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Identification of quantitative trait loci for blast resistance in BC_2F_3 and BC_2F_5 advanced backcross families of rice

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ABSTRACT. Advanced backcross families derived from *Oryza sativa* cv MR219/*O. rufipogon* IRGC105491 were utilized for identification of quantitative trait loci (QTL) for blast resistance using simple sequence repeat markers. Two hundred and sixty-one BC_2F_3 families were used to construct a linkage map, using 87 markers, which covered 2375.2 cM of 12 rice chromosomes, with a mean density of 27.3 cM. The families were evaluated in a greenhouse for resistance to blast disease caused by pathotypes P7.2 and P5.0 of *Magnaporthe oryzae*. Five QTLs (qBL5.1, qBL5.2, qBL6.1, qBL8.1, and qBL10.1) for

pathotype P5.0 and four QTLs (qBL5.3, qBL5.4, qBL7.1, and qBL8.2) for pathotype P7.2 were identified using the BC_2F_3 families. Another linkage map was also constructed based on 31 BC_2F_5 families, using 63 SSR markers, which covered 474.9 cM of 9 rice chromosomes, with a mean density of 8.01 cM. Five suggestive QTLs (qBL11.2, qBL11.3, qBL12.1, qBL12.2, qBL12.3) and one putative QTL (qBL2.1) were identified for pathotype P7.2. Also, seven suggestive QTLs (qBL1.1, qBL2.2, qBL4.1, qBL4.2, qBL5.3, qBL8.3, and qBL11.1) were detected for pathotype P5.0. We conclude that there is a non-race-specific resistance spectrum of *O. rufipogon* against *M. oryzae* pathotypes.

Key words: Quantitative trait loci; Disease resistance; Rice; Advanced backcross; *Oryza rufipogon*

INTRODUCTION

Wild species are valued as unique sources of genetic variation, but they have rarely been used for genetic improvement of quantitative traits. Wild rice (*Oryza rufipogon* Griff.) has many agronomically useful characters, such as resistance to diseases and pests and strong deepwater tolerance, and it produces fertile F_1 hybrids with cultivars (Khush, 1997). It is distributed in the marshy areas of tropical and subtropical Asia. Considerable genetic diversity has been reported within natural populations of *O. rufipogon* in Malaysia (Ngu et al., 2010). *O. rufipogon* is considered the progenitor of *O. sativa*. Therefore, it has been used as an important genetic resource for rice breeding. *O. rufipogon* is resistant to many rice diseases including blast, bacterial blight, and tungro disease (Ram et al., 2007). Several researchers have reported that *O. rufipogon* has resilience to blast disease. *O. rufipogon* has been classified as a promising species that has either the *Pi-ta* or the *Pi-b* allele (Eizenga et al., 2009), and some studies have found that the *Pi-ta* gene in *O. rufipogon* is similar to that of *O. sativa* but significantly different from other *O. rufipogon* genes (Huang et al., 2008). Successful introgression of broad-spectrum blast resistance gene(s) into cultivated rice (*O. sativa* ssp *indica*) from *O. rufipogon* has been reported (Ram et al., 2007).

The introgressed lines in this study showed resistance to 14 isolates and moderate resistance to 2 additional isolates of blast (Abdul Rahim H and Wickneswari R, unpublished results), indicating the introgression of a broad spectrum of resistance to blast from *O. rufipogon*. Geographic distribution surveys of *O. rufipogon* conducted by the International Rice Research Institute demonstrated that *O. rufipogon* accession from Malaysia (OR22) exhibited resistance to blast disease (Huang et al., 2008). Gene flow from cultivated rice also occurs, which can persist in wild rice populations, change its genetic composition, and affect the population genetics of wild rice (Ngu et al., 2010). Among the characteristics of *O. rufipogon* IRGC105491 that implies introgression from *O. sativa* are its upright growth habit and slightly high number of grains per panicle.

The advent of molecular markers and maps has allowed the identification of individual quantitative trait loci (QTLs) associated with yield and its components, environmental stress tolerance, disease and insect resistance, and quality traits in a variety of crop plants

(McCouch et al., 2001). Molecular markers have been used to establish linkage maps for many crop species, which can in turn determine gene number for particular traits and for gene tagging (Paterson et al., 1991). Many important disease resistance genes have been mapped and tagged in various crops (Mohan et al., 1997).

