

Mapping of QTLs Involved in Resistance to Rice Blast (*Magnaporthe grisea*) Using *Oryza minuta* Introgression Lines

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Abstract: Introgression lines derived from *Oryza minuta* and *O. sativa* subsp. *japonica* var. Junambyeo were crossed for a mapping of the population composed of 112 recombinant lines to identify putative QTLs against rice blast disease using the percentage of diseased leaf area. By using 148 Sequence Tagged Site (STS) and Single Sequence Repeat (SSR) markers, five QTLs on chromosomes 6, 7, 9 and 11 and seven epistatic QTLs were identified against two blast isolates (KI307 and KI209). Of them two QTLs (*qKI307-2* and *qKI209-3*) shared a similar position on chromosome 11. *O. minuta* introgression contributed the resistance allele for all of these QTLs. Combined phenotypic variations by QTL and (E-QTL) accounted for 56.9% against KI307, and 53.4% against KI209. Each QTL could account for the resistance variation between 11 and 24.6%. The resistance from wild introgressions was attributable to a combination of QTLs and epistatic effects between different loci, capable of inducing hypersensitive reactions. Our findings are in support of the strategy of pyramiding major QTLs to develop improved rice varieties with durable broad spectrum resistance against the blast fungus.

Keywords: blast; introgression from *Oryza minuta*; quantitative trait locus (QTL); rice

Rice blast, caused by *Magnaporthe grisea* (Hebert) Barr, has been one of the factors limiting rice production worldwide (OU 1985). Rice blast epidemics have been reported in several countries, particularly in Asia, typically resulting in 10–50% yield losses (LOAN *et al.* 2003). The disease has been identified in 85 rice-producing countries in both tropical and temperate zones, decreasing the yield of rice in Asia, Latin America and Africa (ROCA *et al.* 1996). Genetic studies of field resistance have been conducted using rice cultivars since the 1970s. Most studies (HIGASHI & KUSHIBUCHI

1978; MARUYAMA *et al.* 1983; HIGASHI & SAITO 1985; WANG *et al.* 1994; NAGATO & YOSHIMURA 1998; AHN *et al.* 2000; HAYASHI *et al.* 2004; ZHOU *et al.* 2004; WU *et al.* 2005; GOWDA *et al.* 2006; LI *et al.* 2007, 2008) showed that field resistance to blast is complex and controlled by multiple genes with complementary or additive effects (HE *et al.* 1989), as well as their environmental interactions (BONMAN 1992). To characterize field resistance genes, genome information and the materials with a genetic background are necessary (FUKUOKA & OKUNO 2001). However, varieties carrying genes

that confer high levels of resistance (R genes) typically lose their resistance after a few years (CHEN *et al.* 2003). Host-plant resistance based on the hypothesis of gene-for-gene interaction is the most economical and environmentally safe approach to control the disease (JIA *et al.* 2000). However, the pathogenicity of *M. grisea* isolates is highly variable and sometimes a small section of the virulent isolates spreads rapidly and overcomes resistance genes of rice cultivars (WANG *et al.* 1994; FUKUOKA & OKUNO 2001). Blast resistance in rice varieties is generally classified into 2 types: complete and incomplete (field or partial) resistance (YUOKI *et al.* 1970; HIRANO 1994; FUKUOKA & OKUNO 2001; MIYAMOTO *et al.* 2001; ZENBAYASHI *et al.* 2002). In general, field resistance is durable; hence the use of field resistance is one of the most promising measures for blast control (ITO & NAKANE 1973; HIRANO 1994; MIYAMOTO *et al.* 2001; ZENBAYASHI *et al.* 2002). Genetic analyses of resistance have identified resistance QTL in various germplasms and environments (SALLAUD *et al.* 2003; CHEN *et al.* 2003; TALUKDER *et al.* 2004). The detection of QTL represents the first step toward dissecting their molecular basis and their individual phenotypic effects in different environments, and also the first step in their manipulation in breeding materials by selection of linked markers. DNA markers which are closely linked to the targeted genes may contribute to the transfer of resistance genes into a desirable genetic background.

