Mapping Quantitative Trait Loci Responsible for Resistance to Sheath Blight in Rice

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ABSTRACT

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Rice sheath blight (ShB), caused by the soilborne pathogen *Rhizoctonia solani*, annually causes severe losses in yield and quality in many rice production areas worldwide. Jasmine 85 is an indica cultivar that has proven to have a high level of resistance to this pathogen. The objective of this study was to determine the ability of controlled environment inoculation assays to detect ShB resistance quantitative trait loci (QTLs) in a cross derived from the susceptible cv. Lemont and the resistant cv. Jasmine 85. The disease reactions of 250 F₃ recombinant inbred lines (RILs) were measured on the seedlings inoculated using microchamber and mist-chamber assays under greenhouse conditions. In total, 10 ShB-QTLs were identified on chromosomes 1, 2, 3, 5, 6, and 9 using these two methods. The microchamber method identified four of five new ShB-

QTLs, one on each of chromosomes 1, 3, 5, and 6. Both microchamber and mist-chamber methods identified two ShB-QTLs, *qShB1* and *qShB9-2*. Four of the ShB-QTLs or ShB-QTL regions identified on chromosomes 2, 3, and 9 were previously reported in the literature. The major ShB-QTL *qShB9-2*, which cosegregated with simple sequence repeat (SSR) marker RM245 on chromosome 9, contributed to 24.3 and 27.2% of total phenotypic variation in ShB using microchamber and mist-chamber assays, respectively. *qShB9-2*, a plant-stage-independent QTL, was also verified in nine haplotypes of 10 resistant Lemont/Jasmine 85 RILs using haplotype analysis. These results suggest that multiple ShB-QTLs are involved in ShB resistance and that microchamber and mist-chamber methods are effective for detecting plant-stage-independent QTLs. Furthermore, two SSR markers, RM215 and RM245, are robust markers and can be used in marker-assisted breeding programs to improve ShB resistance.

Additional keywords: inoculation method, *Oryza sativa*.

Rice sheath blight (ShB), caused by the soilborne fungal pathogen *Rhizoctonia solani* Kühn, is one of the most destructive diseases worldwide (29,30). In highly intensive production systems in the United States, ShB disease can cause yield losses up to 50% (14,15). ShB management typically has relied on the application of fungicides in combination with cultural practices (36). Utilization of host resistance is the most economical and environmentally sound strategy in managing ShB. The ShB pathogen has a broad host range and no complete resistance has been identified in rice germplasm (17,22). However, substantial differences in the levels of susceptibility to the ShB pathogen among rice cultivars have been observed under field conditions (6,15,16). ShB resistance is believed to be controlled by polygenic quantitative trait loci (QTLs) (22). Li et al. (11) first identified ShB-QTLs using restricted fragment length polymorphism (RFLP) markers and field evaluations. Subsequently, a number of QTLs associated with ShB resistance were identified on rice chromosomes 2, 3, 5, 7, 9, and 11 using segregating F₂ populations, double haploid (DH), and recombinant inbred line (RIL) populations under field conditions (3,5,9,19,22,27,33,38). The populations used were mainly derived from the crosses of the ShB-susceptible cv. Lemont with

the ShB-resistant cvs. Teqing or Jasmine 85. Pinson et al. (22) confirmed the locations and effects of six previously identified ShB-QTLs and identified eight new ShB-QTLs in an RIL population derived from the cross of Lemont × Teqing using a field inoculation method. However, the accurate and reliable measurement of ShB resistance in QTL analysis under field conditions is affected by a number of environmental factors, including light intensity, humidity, temperature (4,10), nitrogen fertilizer rate (28,31), and silica level in soil (25,26) as well as plant morphology such as plant height, growth stage, and plant architecture (1,9,11,26).

