:susceptible ratio. The resistant:susceptible (R:S) segregation in the RIL population is 1:1 to the isolates JS02-43 and JS2004-3 (Supplementary Table 3), showing a major gene effect. These results are completely consistent with the detection of one QTL. Although the R:S segregation in RIL population is also 1:1 to JS2004-183 and JS90-78, two and four QTLs were detected, respectively (Table 2). This could be caused by the united effect of one major QTL and other minor QTLs with lower contributions to phenotypic variance. Like the QTLs conferring resistance to JS90-78, *qtl11-5-5* can explain 80.82% of the phenotypic variance but three other QTLs can only explain 12.28, 45.94, and 25.15% of the phenotypic variance, respectively.

The R:S segregation in the RIL population is 3:1 to the isolates JS2004-180, JS2007-151-1, JS02-95, JS03-44, Hoku1, JS2006-25, and JS2007-2-2 (Supplementary Table 3), indicating that the resistance to these isolates is controlled by two major genes. The detection of two QTLs for these isolates fits this model well, except for Hoku1 (Table 2). The R:S segregation to the isolates JS02-68, JS05-74, and JS2007-22-2 is 7:1 (Supplementary Table 3), and the detection of three QTLs fits the model well. Although

the R:S segregation corresponding to isolates JS2007-11-1, JS2004-185, and JS2007-2-3 were 7:1 or 15:1, two QTLs with lower R^2 were detected. This suggests that the resistance to these isolate may be controlled by many minor QTLs. In addition, for JS2004-141-1, the R:S segregation is intermediate between 1:1 and 3:1 (Supplementary Table 3), indicating that the resistance is controlled by two genes in which one exhibits a predominant effect. For GD10-279a, the R:S segregation is also intermediate between 1:1 and 3:1. Of the four QTLs were detected, *qtl11-10-4* ($R^2 = 52.98\%$) and *qtl11-5-5* ($R^2 = 32.16\%$) may play a major role against GD10-279a.

Comparison of the QTLs identified by three approaches.

Using the QTLnetwork 2.0 approach, which was developed based on the MCIM (56), only nine resistance QTLs were identified from Heikezijing (Supplementary Table 2). Among the nine QTLs detected, one QTL was detected on chromosome 9 (resistance to 11 isolates), seven QTLs were detected on chromosome 11 (resistance to 18 isolates), and one QTL was detected on chromosome 12 (resistance to 1 isolate). The minor QTLs with LOD between 2.5 and 3.0 by CIM could not be identified by the MCIM approach, whereas the major QTLs (LOD >3.0) corresponding to most isolates could be detected by both approaches, except to the isolate JS2004-180. To JS2004-180, the same QTL on chromosome 9 was detected by both approaches whereas the QTL on chromosome 11 was only detected by MCIM (Table 3). This indicates that the results of QTLnetwork 2.0 can verify the major QTLs detected by CIM from Windows QTL Cartographer 2.5.

Mapmaker/QTL was also used to identify putative loci affecting quantitative resistance, based on point and interval analysis (20). Although Mapmaker/QTL is not designed for recombinant inbred populations, the QTLs could be calculated by changing the data format to BC₁ population, as previously described (49). Comparing the results of CIM analysis from Windows QTL Cartographer 2.5, more QTLs were identified based on Mapmaker/QTL analysis (Table 2). The major QTLs on chromosomes 9, 11, and 12 corresponded to the results from the CIM and MCIM methods, and additional minor resistance loci were detected on chromosomes 1, 5, and 7. One QTL (LOD score > 9) on chromosome 4 (between RM335 and RM3367),

Fig. 1. Genomic locations for quantitative trait loci (QTLs) affecting blast resistance of the Heikezijing x Suyunuo recombinant inbred population. Black ellipses are the QTLs detected by composite interval mapping from Windows QTL Cartographer 2.5 (52); black stars are genes that have been reported previously with an imprecise location on our genetic map.