During the last decade, the genetics of blast resistance has been extensively studied and nearly 40 resistance genes have been identified. Most of the resistance genes are from landraces, of *indica* subspecies. Three wild relatives of rice have been used so far for their usefulness against blast resistance, *O. minuta* by LIU *et al.* (2002); *O. australiensis* by JEUNG *et al.* (2007), and *O. rufipogon* by BALLINI *et al.* (2007).

Introgression of agriculturally important genes from wild species and their domesticated relatives by classical hybridization has been practiced for many years, and many new varieties have emerged from such breeding programs. Due to the effect of recombination through repeated backcrossing, only a small fragment containing the desired gene(s) was usually introgressed into the genome of cultivated line (LIU *et al.* 2002). The introgression line from wild species shows stable and high levels of resistance to BPH and blast (BRAR & KHUSH 1997, 2002). Despite the usefulness of *O.*

minuta introgression line against biotic stress, its genetics has not yet been analysed using Korean blast isolates. The objectives of the present study were to identify the number of major resistance QTLs present in *O. minuta* introgression line and their main effect with epistatic interaction that are controlling blast resistance.

MATERIAL AND METHODS

Plant materials and DNA extraction

Introgression line IR71033-121-15 was developed from *Oryza minuta* at the International Rice Research Institute (IRRI) through embryo rescue as well as three back crosses were used and for this IR31917-45-3-2 was used as a recurrent parent. F₂ mapping population was developed from crossing the Korean elite japonica cultivar Junambyeo and *O. minuta* introgression line IR71033-121-15. 112 F₂ lines were grown in 2006 and total DNA was extracted from the leaf blades of each F₂ plant based on the CTAB method (MURRAY & THOMPSON 1980). Self-pollinated F₃ seeds of each F₂ plant were harvested for the evaluation of blast field resistance.

Parental reactions to blast isolates

Two IR lines with the Korean elite *japonica* cultivar Junambyeo were used for parental screening. Table 1 shows the disease scores of the parents (IR71033-121-15, IR31917-15-3-2) against ten Korean isolates of blast. Two isolates KI307 and KI209 among 8 isolates appeared to be the most virulent (Table 1). Therefore these two isolates (KI307 and KI209) were screened against the mapping population.

Evaluation of blast resistance

112 F₃ lines from each F₂ individual were tested for the degree of blast field resistance in an upland nursery according to the method described by ASAGA (1981), with two replications. Artificial inoculation of the two Korean blast isolates, KI 209 and KI307, was conducted in the nursery of Rural Development Administration (RDA), Korea, in 2008. At 21 days after seeding (DAS), a single-

Table 1. Reaction of parents against Korean isolates of *Magnaporthe grisea* using a six grade scale

No.	Name of the Korean races	Symptom grades		
		IR71033-121-15 ^a	IR31917-45-3-2 ^b	Junambyeo ^c
1	KI315b	0	3	2
2	KI1117	1	4	3
3	KJ101	0	3	4
4	KJ401	0	5	2
5	KJ203	0	5	4
6	KI307	0	5	5
7	KI313	0	3	3
8	KI209	0	5	5

^aIntrogression line from *O. minuta*; ^brecurrent parent used for somatic hybridization; ^csusceptible Korean *japonica* cultivar

race spore suspension was inoculated by spray method and spores were adjusted to a cell count as described by CHO *et al.* (2008). The inoculated seedlings were kept inside the incubation chamber at $26 \pm 1^\circ\text{C}$ with saturated humidity for 24 h and then transferred to the greenhouse until scoring time. Disease incidence was evaluated at 8 days after inoculation, using a 0–5 scale according to the method of VALNET *et al.* (1991), in which 0 indicated the absence of infection and 5 indicated most susceptible.