A number of different inoculation methods have been used to infest field plots for screening for resistance to the ShB pathogen. These methods have included the use of colonized toothpicks inserted into leaf sheaths (38), liquid inoculum injection (9,27), and broadcasting solid inoculum onto rice plants (3,5,11,15,19,22,28,33). Other inoculation techniques performed under controlled conditions have included a detached leaf method (7) and a microchamber assay (2,6,23). Different forms of ShB inoculum (grown on agar blocks, liquid cultured mycelia, and mycelia suspension cultures) were also tested in greenhouse studies (20). Jia et al. (6) developed a microchamber method, a simple, rapid, accurate, and reliable method to evaluate varietal resistance to ShB under controlled greenhouse conditions. Compared with inoculated field inoculation screening tests, the controlled environment microchamber assay has the following advantages: (i) younger seedlings used for minimizing the confounding effects of morphological characters on ShB resistance, (ii) 3 to 5 weeks for

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a test, (iii) relatively little experimental space, (iv) year-round greenhouse ShB evaluations, and (v) scoring segregating breeding material or mapping populations by rating individual plants. Similarly, the mist-chamber assay has the advantages in assaying older plants for ShB resistance in a relatively small space. However, an assay takes a longer time.

The objectives of this study were to (i) map ShB-QTLs identified using two greenhouse inoculation chamber methods and (ii) confirm the identified ShB-QTLs in the resistant RILs from Lemont/Jasmine 85 (LJRILs) for future breeding program in ShB resistance.

MATERIALS AND METHODS

Plant materials. The RIL population derived from the cross of Lemont × Jasmine 85 was developed using single-seed descent in a greenhouse at the United States Department of Agriculture–Agricultural Research Service Dale Bumpers National Rice Research Center, Stuttgart, AR. Jasmine 85, a long-grain indica rice cultivar developed at the International Rice Research Institute, Philippines, is moderately resistant to ShB (16). Lemont, an early-maturing, semi-dwarf tropical japonica cultivar commercially grown in the southern United States in the 1980s and 1990s, is susceptible to ShB (1,6). A subset of 256 F₅ RILs from Lemont/Jasmine 85 (LJRILs) was used for constructing a linkage map using 199 simple sequence repeat (SSR) markers. The primer sequences and map positions of the SSR markers were obtained from the Gramene Version 28, September 2008 database (<http://www.gramene.org>). The SSR markers were selected based on the polymorphism in parental screening at ≈10.0 centimorgans (cM) between the two markers. In total, 250 F₅ LJRILs were evaluated under greenhouse conditions for ShB resistance using a microchamber and mist-chamber assay in a greenhouse at the International Center for Tropical Agriculture (CIAT), Colombia. On both assays, the LJRILs were planted in plastic pots (15 cm in diameter) filled with steam-sterilized soil and fertilized with the equivalent of N at 180 kg/ha, 6P at 0 kg/ha, and K at 60 kg/ha. Fertilizer applications were split into three equal amounts at the time of planting and at 1 and 2 weeks after planting for the microchamber assay and, for the mist-chamber assay, at the time of planting, 30 days after planting, and 1 day before inoculation. For each fertilizer application, 230 mg of urea (46% N), 77 mg of triple superphosphate (46% P₂O₅), and 70 mg of potassium chloride (50% K) were applied to one 15-cm pot.

ShB isolate. The ShB isolate Tol 2399-1 of the pathogen *Rhizoctonia solani* AG I-1A is one of most aggressive isolate in the most predominant genetic group, no. 9, in Colombia and has a broad spectrum of virulence, causing >80% of the disease severity in highly susceptible cultivars such as Lemont (6). Therefore, Tol 2399-1 was selected to use in both chamber assays for ShB resistance. Tol 2399-1 was collected from rice cv. Oryzica 1 in Saldana, Tolima, Colombia in 1993. Tol 2399-1 is stored as mycelium grown on filter paper at 0°C and as sclerotia at 4°C, and maintained in the *Rhizoctonia* spp. Collection of the Pathology Laboratory at CIAT.