corresponding to resistance to JS02-95, JS04-180, JS04-183, JS90-78, and JS2004-3; one QTL on chromosome 10 (between RM333 and RM258), corresponding to resistance to JS2004-3 (LOD = 6.57) and JS2004-141-1 (LOD = 9.95); and one QTL (LOD = 9.95) on chromosome 8 (between RM1019 and RM5853), corresponding to resistance to JS2004-141-1 (Table 3) were detected. None of these QTLs were detected by the CIM and MCIM methods (Table 2). Although these QTLs may be major genes according to their LOD scores and R^2 (Supplementary Table 1), it was found that these markers did not correlate with the lesion scores of the corresponding isolates after co-segregation analysis (data not shown). Some pseudo-QTLs may be detected by Mapmaker/QTL due to accumulated R^2 of QTLs to one isolate being far more than 100% (Supplementary Table 1).

DISCUSSION

The *japonica* rice landrace Heikezijing was resistant to all 19 isolates of *M. grisea* tested in this study, indicating that this landrace is a typical first-type broad-resistant accession (41).

Usually, the rice resistance to blast is thought of as a qualitative trait when the reaction to blast is scored on a six-class scale based on lesion types (43). In this system, the plants with scores of 0, 1, and 2 are referred to as resistant and those with scores of 3, 4, and 5 are referred to as susceptible. Some plants with critical scores of 2 or 3 are difficult to clearly classify as resistant or susceptible; therefore, it is necessary to further analyze their resistance or susceptibility using segregation in the F_2 and F_3 generation (1,8, 9,26,30,39). To effectively dissect Heikezijing resistance genes to various isolates of blast, a population of 166 RILs ($F_{2,8}$) was produced from a cross between Heikezijing and a highly blast-susceptible *japonica* cultivar, Suyunuo. Through the population of RILs derived from the resistant $\times$ susceptible accession, the reaction of each line to one blast isolate could be accurately evaluated using an average score from a number of individuals.

The QTL detection approach has been widely used in order to localize major or minor loci involved in resistance at the molecular level (13,43,45). Our results found a number of QTLs in Heikezijing, including several major QTLs on chromosomes 9 and 11 which conferred resistance to most of the isolates, one

TABLE 2. Quantitative trait loci (QTLs) for resistance to blast detected based on lesion scores