One of the strategies used to broaden the genetic diversity of cultivated crops is to identify QTLs associated with beneficial traits such as yield, grain or fruit quality, and disease resistance in interspecific backcross populations involving wild germplasm and elite cultivars (Tanksley and McCouch, 1997). A modification of the advanced backcross QTL strategy (Tanksley and Nelson, 1996) has been undertaken to identify QTLs related to morphology and yield components in an advanced backcross population derived from *O. sativa* cv. MR219 and *O. rufipogon* (Sabu et al., 2006). A total of 261 advanced backcrossed lines have been genotyped with 87 microsatellite markers and evaluated in the field for plant morphology and yield, and this population was used in our experiment. The objectives of this study were to screen BC_2F_3 and BC_2F_5 families from the cross between *O. rufipogon* IRGC105491 and *O. sativa* ssp *indica* cv MR219 for blast resistance using the 2 most virulent blast pathotypes of *Magnaporthe oryzae* (i.e., P7.2 and P5.0) and to detect QTLs for blast resistance in the BC_2F_3 and BC_2F_5 families.

MATERIAL AND METHODS

Mapping populations and blast pathotypes

The BC_2F_3 (Sabu et al., 2006) and BC_2F_5 (Abdullah MZ, unpublished results) mapping populations used in this study were developed at the Rice Gene Bank, Malaysian Agricultural Research and Development Institute (MARDI), Pulau Pinang. The blast pathotypes P7.2 and P5.0 were used to challenge the plants.

DNA isolation

The rice plants belonging to 261 lines of BC_2F_3 and 31 lines of BC_2F_5 and 2 parents (*O. rufipogon* and MR219) were grown at the greenhouse of the Malaysian Nuclear Agency (MNA). Leaf samples of the lines and parents were collected from 30-day-old seedlings. Young leaves of five plants of each line were randomly sampled for pooled DNA extraction. Total genomic DNA was extracted from young, fresh leaves using the cetyltrimethylammonium bromide method (Gawel and Jarret, 1991).

Polymerase chain reaction (PCR) amplification

PCR amplifications were performed using 12.5-µL reaction mixtures containing 5 ng template DNA, 0.2 µM of each primer, 100 µM of each deoxyribonucleotide triphosphate, 1.5 mM $MgCl_2$, 0.5 U *Taq* polymerase, and 1X PCR buffer. The following PCR profile was used: 94°C for 5 min, followed by 35 cycles of 94°C for 30 s, 55°C for 30 s, and 72°C for 30 s, and a final extension of 5 min at 72°C. For some specific complementary DNA-derived microsatellites, 2 annealing temperatures (61° and 67°C) were used (Temnykh et al., 2000).

Genotype determination

Genotypic data of the 261 BC_2F_3 families used in this study were obtained from a previous investigation (Lim, 2007) to cover the whole genome for 12 chromosomes (Chrs) even though some markers were unrelated to blast disease. Sixty-three simple sequence repeat (SSR) markers linked to blast disease were used for genotyping the BC_2F_5 families. The forward primers were fluorescence labelled at the 5'-end with either 6-FAM, VIC, NED, or PET dye from set G5 (Applied Biosystems, Foster city, CA, USA) and incorporated into the PCR. Approximately 1 µL of the pooled amplified products from 4 fluorescent dyes were added to 0.13 µL LIZ-500 internal size standard and topped off to a final volume of 10 µL formamide for fragment detection using capillary separation on an ABI3100 Genetic Analyzer (Applied Biosystems) at MNA. Fragment lengths were estimated with the GeneScan Analysis Software and scored using Genotyper (Applied Biosystems).

Phenotyping for blast disease traits

Two pathotypes of *M. oryzae*, namely P7.2 and P5.0 were used in this study. A pathogenicity assay was performed following standard procedures (Jia et al., 2003). Seeds were soaked for 1 day and sown in a 15 x 30 x 4-cm plastic tray containing sieved topsoil media. The nursery trial on blast was conducted at MARDI in a randomized complete block design with 3 replications and 10 plants per line for each replicate. The rice plants were inoculated with blast pathotype spore suspension (1 x 10^5 spores/mL) 21 days after sowing at 20 mL/tray using an atomizer with control. Plants were incubated in a dark dew chamber for 24 h at 25°-28°C. After 24 h, the plants were returned to the greenhouse with a controlled water sprinkler to maintain the humidity around the plants. Disease reactions were recorded as the number of plants infected by a particular pathotype observed after 7 days of inoculation with the blast spores. Five infected leaves were recorded for each replication. The disease scales were averaged from 3 replications. A trait distribution analysis was carried out using Minitab (v. 11; State College, PA, USA).