Map construction

A total of 148 well distributed polymorphic rice STS (RAHMAN 2008) and SSR (MCCOUCH *et al.* 2002) markers were used to construct a linkage map. The chromosomal location of each marker has been identified by EST mapping (WU *et al.* 2002) using a YAC-based physical map of rice. For all polymorphic markers, we have confirmed the chromosomal location by linkage analysis using 30 randomly selected *indica* and *japonica* back-cross inbred lines (BILs) (LIN *et al.* 1998). Each 25 ml reaction mixture contained 50 ng DNA, 5 pmol of each primer, 2 ml PCR buffer (100mM Tris (pH 8.3), 500mM KCl, 15mM MgCl₂, 2 mg gelatine), 250mM of each dNTPs and 0.5 unit Taq polymerase. All primers and reaction chemicals were used from Bioneer Company (<https://www.bioneer.co.kr>). Amplification was carried out in a PTC220 dual 96-well thermocycler (MJ Research, USA) as follows: 5 min at 94°C , followed by 35 cy-

cles of 1 min at 94°C , 1 min at 48°C (for STS) and 55°C (for SSR), 2 min at 72°C and 5 min at 72°C for final extension. Amplified PCR products were resolved by electrophoresis in 3% agarose gels and for SSR markers PCR products were run on 6% polyacrylamide denaturing gels.

Linkage and QTL analysis

Linkage analysis was performed with MAP-MAKER/EXP 3.0 (LANDER *et al.* 1987). Linkage groups and the order of the markers were determined using Joinmap (STAM 1993). The Kosambi mapping function (KOSAMBI 1944) was used to transform the recombination frequency to genetic distances (cM). Linkage groups were assigned to rice chromosomes according to the published rice map (CHEN *et al.* 1997; TEMNYKH *et al.* 2000). The QGENE 3.06 (NELSON 1997) and SAS were used for statistical analysis. The association between phenotype and genotype was investigated using an interval analysis. To determine empirical significance thresholds for declaring a QTL, 1000 permutations were done to calculate LOD thresholds for each trait at $P = 0.05$ and $P = 0.01$. The disease scores in the derived F₃ lines were used as trait data according to the method of HAYASHI & UKAI (1999); SATO *et al.* (2006). Analysis of epistatic interaction between two loci (E-QTL) was performed by the QTL-mapper 1.0 software (WANG *et al.* 1999). The phenotype variance (PVE) associated with each significant QTL was calculated from the regres-

sion of each marker-phenotype combination. The total phenotypic variation explained was estimated by fitting a model including all putative QTLs for the respective trait simultaneously. QTLs were named according to CHO *et al.* (2004).

RESULTS

Map construction

With the F_2 population, a framework linkage map with 148 STS and RM markers was constructed. The map covered 1455 cM for all 12 chromosomes with an average interval of 9.83 cM between the adjacent markers. The order of the markers in each chromosome was consistent with the order of the Nipponbare/Kasalath map (HARUSHIMA *et al.* 1998).

Variation of resistance to blast disease of F_3 lines

The frequency distribution of resistance levels of 112 $F_{2:3}$ segregating progenies exhibited continuous distribution (Figure 1), which indicated that blast resistance in IR71033-121-15 against two isolates (KI307 and KI209) was controlled by QTLs.

QTL affecting blast resistance

In the analysis of Junambyeo/IR71033 $F_{2:3}$ populations five putative QTLs were detected on chromosome 6, 7, 9 and 11 by single locus QTL analysis which could account for the variation of 55.3%. Seven epistatic QTLs were detected by using the random pair epistatic model analysis which could also account for the phenotypic variation of 55.3%.