Microchamber assay. A microchamber inoculation assay was used as previously described (6). Actively growing mycelia of Tol 2399-1 from potato dextrose agar (PDA) plates were transferred to fresh PDA medium and cultured for 5 days at 27°C under darkness. Mycelial discs (7 mm in diameter) were excised from the culture plate using a pipette tip and were used to inoculate 3-week-old rice seedlings (four-leaf stage). A colonized disc was placed at the base of each of three rice seedling and the pot was covered with a 3-liter clear plastic bottle whose bottom and cap had been previously removed, forming a microchamber. The inoculated plants were kept at 24 to 28°C, and evaluated at 8 to 10 days after inoculation or when the susceptible check Lemont exhibited a highly susceptible reaction, where the leaves of in-

fectured seedlings contained brown lesions and the entire plant became blighted and died, turning straw-colored or light brown. At this time, the resistant cv. Jasmine 85 exhibited a few green-gray, water-soaked spots on the leaves and stems. The perforated pots were maintained in flats containing shallow water (≈5 cm deep) to keep the soil moist during the evaluation period. In a single assay, each pot contained a given LJRIL with three plants per pot. Lemont and Jasmine 85 were included in all experiments as the susceptible and resistant checks, respectively. For each replication, 250 LJRIL entry pots and 4 pots from each of the susceptible and resistant checks were arranged in a completely randomized design. Each assay was then repeated four times (time serves as the random block effect) for a total of four replications. The replications were conducted at different dates during February and March 2007. The greenhouse temperatures for day and night averaged at 30 and 21°C, respectively.

ShB severity of an individual test plant was estimated using the percentage of the diseased plant area affected (DPAA). For this purpose, on a 3-week-old seedling containing three leaves, a maximum disease severity value of 30% was assigned to the first or oldest leaf; a maximum disease severity of 25% was assigned to each of the two following leaves, and a maximum of 20% to the stem, for a possible total of 100%. For example, if the oldest leaf was half covered with ShB symptoms, a disease severity of 15% was assigned to this leaf, and if the stem was only half covered with ShB symptoms, a disease severity of 10% was assigned to the stem. The disease severity of a test plant was estimated as the sum of the individual severities assigned to each one of the three leaves and the stem of the seedling.

Mist-chamber assay. Inoculum was prepared using the same procedure as described above for the microchamber assay. Rice plants were grown in 15-cm plastic pots placed in a walk-in plastic chamber (4-by-3-by-2 m with a capacity of 500 pots) at >95% relative humidity. The high humidity was generated by three Humidifier 707-U humidifiers (Effingham, IL) in a chamber. Fifty days after sowing (flag leaf well developed), a colonized mycelial disc was placed at the base of each rice plant in each pot. The inoculated plants were incubated in the mist chamber for 10 days, transferred onto a greenhouse bench, and left for 5 days. The plants were moved back to the mist chamber for another 5 days in the second incubation period. In a single assay, each pot contained a given LJRIL with five plants per pot. Lemont and Jasmine 85 were included in all experiments as the susceptible and resistant checks, respectively. In a replication, 250 LJRIL entry pots and 2 pots from each of the susceptible and resistant checks were arranged in a completely randomized design. Each assay was then repeated five times (time serves as the random block effect), making for a total of five replications. The replications were conducted at different dates during February and June 2007. The average temperature within the mist chamber was maintained at 21 to 35°C.

ShB severity of an individual test plant was also estimated using DPAA. For this purpose, on a 50-day-old plant containing five leaves on the main tiller, a maximum disease severity value of 30% was assigned to the first leaf; 15% to each of the second and third leaves, 10% to each of the fourth and fifth leaves, and 20% to the stem, for a possible total of 100%. For example, if the oldest leaf was half covered with ShB symptoms, a disease severity of 15% was assigned to this leaf and, if the stem was only half covered with ShB symptoms, a disease severity of 10% was assigned to the stem. The disease severity of the test plant was then estimated as the sum of the individual severities assigned to each one of the five leaves and the stem of the plant.