Linkage map construction

An SSR linkage map was constructed using Map Manager QTX (Kim and Kenneth, 2002). Linkage evaluation was carried out using the Advanced Backcross-2 option in the program. Map distances were estimated with the Kosambi map function following a Search Linkage Criterion of 0.05. The SSR markers were distributed over 12 linkage groups (Chrs) based on information from previous studies (Moncada et al., 2001; Brondani et al., 2002; Monosi et al., 2004).

QTL analysis

QTL identification was performed using Map Manager QTX version b19, and QGENE version 4.2 was used to detect significant QTLs through permutation analysis and trait analysis to determine trait distribution in both populations. The mean value of the phenotypic score for the BC_2F_3 and BC_2F_5 families and the genotype data of 261 BC_2F_3 and 31

BC_2F_5 were used in the analysis. The text file was converted into a qdf file before loading into QGENE to detect any significance of the QTLs through interval mapping and permutation analysis.

The associations between individual marker loci and QTLs for blast disease lesions of pathotypes P7.2 and P5.0 were evaluated with a single-marker analysis using Map Manager. The simple interval analysis via a flanking-marker regression method (Haley and Knott, 1992) was also performed under this program to identify QTLs on the genetic maps of 12 Chrs in the BC_2F_3 and BC_2F_5 populations. The interval size was set at 2 cM, and the threshold value of the logarithm of odds (LOD) score was set at 2.0 to detect the presence of a QTL (Shindo et al., 2003). The significant level of the LOD threshold was examined by permuting the data using the permutation analysis module (Churchill and Doerge, 1994). The parameters, BC_2 population structure, 2-cM intervals, and Kosambi function were selected for the interval mapping analysis. To identify an accurate significant threshold for each trait, an empirical threshold was determined for interval mapping using 1000 permutations for each trait across all 12 Chrs (Churchill and Doerge, 1994).

To identify additional QTLs and to increase the resolution of QTL locations, we used composite interval mapping (Zeng, 1994). Automatic cofactor selection using a forward/backward regression (forward, $P < 0.01$; backward, $P < 0.01$) was also performed with QGENE (Nelson, 1997). Significant thresholds for composite interval mapping were determined using 1000 permutations for each trait. A putative QTL was reported if detected by single-marker analysis and either interval mapping or composite interval mapping at an LOD >3.0. Identified QTLs were named according to accepted methods (McCouch et al., 1997).

RESULTS

Trait distributions

The frequency distribution for the 2 phenotypic traits in BC_2F_3 and BC_2F_5 families are shown in Figures 1 and 2, respectively.

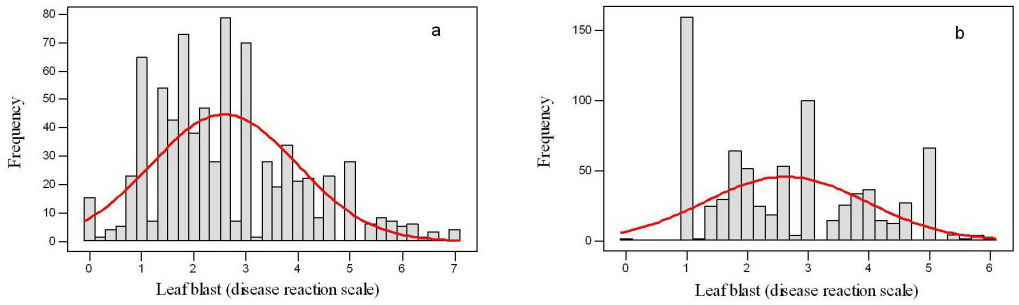

Figure 1. Histograms of 261 BC_2F_3 families showing normal curve for disease lesion against pathotype P7.2 (**a**) and P5.0 (**b**).