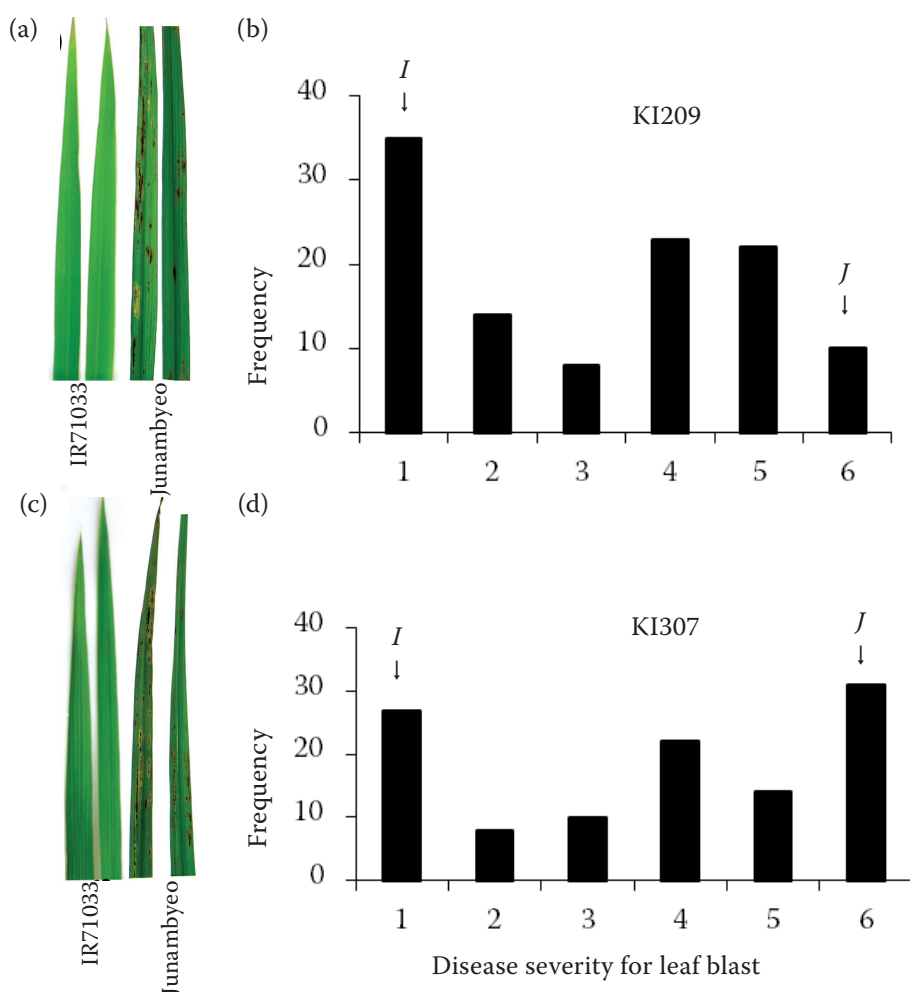


Figure 1. Disease reaction and frequency distribution of the scores for field resistance against two blast isolates (a, b for KI209 and c, d for KI307) in F_3 lines from a cross between introgression line and Junambyeo; I and J indicate the score of the parents IR71033 and Junambyeo, respectively

Table 2. Single locus QTL analysis against two isolates in a mapping population

Isolates ^a	QTLs	Chr	Linked marker	Interval (cM)	<i>F</i>	LOD	<i>R</i> ² (%) ^b	Permutation ^c (%)		Additive effect
								95	99	
KI307	<i>qKI307-1</i>	9	S9026	0–26	17.4	6.8	24.6	2.3	3.1	1.5
	<i>qKI307-2</i>	11	S11077.4	65–73	8.5	3.5	13.5	2.2	3.1	1.1
Total							<i>31.1</i>			
KI209	<i>qKI209-1</i>	6	S6065	80–84	7.3	3.1	11.9	2.5	3.1	1
	<i>qKI209-2</i>	7	S7048	37–40	6.5	2.8	10.9	2.3	3.5	0.9
	<i>qKI209-3</i>	11	S11077.4	68–73	9.1	3.8	14.5	2.4	3.3	1.2
Total							<i>24.2</i>			

^aIsolates compatible with the parents are considered for genetic analysis; ^bquantitatively acquired data sets for blast-resistance levels of each line; the explainable phenotypic variation explained (PVE) portion at the tested locus (*R*²) and the total value of *R*² is the multiple regression of all QTL loci PVE value indicated in italics; ^cempirical significance thresholds for declaring a QTL at *P* = 0.05 and *P* = 0.01