Statistical analysis of phenotypic data. The ShB severity ratings from three individual plants (microchamber assay) or five individual plants (mist-chamber assay) in a pot were averaged as a replicate for calculating the least square means (LSMs) and standard errors. The LSMs of the ShB severity of the LJRILs

were determined using the GLIMMIX procedure in SAS (version 9.1.3; SAS Institute, Cary, NC). The LSMEANS option was used to calculate the estimated means and standard errors of the LJRILs and checks. The statistical model for each assay included the fixed effect of the LJRILs and checks and the random block effect of time. Within a chamber assay, the distribution of measured variable and residuals were assessed and determined to fit a Gaussian (normal) curve. Correlation analysis of the two chamber assays was performed using the LSMs in the CORR procedure of SAS (version 9.1.3).

DNA marker genotyping and the construction of an SSR map. DNA extraction of 256 LJRILs followed the method as described by Tai and Tanksley (32). Five to nine fresh rice leaves of each LJRIL were collected and ground. Total genomic DNA was extracted and normalized to 5 ng/μl for SSR amplification. The conditions of SSR amplification and allele determination were previously described by Liu et al. (12). More than 270 SSR markers which evenly covered 12 rice chromosomes at ≈10 cM/marker were screened to identify polymorphism between the parents of Lemont and Jasmine 85, and 199 polymorphic SSR markers were found. The amplified polymerase chain (PCR) reaction products were pooled and separated on an ABI Prism 3700 DNA analyzer. The sizes of SSR fragments were determined using the software GeneScan and Genotyper, version 3.7NT (Applied Biosystem Inc., Foster City, CA).

An SSR linkage map comprising 199 SSR markers was constructed using JoinMap 4 based on the Kosambi function (8). Loci were assigned to linkage groups by the program default settings and likelihood odds ratio (LOD) scores were ≥3.0. The “fix order” command was used to identify the most likely marker order within a group when needed. The constructed SSR map represents a total of 1,684.2 cM of the genetic distance at an average of 8.5 cM between linked marker loci.

Data analysis and QTL mapping. The mean ShB severity for the LJRILs was used for the composite interval mapping (CIM). QTL analysis was performed using Windows QTL Cartographer, version 2.5 (35), with the default CIM control parameters model 6 of standard model, five control markers, 10 cM of window size, and forward regression method. The LOD threshold of ≥2.4 was used to declare the presence of a putative QTL in order to compare with the previously identified ShB-QTLs on rice chromosomes (3,5,9,19,22,27,33,38). Additive effect and percentage of variation explained by individual ShB-QTLs were estimated.

RESULTS

Variability in ShB severity in the LJRIL population. The resistant check Jasmine 85 and susceptible Lemont had significantly different ShB ratings in the microchamber assay ($P = 0.0004$) and mist-chamber assay ($P < 0.0001$) (Table 1). Jasmine 85 and Lemont had consistent reactions to the pathogen in both chamber assays. The DPAA of each replicate varied at 90.0 to 94.0 and 60.0 to 90.0% for Lemont and 45.0 to 55.0 and 36.4 to 46.4% for Jasmine 85 in the microchamber and mist-chamber assays, respectively. Jasmine 85 had a similar deviation of LSMs,

indicating that it had more stable performance in both assays than Lemont. The relatively small standard errors for the means of the LJRIL population indicated that the phenotypic variation in ShB severity ratings between replicates was acceptable for QTL mapping (Table 1). This finding ruled out the potential artifacts contributed by the small percentage of the heterozygosity in the F_5 LJRIL population used for this study. In comparison with the microchamber assay, the lower standard error for the population mean in the mist-chamber assay demonstrated a higher precision between replicates, likely due to more replicates and individual rice plants being used (Table 1). The fixed effects from the LJRILs were statistically significantly different in both chamber assays. The random effect due to the time block (replication) was more variable in the mist chamber assay than in the microchamber assay (data not shown).