Isolates (race)	QTLs ^a	Chr. no. ^b	Position (cM) ^c	LOD ^d	Additive ^e	R^2 (%) ^f	Marker left	Marker right
JS2004-180(ZG1)	<i>qtl1-1-1*</i>	1	0.01	2.61	-0.3621	4.4494	RM3482	RM3403
	<i>qtl9-1-9</i>	9	0.01	9.25	-0.716	17.3416	RM24019	RM285
JS2007-51-1(ZD5)	<i>qtl9-5-1*</i>	9	96.81	2.83	-0.3352	7.4953	RM7175	RM2144
	<i>qtl11-8-2</i>	11	143.81	11.43	-0.6827	31.3272	RM27187	RM27389
JS2004-183(ZD5)	<i>qtl11-10-4</i>	11	149.81	15.38	-1.115	34.1111	RM27389	RM144
	<i>qtl12-1-1</i>	12	56.81	6.98	-0.6809	12.3970	RM28089	RM3326
JS02-68(ZC1)	<i>qtl9-1-9</i>	9	0.01	6.2	-0.4647	12.9744	RM24019	RM285
	<i>qtl11-4-1</i>	11	131.61	4.5	-0.5378	12.4631	RM27088	RM27154
JS05-74(ZG1)	<i>qtl11-10-4</i>	11	149.81	9.81	-0.685	25.9149	RM27386	RM144
	<i>qtl1-2-1*</i>	1	6.01	2.69	-0.3	7.8450	RM3482	RM3403
	<i>qtl9-1-9</i>	9	0.01	3.76	-0.2965	7.4278	RM24019	RM285
	<i>qtl11-7-5</i>	11	139.91	8.92	-0.4998	19.4588	RM27154	RM27381
JS2007-2-3(ZC13)	<i>qtl9-1-9*</i>	9	0.01	2.65	-0.2231	7.9592	RM24019	RM285
	<i>qtl11-7-5</i>	11	139.91	3.21	-0.2701	10.6505	RM27088	RM27187
JS02-95(ZE3)	<i>qtl7-1-1*</i>	7	78.91	2.54	0.4027	5.0494	RM11	RM336
	<i>qtl9-4-1</i>	9	14.01	14.57	-1.7814	68.6169	RM24019	RM285
JS2007-11-1(ZG1)	<i>qtl9-1-9</i>	9	0.01	4.38	-0.4246	10.0918	RM24019	RM285
	<i>qtl11-6-1</i>	11	135.61	5.75	-0.5957	17.9911	RM27088	RM27154
JS02-43(ZD7)	<i>qtl11-9-2</i>	11	146.91	15.65	-1.1933	42.8057	RM27386	RM144
JS03-44(ZA13)	<i>qtl9-3-1</i>	9	4.01	5.07	-0.6146	14.2235	RM24019	RM285
	<i>qtl11-9-2</i>	11	146.91	5.75	-0.5904	13.2858	RM27386	RM144
JS90-78(ZF1)	<i>qtl1-4-1*</i>	1	72.61	2.54	-0.7186	12.2871	RM1297	RM5853
	<i>qtl11-2-2</i>	11	115.31	14.95	-1.7172	45.9418	RM6680	RM27088
	<i>qtl11-5-5</i>	11	133.61	20.44	-1.8804	80.8162	RM27088	RM27154
	<i>qtl11-10-4</i>	11	149.81	8.03	-1.1887	25.1497	RM27389	RM144
JS2004-3(ZB5)	<i>qtl11-8-2</i>	11	143.81	9.19	-0.8612	23.3719	RM27189	RM27389
JS2004-141-1(ZC5)	<i>qtl11-2-2</i>	11	115.31	5.47	-1.4067	39.3976	RM6680	RM27088
	<i>qtl11-5-5</i>	11	133.61	9.93	-1.0887	38.3425	RM27088	RM27154
JS2007-22-2(ZB29)	<i>qtl9-1-9</i>	9	0.01	5.3	-0.4232	15.3173	RM24019	RM285
	<i>qtl11-1-1*</i>	11	14.01	2.7	0.3399	9.9050	RM286	RM1812
	<i>qtl11-7-5</i>	11	139.91	4.6	-0.4087	13.3469	RM27154	RM27381
	<i>qtl11-3-1</i>	11	119.31	13.98	-1.5197	33.5064	RM6680	RM27088
Hoku1(ZG1)	<i>qtl11-5-5</i>	11	133.61	38.15	-1.6211	74.3417	RM27088	RM27154
	<i>qtl11-11-1</i>	11	151.81	17.55	-1.5763	47.3126	RM27385	RM144
JS2006-25(ZE3)	<i>qtl9-1-9</i>	9	0.01	6.9	-0.5819	13.2528	RM24019	RM285
	<i>qtl11-7-5</i>	11	139.91	9.57	-0.7456	19.9360	RM27154	RM27381
JS2004-185(ZE3)	<i>qtl9-1-9</i>	9	0.01	4.76	-0.4364	9.5439	RM24019	RM285
	<i>qtl11-5-5</i>	11	133.61	7.9	-0.6905	21.6825	RM27088	RM27154
GD10-279a(ZB5)	<i>qtl1-3-1*</i>	1	8.01	2.82	-0.4386	6.0319	RM3482	RM3403
	<i>qtl9-2-1</i>	9	2.01	8.62	-0.6878	15.1849	RM24019	RM285
	<i>qtl11-5-5</i>	11	133.61	9.8	-1.3341	32.1659	RM27088	RM27154
	<i>qtl11-10-4</i>	11	149.81	23.3	-1.3505	52.9777	RM27385	RM144
JS2007-2-2(ZF1)	<i>qtl9-1-9</i>	9	0.01	4.6	-0.5112	11.8846	RM24019	RM285
	<i>qtl11-7-5</i>	11	139.91	7.03	-0.686	19.6855	RM27189	RM27381