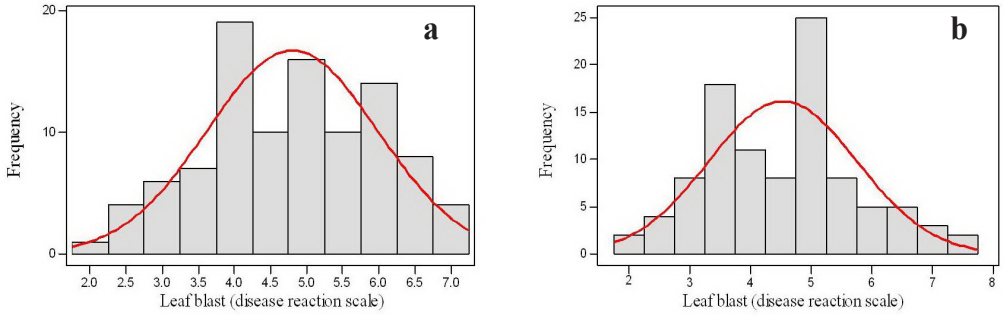

Figure 2. Histograms of 31 BC_2F_5 families showing normal curve for disease lesion against pathotype P7.2 (**a**) and P5.0 (**b**).

Linkage map construction

The 87 SSR markers genotyped in 261 families were used in the linkage analysis of the BC_2F_3 population. Each of the 12 linkage groups (Chrs) contained, on average, 8 loci: a minimum of 3 (e.g., Chr 9) and a maximum of 13 (e.g., Chr 2). The 87 SSR markers covered 2375.2 cM of the rice genome; the mean SSR density was 27.3 cM (Figure 3).

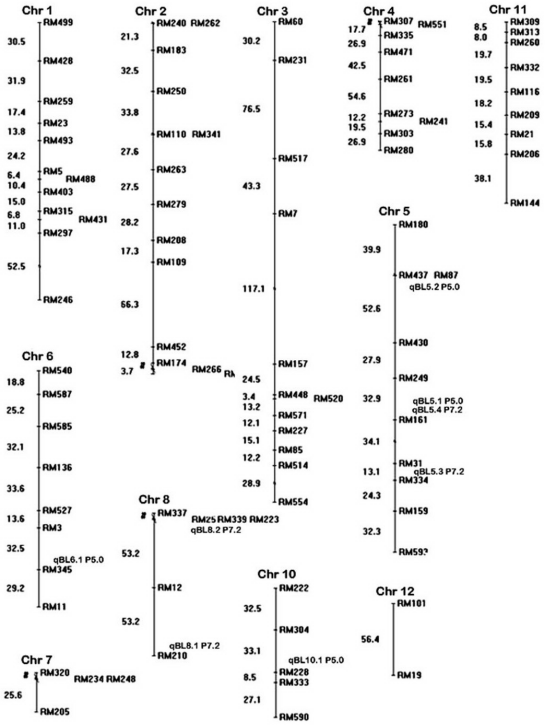

Figure 3. Rice simple sequence repeat (SSR) linkage map constructed using 87 SSR markers - nine suggestive QTLs were detected from the BC_2F_3 families from cross *Oryza rufipogon* x *O. sativa* cv. MR219.

A total of 63 SSR markers genotyped in 31 families were used in the linkage analysis of the BC_2F_5 population. Only 9 Chrs (1-6, 8, 11, and 12) of the SSR loci studied had linkages. No linkages were found for Chrs 7, 9, and 10. Each of the 9 linkage groups (Chrs) contained an average of 6 loci: a minimum of 2 (e.g., Chr 4) and a maximum 14 (e.g., Chr 1). The 63 SSR markers covered 456 cM of the rice genome; the mean SSR density was 8.6 cM (Figure 4).