The mean ShB severity on the LJRILs in the microchamber and mist-chamber assays was distributed normally, with the resistant and susceptible parents at the extreme ends (Fig. 1). The microchamber assay for evaluating ShB resistance was very comparable with the mist-chamber assay. The linear correlation for the mean severity of the LJRILs between two assays was positive, with the correlation coefficient of $r = 0.62$ ($P < 0.0001$, $n = 252$) (Fig. 2).

QTL mapping for ShB resistance. An SSR map was constructed for QTL analysis using 256 LJRILs and 199 SSR markers evenly distributed over the 12 rice chromosomes. The identified ShB-QTLs responsible for ShB resistance using the two types of incubation assays are summarized in Table 2 and Figure 3. Six ShB-QTLs of *qShB1*, *qShB3-1*, *qShB5*, *qShB6*, *qShB9-1*, and *qShB9-2* identified using the microchamber method were located on chromosome 1, 3, 5, 6, and 9. Using the mist chamber method, six ShB-QTLs were mapped on chromosome 1, 2, 3, and 9, referred to as *qShB1*, *qShB2-1*, *qShB2-2*, *qShB3-2*, *qShB3-3*, and *qShB9-2*. Except for *qShB9-2*, all the individual ShB-QTLs in both chamber assays could explain a range of 3 to 6.9% of total phenotypic variation (Table 2). However, one major ShB-QTL of *qShB9-2* was identified using both the micro- and mist-chamber assays and had LOD values of 17.3 and 19.9, respectively. The resistant allele of *qShB9-2* from Jasmine 85 explained 24.3% of total phenotypic variation, with an additive effect of 8.06 when ShB was evaluated using the microchamber assay, and 27.2% of total phenotypic variation with an additive effect of 5.75 using the mist-chamber assay (Table 2). RM215 and RM245 flanked *qShB9-2* (Fig. 3). Similarly, *qShB1* was also identified by both incubation chamber assays on chromosome 1, and was flanked by RM1361 and RM104 (Fig. 3). *qShB3-1* detected by the microchamber and *qShB3-2* by the mist chamber were located in a QTL region between RM16 and RM426 on chromosome 3. Resistant alleles at *qShB5*, *qShB6*, and *qShB9-1* from the ShB-susceptible parent Lemont were identified only using the microchamber method, and accumulated 19.0% of total phenotypic variation and -14.51% of additive effects (Table 2).

Confirmation of identified ShB-QTLs in LJRILs for marker-assisted breeding. All the resistant LJRILs selected were found to contain the major ShB-QTL *qShB9-2* (Table 3). Six

TABLE 1. Summary of the diseased plant area affected (DPAA) (%) by rice sheath blight (ShB) on the recombinant inbred lines of Lemont × Jasmine 85 and the susceptible and resistant checks assayed in Colombia, 2007^x

ShB assay	Parental DPAA ^y				DPAA of RIL population ^z	
	Range		LSM ± SD		Range	Mean ± SD
	Lemont	Jasmine 85	Lemont	Jasmine 85		
Microchamber	90.0–94.0	45.0–55.0	93.0 ± 1.8 a	54.5 ± 4.5 b	30.8–100.0	73.2 ± 8.3
Mist chamber	60.0–90.0	36.4–46.4	72.7 ± 12.9 A	46.5 ± 4.1 B	37.6–95.0	63.7 ± 5.7

^x Within an assay method, means followed by the same letters are not significantly different at $P = 0.0004$ (micro-chamber assay) and $P < 0.0001$ (mist chamber assay). Averages from three ShB severity ratings in a pot (replicate).

^y Shown are range of Lemont (susceptible check) and Jasmine 85 (resistant check) and least square means (LSD) ± standard deviation (SD).