Table 2 and Figure 2 show five QTLs affecting resistance against two Korean blast isolates. All these QTLs were detected based on the LOD scores. Two

QTLs for KI307 were detected and designated as *qKI307-1* and *qKI307-2* located on chromosome 9 and 11, respectively. Three major QTLs for KI209

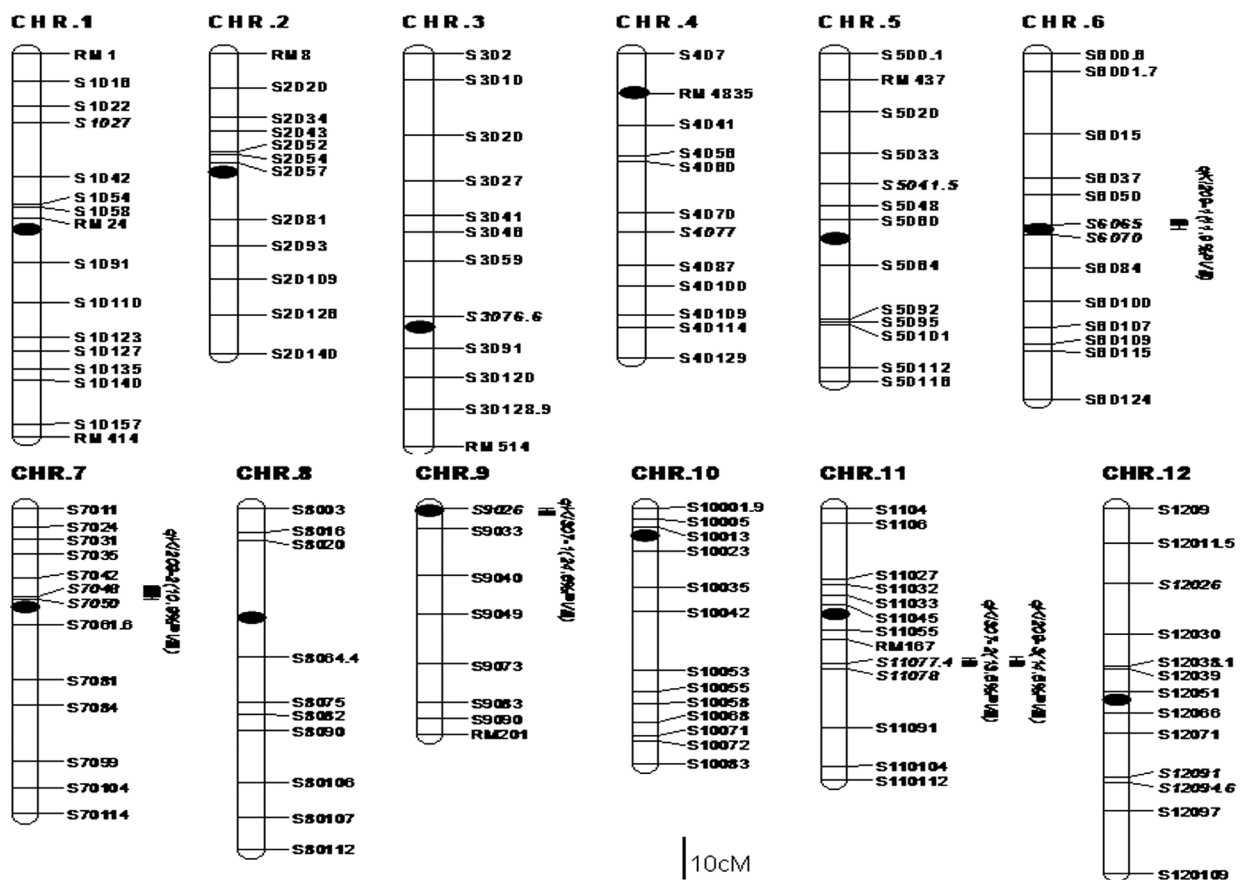


Figure 2. Mapping position of major QTLs and percentage of value explained (PVE%) detected from *O. minuta*; markers shown positive for alien introgression are in italics and black solid circles represent centromere positions; bar = 10 cM