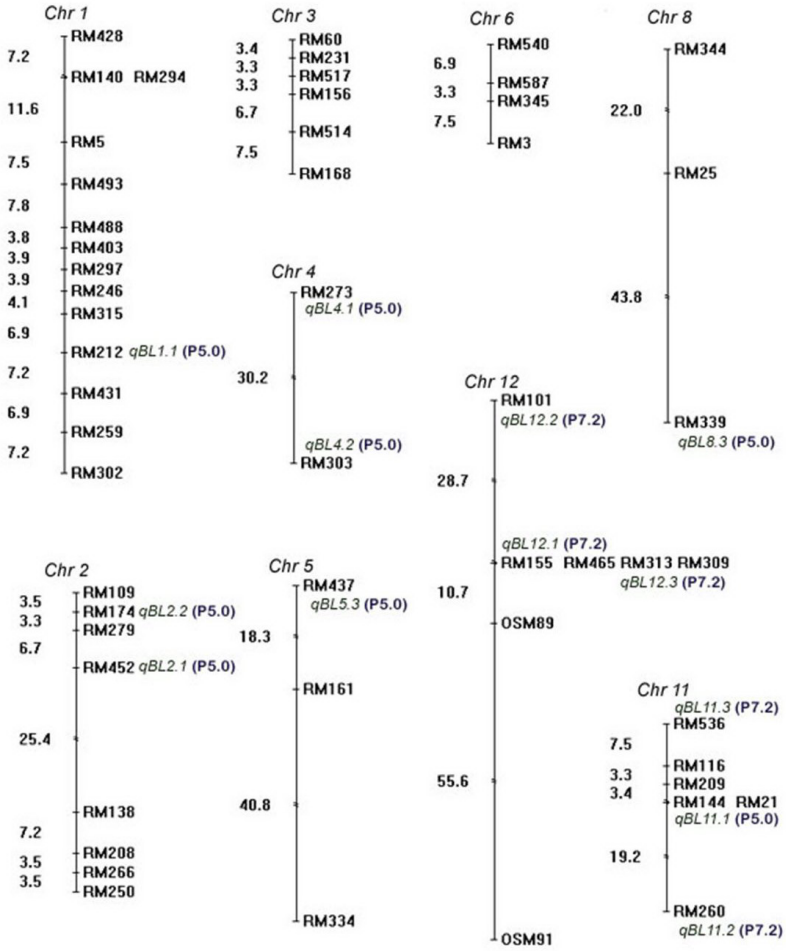

Figure 4. Rice simple sequence repeat (SSR) linkages map constructed using 63 SSR markers - 12 suggestive and 1 significant QTL were detected from the BC_2F_5 families from cross *Oryza rufipogon* x *O. sativa* cv. MR219.

QTL identification in BC_2F_3 population

A single-point marker analysis by means of marker regression resulted in 9 QTLs (suggestive) for each of the traits studied (Table 1).

Table 1. Nine quantitative trait loci (QTL) detected in the BC_2F_3 families from *Oryza rufipogon* 'IRGC105491' x *O. sativa* 'MR219'.

Trait	Locus	QTL	Chr	LRS	Trait variance (%)	P	A	P/N	Advantage (%)
BL (P7.2)	RM334	qBL5.3	5	4.3	2	0.037	-0.3	-	-8.25
	RM161	qBL5.4	5	3.9	1	0.048	0.2	+	6.35
	RM180	qBL7.1	7	4.4	2	0.036	0.2	+	6.03
	RM25	qBL8.2	8	4.4	2	0.036	-0.2	-	-6.35
BL (P5.0)	RM161	qBL5.1	5	6.5	2	0.011	0.2	+	7.34
	RM437	qBL5.2	5	5.4	3	0.02	-0.3	-	-9.17
	RM345	qBL6.1	6	4.2	2	0.041	-0.2	-	-6.42
	RM210	qBL8.1	8	4.6	2	0.032	-0.2	-	-4.89
	RM228	qBL10.1	10	4.9	2	0.026	-0.2	-	-6.12

BL = blast lesion; Chr = chromosomal assignment of SSRs; LRS = likelihood ratio statistics for the association of the trait with this locus at P = 0.05; Trait variance (%) = amount of the total trait variance that would be explained by a QTL at this locus, as a percent; P = probability of an association with this strong happening by chance; A = additive regression coefficient for the association; P/N = positive (+) or negative (-) effect of the QTL-associated *O. rufipogon* allele; Advantage (%) = trait advantage due to the QTL-associated *O. rufipogon* allele calculated as the percentage of additive regression coefficient to the trait effect of 'MR219'. The QTL nomenclature is according to McCouch et al. (1997).

Resistance to blast infection due to pathotype P7.2

Four suggestive QTLs were located for P7.2. Two suggestive QTLs, RM161 (Chr5) and RM180 (Chr 7) increased resistance to leaf blast infection by 6.35 and 6.03%, respectively. The explained trait variances due to these QTLs were 1 and 2%, respectively.