^z Recombinant inbred line (RIL) population sizes were 252 for microchamber assay and 258 for mist-chamber assay (including 6 local ShB-resistant checks).

ShB-QTLs of *qShB1*, *qShB2-1*, *qShB3-1*, *qShB3-2*, *qShB3-3*, and *qShB6* were commonly presented in most of the LJRILs. Six LJRILs of LJRIL142, LJRIL158, LJRIL186, LJRIL190, LJRIL215, LJRIL221, and LJRIL246 were homozygous at all loci. Nine haplotypes were found in the LJRILs based on allelic comparison (Table 3). LJRIL158 and LJRIL 215 belonged to one haplotype.

DISCUSSION

The absence of complete resistance to ShB in *Oryza sativa* has prevented the use of such genes for managing ShB (17). ShB resistance is considered to be a multigenic trait, making it difficult to evaluate the effect of individual QTLs on resistance. Inoculated field trials have been used for phenotypic evaluations of ShB

resistance in previous QTL mapping studies (3,5,9,19,22,27, 33,38). The accuracy of such an approach for QTL mapping can be confounded by a number of variables, including environmental factors under field conditions (13,21). Pinson et al (22) suggested that the accuracy of ShB phenotyping could be confounded by variable humidity and temperature in the plant microenvironment under field conditions during ShB disease development. Factors such as plant density, tiller number, lodging, and water depth can all affect the plant microenvironment. Plant height and heading date were also reported as confounding factors associated with mapping ShB-QTLs (9,11,13,22,27,38). In the present study, such confounding factors that may influence the accuracy of mapping ShB-QTLs can be ruled out because young rice plants were used for both assays under controlled environmental conditions.

Fig. 1. Frequency distribution of the least square means of the diseased plant area affected in percentage of 250 recombinant inbred lines from the cross of Lemont (LMNT) and Jasmine 85 (JSMN) assayed in greenhouse for sheath blight resistance using microchamber and mist-chamber methods. Arrows show the relative positions of the resistant JSMN and susceptible LMNT parents.

Fig. 2. Scatter plot and Pearson's correlation coefficient based on the correlation analysis of the two chamber assays for sheath blight (ShB) resistance. Least square means from the recombinant inbred lines population of Lemont/Jasmine 85 were used for plotting.

TABLE 2. Summary of quantitative trait loci (QTLs) analysis for the resistance to rice sheath blight (ShB) using composite interval mapping^x

ShB evaluation assay, ShB-QTL	Chromosome	Marker interval	Nearest marker locus	LOD value ^y	Phenotypic variation (%)	Additive effect ^z
Microchamber						
<i>qShB1</i>	1	RM1361–RM104	RM104	3.2	3.4	2.98
<i>qShB3-1</i>	3	RM16–RM426	RM5626	2.8	3.7	3.24
<i>qShB5</i>	5	RM507–RM7349	RM13	4.4	5.1	–3.67
<i>qShB6</i>	6	RM435–RM190	RM190	2.7	3.4	–2.90
<i>qShB9-1</i>	9	RM409–RM257	RM434	4.7	5.4	–4.27
<i>qShB9-2</i>	9	RM215–RM245	RM245	17.3	24.3	8.06
Mist chamber						
<i>qShB1</i>	1	RM1361–RM104	RM104	3.0	3.6	2.13
<i>qShB2-1</i>	2	RM424–RM5427	RM341	6.1	6.9	2.95
<i>qShB2-2</i>	2	RM112–RM250	RM250	2.6	3.2	1.96
<i>qShB3-2</i>	3	RM5626–RM426	RM426	2.6	3.3	2.19
<i>qShB3-3</i>	3	RM514–RM85	RM85	2.8	3.0	2.07
<i>qShB9-2</i>	9	RM215–RM245	RM245	19.9	27.2	5.75

^x ShB resistance was phenotyped using micro-chamber and mist chamber assays in Colombia, 2007.^y ShB-QTLs were determined using the likelihood odds ratio (LOD) value of 2.4 to compare the previously identified ShB-QTLs under field condition based on the reports on ShB-QTL mapping (3,5,9,19,22,27,33,38).^z Additive effect is the effect associated with substitution of a Jasmine 85 allele by its corresponding Lemont allele.