^a QTLs with “*” were detected when logarithm of the odds (LOD) threshold down to 2.5.

^b Chromosome number.

^c Peak LOD score in the QTL.

^d Position in centimorgans (cM) of peak LOD score.

^e Additive effect explained at peak LOD score.

^f Explained the contribution to the phenotypic variance.

QTL on chromosome 12 which was effective against the isolate JS2004-183, and a number of minor loci on chromosomes 1 and 9 (Fig. 1). In general, the *R* genes fall into six distinct classes, the most prevalent of which encodes an NBS and a series of carboxy-terminal LRRs (33). In all, ~600 NBS-LRR genes have been identified in the rice genome but >100 of these were predicted to be pseudogenes in rice cv. Nipponbare (3,37). Most putative *R* genes are present in clusters. Rice chromosome 11 is rich in disease-resistance genes and has at least 201 putative *R* genes (42,62). In fact, it has been reported that there are many genes resistant to blast that are located on the long arm of chromosome 11 (Fig. 1), such as *Pil(t)* (31), *Pik* (34), *Pi-lm2* (46), *Pi-18* (1), *Pi44(t)* (8), *Pi-30(t)* (43), *Pi34* (57,58), *Pi-y(t)* (60), *Pik-h* (44), *Pif*, and *Pi7* (12). Recently, *Pik-m* and *Pi-hkl1(t)* were reported to be localized in this region (22,23).

The QTLs in this study were mapped to the chromosomal locations where other blast resistance genes have been located, except for two minor QTLs (*qtl1-4-1* and *qtl9-5-1*) (Fig. 1). The QTLs may correspond to the specific resistance genes already described at these map positions or to different alleles. However, allelism tests between reference cultivars with known resistance genes will be necessary to demonstrate whether these QTLs are new resistance gene alleles. Twenty QTLs were detected that confer resistance to 19 blast isolates (Table 2). A cluster of 10 blast resistance loci on chromosome 11 was detected between SSR markers RM6680 and RM144, close to a series of blast resistance genes (Fig. 1) The QTLs effective against more isolates, such as

qtl11-2-2 (resistant to two isolates), *qtl11-5-5* (resistant to five isolates), and *qtl11-7-5* (resistant to five isolates), may confer the broad-spectrum resistance. However, none of these loci were effective against all 19 isolates tested in this study. It can be concluded that there is an *R*-gene cluster found on the long arm of chromosome 11 in the *japonica* landrace Heikezijing, and QTLs detected here likely function to confer race-specific resistance.

Qtl1-1-1, *qtl1-2-1*, and *qtl1-3-1*, identified on chromosome 1, mapped closely to the blast resistance genes *Pi35(t)* and *Pi37* (9,38). *Qtl9-1-9*, *qtl9-2-1*, *qtl9-3-1*, and *qtl9-4-1*, located on chromosome 9, are close to the blast resistance genes *Pi5* and *Pi15* (16,39). *Qtl12-1-1* located on chromosome 12 mapped closely to a blast resistance gene cluster including *Pi-ta(6)*. The QTLs on chromosomes 1 and 9 were also distributed in a cluster (Fig. 1). The QTLs on chromosome 1 were detected with a lower LOD threshold, suggesting that they may confer partial resistance to blast. Four QTLs on chromosome 9 correspond to the resistance to 12 isolates and *qtl9-1-9* corresponds to 9 isolates, suggesting that *qtl9-1-9* may be a broad-spectrum resistance gene.