Resistance to blast infection due to pathotype P5.0

Five suggestive QTLs were found for P5.0. All suggestive QTLs were distributed on Chrs 5, 6, 8, and 10. Of these, only 1 showed positive effect for resistance to blast associated with the *O. rufipogon* allele at RM161 (Chr 5), and it increased resistance to blast infection by 7.34%. The explained trait variance due to this QTL was 2%.

Nine QTLs were mapped on the BC_2F_3 population, 4 of which were associated with pathotype P7.2 and 5 with pathotype P5.0. Four QTLs (qBL5.1, qBL5.2, qBL5.3, and qBL5.4) were identified on Chr 5, 2 (qBL8.1, qBL8.2) were identified on Chr 8, and 3 (qBL6.1, qBL7.1, and qBL10.1) were identified on Chrs 6, 7, and 10. These loci control 1-3% of the phenotypic variance and show a resistance to leaf blast infection that is increased by 6.03 to 7.34%. All QTLs observed in BC_2F_3 were insignificant, as the likelihood ratio values were below 15.

QTL identification in the BC_2F_5 population

A single-point marker analysis and interval mapping were carried out for QTL analysis of the BC_2F_5 population. QTL localization performed using a marker regression method resulted in 11 (suggestive) and 2 (significant) QTLs for each of the traits studied (Table 2).

Table 2. List of 13 quantitative trait loci (QTLs) detected from the BC_2F_5 families from *Oryza rufipogon* x 'MR219'.

Trait	Locus	QTL	Chr	LRS		Variance (%)	P	A	P/N	Advantage (%)
				SMA	IM					
BL (P7.2)	RM260	qBL11.2	11	9.3		27	0.00232	-0.37	-	-9.74
	RM155	qBL12.1	12	5.1		15	0.0234	-0.39	-	-10.26
	RM101	qBL12.2	12	10.6		29	0.00111	-0.42	-	-11.05
	RM313	qBL12.3	12	4.9		14	0.02762	-0.31	-	-8.16
	RM536	qBL11.3	11	3.9		13	0.04693	-0.5	-	-13.16
BL (P5.0)	RM273	qBL4.1	4	4.7	7.5	22	0.03084	-0.59	-	-17.35
	RM303	qBL4.2	4	4.9		15	0.02705	-0.49	-	-14.41
	RM437	qBL5.3	5	7.1	7.9	23	0.00783	1.03	+	30.29
	RM339	qBL8.3	8	3.9		12	0.04821	0.28	+	8.24
	RM212	qBL1.1	1	6.1		18	0.01335	-0.36	-	-10.59
	RM452	qBL2.1	2	14.6	15	40	0.00013	-0.56	-	-16.47
	RM174	qBL2.2	2	11.5		31	0.00068	-0.4	-	-11.76
	RM144	qBL11.1	11	4.6		14	0.03154	0.56	+	16.47

SMA = single-marker locus analysis; IM = interval mapping. For other abbreviations, see legend to Table 1. The QTL nomenclature is according to McCouch et al. (1997).

Resistance to blast infection due to pathotype P7.2

Five QTLs distributed on Chrs 11 and 12 were located for P7.2. Three were suggestive and 2 were significant. The significant QTLs were found at RM260 (Chr11) and RM101 (Chr12). However, no QTL was found with the positive effect of resistance to blast.

Resistance to blast infection due to pathotype P5.0

Eight QTLs were located for P5.0; 6 were suggestive and 2 were significant. All suggestive QTLs were distributed on Chrs 1, 4, 5, 8, and 11, whereas significant QTLs were on Chr 2. Of these, only 3 had the positive effect of resistance to blast associated with the *O. rufipogon* allele. The positive effects were found at RM437 (Chr 5), RM339 (Chr 8), and RM144 (Chr11), increasing resistance to blast infection by 30.29, 8.24, and 16.47%, respectively. The explained trait variances owing to these QTLs were 23, 12, and 14%.

Thirteen QTLs were detected in the BC_2F_5 population, in which 5 QTLs were associated with pathotype P7.2 and 8 with pathotype P5.0 on Chrs 1, 2, 4, 5, 8, 11, and 12. Three QTLs (qBL11.2, qBL11.3, and qBL11.1) were identified on Chr 11 (see Figure 4). These loci controlled 13-27% of the phenotypic variance. Three QTLs (qBL12.1, qBL12.2, and qBL12.3) were identified on Chr 12, the loci of which controlled 14-29% of the phenotypic variance. Four QTLs (qBL2.1, qBL2.2, qBL4.1, and qBL4.2) were identified on Chrs 2 and 4. These loci controlled about 15-22 and 31-40% of the phenotypic variance, respectively. Three QTLs (qBL1.1, qBL5.3, and qBL8.3) were detected on Chrs 1, 5, and 8, respectively. These loci controlled 12-23% of the phenotypic variance.