Fig. 3. Chromosomal locations of the rice sheath blight quantitative trait loci (ShB-QTLs) mapped in this study using a microchamber assay (solid boxes) and mist-chamber assay (hollow boxes) and estimated locations of the ShB-QTLs identified previously using Lemont/Jasmine 85 (Jm) population by Zou et al. (38) and using Lemont/Teqing (Tq) population by Li et al. (11), Pinson et al. (22), and Tan et al. (33). The newly identified ShB-QTLs or ShB-QTL regions were indicated on chromosome (Chr.) bars using a cross-hatched pattern.

The resistant alleles at the *qShB3-1* and *qShB3-2* loci from Jasmine 85 detected using both assay methods confirmed the existence of the ShB-QTL *qSB-3* identified using an F₂ clonal population derived from the cross of Lemont × Jasmine 85 by Zou et al. (38) (Fig. 2). Furthermore, the location of *qShB2-1* on chromosome 2 detected using the mist chamber was near the locus of *qSB-2* previously identified by Zou et al. (38). In addition, *qShB9-1* originating from Lemont was located on chro-

mosome 9 in the present study. Zou et al. (38) previously mapped Lemont-originated *qSB-9-2* at different location on chromosome 9 using a different fungal isolate (Fig. 2). Whether *qShB9-1* is allelic to *qSB-9-2* needs further verification. However, our results confirm the existence of the major ShB-QTL *qShB9-2* conferring partial resistance to ShB in seedling (chamber assays) and adult plant (field evaluation) stages, which has been reported from the analyses of various mapping populations at different locations

TABLE 3. Confirmation of the presence of rice sheath blight quantitative trait loci (ShB-QTLs) with the resistant phenotype in selected recombinant inbred lines from Lemont/Jasmine 85 (LJRILs) for marker-assisted breeding

				Alleles at ShB-QTLs ^z											
				DPAA (%) ^y		<i>qShB1</i> Chr. 1 QTL ^{Jm}	<i>qShB2-1</i> Chr. 2 QTL ^{Jm}	<i>qShB2-2</i> Chr. 2 QTL ^{Jm}	<i>qShB3-1</i> Chr. 3 QTL ^{Jm}	<i>qShB3-2</i> Chr. 3 QTL ^{Jm}	<i>qShB3-3</i> Chr. 3 QTL ^{Jm}	<i>qShB5</i> Chr. 5 QTL ^{Lm}	<i>qShB6</i> Chr. 6 QTL ^{Lm}	<i>qShB9-1</i> Chr. 9 QTL ^{Lm}	<i>qShB9-2</i> Chr. 9 QTL ^{Jm}
				Micro	Mist	RM104	RM341	RM250	RM5626	RM426	RM85	RM13	RM190	RM434	RM245
Haplotype ^x	LJRIL														
1	51	46.0	46.4	L	L	J	h	J	J	L	L	L	J		
2	101	43.3	43.2	J	h	L	J	J	J	J	L	J	J		
3	103	33.3	42.5	J	J	L	J	J	J	h	L	J	J		
4	142	45.1	54.2	J	J	L	J	J	J	J	L	L	J		
5	186	41.3	47.4	J	J	J	J	J	J	L	L	J	J		
6	190	42.5	50.8	J	J	L	J	J	J	J	L	L	J		
7	221	54.0	53.6	J	J	J	L	h	J	L	L	J	J		
8	246	47.5	53.3	J	J	J	J	J	L	L	J	L	J		
9	158	35.6	48.0	J	J	L	J	J	J	L	L	L	J		
9	215	50.3	47.3	J	J	L	J	J	J	L	L	L	J		

^x Haplotype was selected based on <55% of the DPAA percentages and 100% SSR amplification.