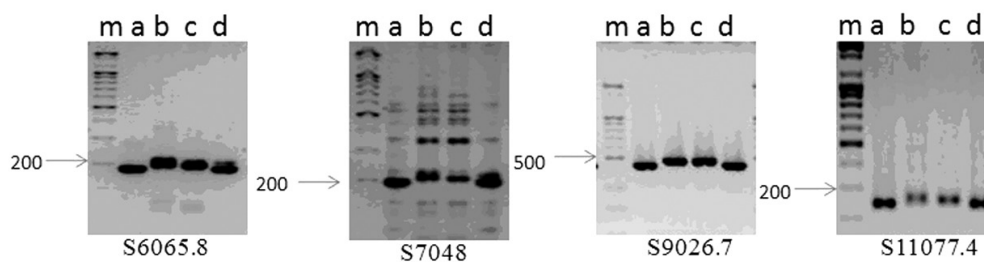


Figure 3. Positive introgression shown by linked markers where m – marker size, a – IR71033, b – Junambyeo, c – IR31917 and d – *O. minuta*; introgression line produced the same amplicon as the wild type but the difference from its recurrent parent indicates positive introgression in those specific regions

were detected and designed as *qKI209-1*, *qKI209-2*, *qKI209-3*, located on chromosome 6, 7 and 11, respectively. QTLs which were linked to STS markers, S9025 and S11077, on chromosome 9 and 11 held 31.1% of the variance for resistance and QTLs which were linked to markers, S6065, S7048 and S11077, on chromosome 6, 7 and 11 held 24.2% of the variance for resistance (Table 3). Among the 5 QTLs two QTLs, *qKI307-2* and *qK209-3*, shared a similar position on chromosome 11. The IR71033 alleles from wild introgression to all QTLs reduced the disease severity, as anticipated on the basis of IR71033 phenotype (Figure 1).

Table 3 and Figure 2 show 7 pairs of digenic epistatic QTLs (E-QTLs) for resistance against two blast isolates. Among them, two E-QTLs shared a similar position with the markers associated with the markers for QTLs for blast resistance. Positive epistatic effects indicated that the recombinant types of alleles at the interaction loci were expected to result in resistance. Above all, the contribution of E-QTLs to blast resistance was higher than that of QTLs.

Evidence of *O. minuta* chromosome segment integration

To test whether the two chromosomal regions containing resistant genes originated from the wild species *O. minuta*, the alleles of four parental lines were compared with the candidate markers (S6065, S7048, S9026.7, and S11077.4 on chromosome 6, 7, 9 and 11) that showed blast resistance. On 4 regions, the developed markers produced the same PCR amplicon between *O. minuta* (a) and IR71033 (d), which was different from the amplicon generated in the recurrent parent IR31917-45-3-2 (b) and Junambyeo (c). This phenomenon showed evidence of positive introgression in those particular positions (Figure 3).

DISCUSSION

Qualitatively expressed data may cause serious bias per se, due to phenotypic mis-scoring as well

Table 3. Epistatic interaction between random markers

Isolates	Marker A	Chr	Marker B	Chr	<i>F</i>	<i>R</i> ² (%) ^a	<i>P</i>
KI307	S3076.6	3	RM4835	4	17.9	7.7	0.0001
	S30128.9	3	S12026	12	27.9	12.1	0.0000
	<i>qKI209-2</i>	7	<i>qKI307-2</i>	11	11.7	5.1	0.0009
Total						25.9	
KI209	<i>qKI209-2</i>	7	S12026	12	18.8	8.7	0.0000
	S30120	3	S5041.5	5	22.1	10.2	0.0000
	S500.1	5	S12094.6	12	11.5	5.3	0.0009
	S8064.4	8	S12038.1	12	11.1	5.1	0.0012
Total						29.4	

^aTotal value of *R*² is the multiple regression of E-QTL loci PVE value indicated in italics

as environment fluctuations during evaluation (TABIEN *et al.* 2002). To overcome these practical problems, we made a quantitative interpretation of the disease reaction data, since it is less sensitive to even modest numbers of phenotypic mis-scores. Based on the principles of linkage disequilibrium (REMINGTON *et al.* 2001), and the observed abnormality of frequency distribution of resistance levels from $F_{2.3}$ segregating progenies indicated that blast resistance from *O. minuta* is controlled by multiple loci.