DISCUSSION

The Kolmogorov-Smirnov normality test of the BC_2F_3 families showed a P value of <0.01 for the blast lesion trait for pathotype P7.2, indicating that the observed data are not normally distributed. The leaf blast disease reaction scale showed the widest range of variation in

both traits (P7.2 and P5.0). The plant score for P7.2 was less than that of P5.0. Approximately 15 and 20% of the plant frequency scores were <1 for P7.2 and P5.0, respectively. The normality test in the BC_2F_5 families for the leaf blast trait for pathotypes P7.2 and P5.0 showed non-significance at $P > 0.05$, with a P value of 0.133 and 0.15, respectively. These results imply that the leaf blast trait data observed in this experiment were normally distributed (Wardlaw, 1999).

QTL analysis detected only suggestive QTLs in the BC_2F_3 population. Several factors influence the detection of QTLs in a population. The size of the mapping population (Haley and Andersson, 1997) and the marker coverage within a linkage map (Hackett, 2002) can affect the detection of highly significant QTLs. The genome of the advanced lines (BC_2F_5) varied because they belong to a segregating population. Furthermore, disease infection is influenced by environment, as is the case with yield-related traits in segregating populations (Sabu et al., 2009); hence, variation in the genotypic and phenotypic evaluation may occur, making the detection of significant QTLs during linkage mapping analysis difficult.

The same population used in the present study has been studied earlier (Wickneswari et al., 2012) to identify QTLs related to agronomic traits. The backcross population of the F_2 generation was 75% heterozygous. Similar results of heterozygosity in the early generation of the backcross population have also been reported elsewhere (Xiao et al., 1998). The accuracy of phenotypic evaluation is of utmost importance for the accuracy of QTL mapping (Haley and Andersson, 1997).

Validation of significant QTLs detected on Chr 2 in BC_2F_5

A higher number of QTLs and different values between the QTLs were detected with various mapping methods in other studies (Moncada et al., 2001). Those mapping methods, when used together with single-marker analysis, suggest the presence of putative QTLs that can be further tested for confirmation. In the present study, a QTL (qBL2.1 for the P5.0 trait) around RM452 on Chr 2 with a likelihood ratio statistics **(**LRS) value of 14.6 on single-marker analysis obtained using Map Manager was analyzed with composite interval mapping using QGENE (Nelson, 1997). The LOD score was 2.8, which was calculated after 1000 iterations (alpha 0.05 = 2.5; i.e., 5% level) upon permutation analysis. The threshold value of the LOD score was set at 2.0 for the detection of a QTL (Shindo et al., 2003). Therefore, the QTL region found to be significant after permutation analysis could have 1 putative QTL for blast resistance against the P5.0 pathotype.

QTLs of agronomic traits identified previously

The population of BC_2F_2 derived from *O. rufipogon* and MR219 has been used in a previous study to identify QTLs related to yield (Wickneswari et al., 2012). A total of 261 lines were genotyped with 96 DNA microsatellite markers and evaluated in the field for morphology, yield components, and growth period. A total of 41 QTLs with LODs of >3.0 were identified using single-marker analysis and underwent either interval mapping or composite interval mapping (at LOD >3.0).

When we compared the locations of the QTLs detected in the present study with the results of traits reported previously, we noted that the QTLs on Chr 8 in the BC_2F_3, qBL8.2 of pathotype P7.2 traits, appeared to reside in the same marker, RM25, as those detected in previous studies for spikelet per plant (qSPL-8). Blast resistance QTLs in the BC_2F_5 population

qBL12.2 qBL11.3, qBL4.1 qBL4.2, and qBL2.2 corresponded to QTLs in the BC_2F_2 population for qSPP-12-1, qDTH-11, qDTM-4, qYLD-4, and qPL-2, respectively.