Through comparing the results of three analysis approaches, we found that Mapmaker/QTL could detect more QTLs, the QTL network 2.0 based on MCIM could detect most of the major QTLs, and WinQTLCartographer 2.5 based on CIM could detect both major QTLs and the minor loci. The QTLs estimated by the CIM method were used for further analysis. Two QTLs from the susceptible parent Suyunuo, corresponding to resistance to JS02-

TABLE 3. Comparison of the results of different analysis approaches

Isolates	Chromosome no.	Position	Mapmaker	CIM ^a	MCIM ^b
JS2004-180	1	RM3482-RM3403	X	X*	...
	4	RM335-RM3367	X	...	...
	9	RM24019-RM285	X	X	X
	11	RM27088-RM27154	X	...	...
	11	RM27385-RM144	...	...	X
JS2007-51-1	1	RM3482-RM3403	X	...	...
	9	RM7175-RM2144	...	X*	...
	11	RM27187-RM27389	X	X	X
JS2004-183	1	RM3482-RM3403	X	...	...
	4	RM335-RM3367	X	...	...
	11	RM6680-RM27088	X	...	...
	11	RM27187-RM7654	X	...	...
	11	RM27389-RM144	X	X	X
JS02-68	12	RM1261-RM3326	X	X	X
	9	RM24019-RM285	X	X	X
	11	RM27088-RM27154	X	X	...
	11	RM27386-RM144	...	X	X
JS05-74	1	RM3482-RM3403	...	X*	...
	9	RM24019-RM285	X	X	X
	11	RM27154-RM27381	X	X	...
	11	RM27389-RM27385	...	...	X
JS2007-2-3	9	RM24019-RM285	...	X*	...
	11	RM27088-RM27381	X	X	X
JS02-95	4	RM335-RM3367	X	...	...
	7	RM11-RM336	...	X*	...
	9	RM285-RM3700	X	X	X
	11	RM27088-RM27154	X	...	...
JS2007-11-1	9	RM24019-RM285	X	X	X
	11	RM27088-RM27154	X	X	...
	11	RM27381-RM27386	...	...	X
JS02-43	11	RM6680-RM27088	X	...	...
	11	RM27088-RM27154	X	...	...
	11	RM27386-RM144	...	X	X
JS03-44	4	RM16616-RM3471	X	...	...
	9	RM24019-RM285	X	X	X
	11	RM27189-RM27187	X	...	...
	11	RM27386-RM144	...	X	X
JS90-78	1	RM3482-RM3403	X	...	...

(continued on next page)

^a Asterisk (*) indicates the minor quantitative trait loci detected by the composite interval mapping (CIM) of Windows QTL Cartographer 2.5 (52).

^b Mixed-model-based CIM.

95 and JS2007-22-2, were detected on chromosome 7 (LOD score = 2.54) and chromosome 11 (LOD score = 2.70), respectively (Table 2), implying that minor genes conferring blast resistance might exist in Suyunuo.

In addition, the results of QTL analysis do not correlate with the class of isolates based on the Chinese rice blast identification system (25). Some QTLs, such as *qtl11-5-5*, could be responsible for the resistance to multiple isolates, including B group (GD10-279a), C group (JS2004-141-1), E group (JS2004-185), F group (JS90-78), and G group (Hoku 1), but the resistance to isolates in the same group did not correspond to the same QTLs detected here (Table 2).