^y Percent diseased plant area affected (DPAA) for average of four and five replicates for microchamber (Micro) and mist-chamber (Mist) assays, respectively.

^z Letters L, J, and h stand for the parents Lemont and Jasmine 85 and heterozygous alleles, respectively. Each ShB-QTL is followed by its chromosome (Chr.) number, the indication of the resistant allele origination from either Jasmine 85 (QTL^{Jm}) or Lemont (QTL^{Lm}), and the nearest simple sequence repeat (SSR) marker.

using different evaluation methods (22,33). These findings suggest that *qShB9-2* identified in different plant growth stages is a plant-stage-independent QTL. ShB-QTL *qShB2-2*, mapped using the mist-chamber assay on chromosome 2 in the present study, was close to *qSbr2a* identified using bulk F₄ population of Lemont × Teqing (11). The major QTL *qShB9-2* on chromosome 9 was mapped between markers RM215 and RM245, representing a region of ≈12 cM in the present study. The same QTL was also identified from Teqing using the F₁₀₋₁₁ RIL population of Lemont × Teqing (22) and using the F₂ clonal population of Lemont × Teqing (33). These observations suggest that the same major QTL derived from these two indica genotypes.

In the present study, some of ShB-QTLs were identified using the microchamber assay (*qShB3-1*, *qShB5*, *qShB6*, and *qShB9-1*) and others were identified using the mist-chamber assay (*qShB2-1*, *qShB2-2*, *qShB3-2*, and *qShB3-3*). Using both chamber assays, four new ShB-QTLs—*qShB1*, *qShB3-3*, *qShB5*, and *qShB6* on chromosomes 1, 3, 5, and 6—were identified and some of these new QTLs may be plant-stage dependent. The discovery of these QTLs suggests that each of the inoculation methods can detect the major, unique ShB-QTLs and ones which may not be detected under field evaluations, such as the plant-stage-dependent QTLs. Similarly, the plant-stage dependent QTLs were previously identified in partial resistance to leaf rust in barley (24) and tiller number of rice (37). Hence, both micro- and mist-chamber assays are useful for identifying the major or plant-stage-independent ShB-QTLs in early growth stages of rice plants. Taken together, the greenhouse and field ShB evaluation methods are recommended for tagging all possible ShB-QTLs.

Fine mapping and cloning the genes for ShB resistance is the next important step for understanding resistance mechanisms and increasing selection precision using marker-assisted selection (MAS). A number of defense genes have recently been identified in cv. Jasmine 85 within 48 h of infection by the pathogen *R. solani* using the robust long-serial analysis of gene expression and microarray analysis (34). A number of nucleotide binding site leucine-rich repeat candidate genes for ShB resistance and 400 highly expressed rice genes following pathogen infection were also recently identified on several chromosomes where ShB-QTLs were determined using the present greenhouse methods (M. Jia and Y. Jia, unpublished data).

Two common problems for using MAS for ShB QTL are (i) DNA markers may not be distinguishable in different germplasm and (ii) phenotypic evaluation methods are too inaccurate to verify genetic resistance (18). Mapping and confirmation of a major ShB-QTL on chromosome 9 and resistant LJRILs with the

majority of ShB-QTLs identified in the present study are important advances for using MAS in breeding for ShB resistance (18). Recently, Zuo et al (39,40) demonstrated the potential for deploying indica resistance ShB-QTLs of *qSB-9* and *qSB-11* to improve japonica germplasm. The markers RM215 and RM245 identified in this study, in combination with microchamber and mist-chamber assays, are excellent new tools for implementing MAS for improving ShB resistance in rice-breeding programs. In addition, we have identified other minor ShB-QTLs, some even contributed by the susceptible parent, which can be used to develop new cultivars with improved resistance to this important disease.

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