QTLs for blast resistance in various mapping populations

Several QTLs related to blast resistance have been detected using various markers, populations, and environments. Ten putative QTLs for blast resistance in 12 rice Chrs have been mapped by McCouch et al. (1994). Two QTLs have been mapped on Chrs 2 and 6, and one each on Chrs 3, 5, 7, 8, 11, and 12. Furthermore, 9 QTLs have been mapped using RFLP markers on Chrs 1-4, 6, 7, and 9, with 2 loci on Chr 12 (Tabien et al., 2002). QTL analyses using RFLP and SSR markers have been carried out in F_4 progeny lines from the cross between Nipponbare (moderately susceptible, lowland) and Owarihatamochi (resistant, upland). Two QTLs were detected on Chr 4 and one each on Chrs 9 and 12. The phenotypic variation explained by each QTL ranged from 7.9 to 45.7%, and the 4 QTLs explained 66.3% of the total phenotypic variation (Fukuoka and Okuno, 2001).

In another study, microsatellite marker RM25 was found to be associated with blast resistance in rice cultivar Laxmi, in which it showed linkage with resistance and susceptibility in all 20 progenies derived from Laxmi and Mahsuri (Sharma et al., 2007). Laxmi has also been reported to show blast resistances *Pi-1*, *Pi-2*, *Pi-3*, and *Pi-4* (Chaudhary et al., 2004). RM25 was also linked to blast resistance genes *Pi-1*, *Pi-2*, and *Pi-3* from rice genotypes Lac 23, 5173, and Pai-kan-tao, respectively (Mackill and Bonman, 1992). Another study showed that RM25 was close to the *Pi* genes (*Pi-33*, *Pi-29*, and *Pi-11*) on Chr 8 (Monosi et al., 2004).

Two QTLs on Chrs 3 and 11 (qBFR3 and qBFR11) were identified from an F_3 population derived from URN12 (resistant) and Koshihikari (susceptible) (Sato et al., 2006). The phenotypic variances explained were 8.3 and 39%, respectively. Five QTLs relating to leaf blast resistance have been detected on Chrs 4, 6, 8, 11, and 12 from the BC_2F_2 population derived from the backcross of Koshihikari/*O. rufipogon* (Hirabayasi et al., 2005). The QTL on Chr 4 was found to be most effective and considered a complete resistance gene. The same study was conducted to analyze blast resistance in rice using QTL mapping from an interspecific population originating from the backcrossing of *O. rufipogon* and cultivated rice IR64 (Dwinita et al., 2008). Results of the experiment showed that based on blast QTL mapping, 2 resistance genes, *Pirf2-1*(t) and *Pir2-3*(t), were mapped on Chr 2. The *Pirf2-1*(t) gene was isolated from Chr 2 of *O. rufipogon* and encoded resistance to *M. oryzae* race 001, whereas the *Pir2-3*(t) gene that was isolated from rice cultivar IR64 was encoded for resistance to *M. oryzae* race 173. The study also showed the resistance spectrum of *O. rufipogon* to be non-race-specific to *M. oryzae* pathotypes.

The results obtained from previous studies have shown that QTLs for blast disease resistance can be found in all 12 linkage groups of rice. In this study, 9 loci distributed on 4 rice Chrs in the BC_2F_3 population and 13 loci distributed on 7 rice Chrs in the BC_2F_5 population were identified. These loci were associated with quantitative expression of 1 or 2 resistance traits (BLP7.2 and BLP5.0) to *M. oryzae* isolates. The QTL with the largest effect for the BC_2F_3 population was on Chr 5 (LRS 6.5 or LOD 1.4, explaining 2% of the phenotypic variance). The largest effect for the BC_2F_5 population was on Chr 2 (LRS 15 or LOD 3.3, explaining 40% of the phenotypic variance). QTLs qBL5.3, qBL5.4, qBL7.1, qBL8.2, qBL5.1, qBL5.2, qBL6.1, qBL8.1, and qBL10.1 in BC_2F_3 (LRS 3.9-5.4) explained 1-2% of the phe-

notypic variance, whereas QTLs qBL11.2, qBL12.1, qBL12.2, qBL12.3, qBL11.3, qBL4.1, qBL4.2, qBL5.3, qBL8.3, qBL1.1, qBL2.1, qBL2.2 and qBL11.1 in BC_2F_5 (LRS 3.9-11.5) explained 12-31% of the phenotypic variance.

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