Many resistance genes have been mapped as a qualitative trait by using bulked-segregate analysis (BSA) in order to find tightly linked molecular markers (35). Through the BSA approach, several genes resistant to blast—*Pi5(t)*, *Pi36*, and *Pi27(t)*—were mapped onto chromosomes 8, 1, and 9, respectively (16,29,63). Recently, a new rice blast resistance gene, *Pi40*, was mapped onto chromosome 6 using the single-locus analysis of variance (ANOVA) test (17). QTL identification using the CIM approach, based on genetic a linkage map, can detect more than one gene and not only major loci but also minor loci associated with resistance (43). Li et al. (23) identified the resistance gene *Pi-hk1(t)* from Heikezijing using the BSA method (36). With 166 RILs and the CIM approach used in this study, three major tightly linked QTLs to Hoku 1 were detected, with LOD scores of 13.98, 38.15, and 17.55 and 33.5, 74.3, and 47.3% of the phenotypic

variance, respectively (Table 2). Thus, the major QTL (*qtl11-5-5*) with the highest LOD score might be *Pi-hk1(t)* identified by BSA (23). BSA may be considered suitable for finding a single pathogen resistance gene in a plant but it difficult to find gene-linked markers when there are two or more major genes corresponding to resistance to the same pathogen or when minor loci near the single gene could affect linkage analysis.

The frequency of polymorphisms between Heikezijing and Suyunuo was not as high as for the *indica/japonica* crosses. There are some crossover-cold regions that exist in this linkage map. One strategy to clone resistance genes in Heikezijing is to choose a line with a single resistant locus from RILs and cross with a susceptible *indica* cultivar.

In summary, we mapped rice blast resistance QTLs using RILs from the cross of Heikezijing × Suyunuo. The results showed that several QTLs with various contribution rates were responsible for the blast resistance in Heikezijing. In particular, one blast-resistance-gene cluster was found on chromosome 11. It will be valuable to further characterize and clone genes resistant to blast in this *japonica* landrace in order to breed new rice cultivars with resistance to blast using genetic engineering or molecular assistance selection approaches.

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TABLE 3. (continued from preceding page)

Isolates	Chromosome no.	Position	Mapmaker	CIM ^a	MCIM ^b
	1	RM1297-RM5853	...	X*	...
	1	RM5853-RM11179	X	...	...
	4	RM335-RM3367	X	...	...
	11	RM6680-RM27088	X	X	...
	11	RM27088-RM27381	X	X	X
	11	RM27389-RM144	X	X	X
JS2004-3	4	RM335-RM3367	X	...	...
	10	RM333-RM258	X	...	...
	11	RM6680-RM27088	X	...	...
	11	RM27088-RM27154	X	...	...
	11	RM27189-RM27389	X	X	X
JS2004-141-1	1	RM3482-RM3403	X	...	...
	8	RM1019-RM5853	X	...	...
	10	RM333-RM258	X	...	...
	11	RM6680-RM27088	X	X	...
	11	RM27088-RM27154	X	X	X
	11	RM27385-RM144	X	...	...
JS2007-22-2	9	RM24019-RM285	...	X	X
	11	RM2286-RM1812	...	X	...
	11	RM27154-RM27381	X	X	X
Hoku1	5	RM18932-RM480	X	...	...
	11	RM6680-RM27088	X	X	...
	11	RM27088-RM27154	X	X	...
	11	RM27187-RM7654	X	...	...
	11	RM27385-RM144	X	X	X
JS2006-25	7	RM3555-RM172	X	...	...
	9	RM24019-RM285	...	X	X
	11	RM27088-RM27154	X	X	...
	11	RM27389-RM27385	...	...	X
JS2004-185	9	RM24019-RM285	...	X	X
	11	RM27088-RM27154	X	X	X
	11	RM27385-RM144	...	...	X
GD10-279a	1	RM3482-RM3403	X	X*	...
	5	RM18932-RM480	X	...	...
	9	RM24019-RM285	X	X	X
	9	RM285-RM3700	X	...	...
	11	RM6680-RM27088	X	...	...
	11	RM27088-RM27154	X	X	...
	11	RM27187-RM7654	X	...	...
	11	RM27385-RM144	X	X	X
JS2007-2-2	9	RM24019-RM285	X	X	X
	11	RM27189-RM27381	X	X	X

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