Our study by the classical molecular approach of QTL mapping could be used in linked DNA markers as a tool for identifying and mapping a new blast resistance gene. Comparing blast resistance genes identified in different countries has been difficult mainly due to problems associated with obtaining blast fungal isolates from abroad. With the advent of molecular markers it became possible to compare resistance genes based on their map locations. One QTL named *qKI-209-1* was also detected on chromosome 6, linked with the marker S6065. The blast resistance genes *Pi2(t)* and *Pi9(t)* were previously mapped in a similar position on chromosome 6, of them *Pi9(t)* was reported from *O. minuta* introgression against Philippine isolates (LIU *et al.* 2002), suggesting that *O. minuta* might confer resistance to the broad range of rice blast spectrum. Another resistance gene *Pi40(t)* from *O. australiensis* shared a similar position against Korean isolates (JEUNG *et al.* 2007). It is notable that blast resistance of different wild origin like *Pi9(t)* from *O. minuta* covers the region between two RFLP markers RG64 and R2131 (LIU *et al.* 2002) and *Pi40(t)* from *O. australiensis* covers the region between two SSR markers RM527 and RM3330 (JEUNG *et al.* 2007) in a similar position on chromosome 6. The physical distance between two candidate markers (RG64 and RM527) is 337kb by (http://www.gramene.org/Multi/blastview/BLA_hXJKZNXea). QTL for lesion size resistance was also reported in a similar position on chromosome 6 by JIEYUN *et al.* (1999). Another QTL for leaf blast against the Thailand isolate THL84 was also reported by SIRITHUNYA *et al.* (2002), in a similar position on chromosome 6. The QTL for blast resistance *qK209-2* was not reported indicating a novel QTL from *O. minuta* although TABIEN *et al.* (2002) reported a QTL vicinity with it on chromosome 7 but not similar to *qK209-2*. Two QTLs when using Korean isolates, KJ-301 and KI313 (CHO

et al. 2004), were reported in a similar position with QTL, *qKI307-1* on chromosome 9. PAN *et al.* (2003) reported four genes *Pii*, *Pi3(t)*, *Pi5(t)* and *Pi15(t)* that were located in a similar position on chromosome 9. Two QTLs (*qKI209-3*, *qKI307-2*) shared a similar region on chromosome 11 linked with the marker S11077.4, previously studied by other groups (MEW *et al.* 1994; YU *et al.* 1996) on a distal region of chromosome 11. This region of rice chromosome 11 carrying *Pi-1(t)* also harbours the blast resistance gene *Pi-K* (KIYOSAWA 1981). The map locations on chromosome 6 and 9 for leaf blast resistance corresponded with other published reports indicating the complexity of this disease and its genetic control (SIRITHUNYA *et al.* 2002). The significant interactions between random markers indicated the importance of epistasis in determining the broad range of resistance spectrum. Significant epistatic interactions between random markers were found to play a certain role in the expression of resistance to rice blast.

CONCLUSIONS

Rice blast continues to be one of the most devastating diseases in rice production worldwide, and breeding of cultivars with durable resistance is one of the major objectives of current breeding programs. The markers linked to QTL identified in this study can be directly used in marker-aided selection (MAS), although phenotypic variation explained (PVE) by *O. minuta* was not as great as explained resistance against brown planthopper (BPH) (RAHMAN *et al.* 2009). Pyramiding these QTLs into existing cultivars will lead to the development of highly resistant rice cultivars. The wild origin of the resistance gene may also contribute to its durability because it has been under the pressure of natural selection in the wild for many years (LIU *et al.* 2002).

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