

## Mapping quantitative trait loci for sheath blight disease resistance in Yangdao 4 rice

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**ABSTRACT.** Rice sheath blight (ShB), which is caused by *Rhizoctonia solani*, has become the most serious rice disease in China. Yangdao 4, a cultivar with partial resistance to ShB, was crossed with Lemont, a susceptible cultivar, to develop mapping populations that were used to analyze quantitative trait loci (QTL) that confer resistance to ShB. QTL analysis were performed in 3 environments (E1-E3) using 2 F<sub>2</sub> and 1 F<sub>2:3</sub> populations, respectively. Three traits were recorded to evaluate ShB resistance, including disease rating (DR), lesion height (LH), and percentage of lesion height (PLH). Based on field evaluation of ShB resistance and the 2 genetic maps constructed, we identified a total of 8 QTLs for DR (4 in E1, 4 in E2, and 3 in E3), 6 QTLs for LH (1 in E1, 3 in E2, and 2 in E3), and 7 QTLs for PLH (1 in E1, 4 in E2, and 2 in E3). Sixteen of the ShB-QTLs co-localized as 6 clusters on chromosomes 3, 7, 11, and 12. Four of the 6 clusters contained ShB-QTLs that were detected in 2 environments, while the other 2 clusters with ShB-QTLs were detected in 1 environment. Three ShB-QTLs (*qSBD-3-2*, *qSBL-3-1*, and *qSBPL-3-1*) were delimited to a 581-kb region flanked by markers D333B and D334 on chromosome 3. The resistance alleles of

Yangdao 4 at the *qSBD-3-2* locus decreased DR by 0.68 and 0.79 in E2 and E3, respectively.

**Key words:** Quantitative trait locus; Rice (*Oryza sativa* L.); Sheath blight (ShB); ShB-resistant cultivars

## INTRODUCTION

Rice sheath blight (ShB) is one of the 3 major rice diseases and has become the most serious disease causing rice yield loss in China (Zou et al., 2000; Zeng et al., 2011). The soil-borne pathogen *Rhizoctonia solani* is the causal agent of rice ShB. *R. solani* can infect a wide range of hosts and infects more than 200 plant species, including some of the most important crops worldwide (Lehtonen et al., 2008). A large number of rice accessions from different countries have been examined to identify resources for ShB resistance (Pan et al., 2001; Srinivasachary et al., 2011; Jia et al., 2012). Thus far, no rice germplasm has been identified that is immune to this fungus. However, some resistance varieties and lines have been shown to offer sufficient partial resistance against the pathogen under field conditions (Sato et al., 2004), showing potential for the breeding of ShB-resistant cultivars.

Rice ShB resistance is generally considered to be a typical quantitative trait controlled by quantitative trait loci (QTL), but a few studies proposed that ShB resistance in some rice varieties is controlled by major genes (Che et al., 2003; Xiang et al., 2007). ShB-QTL have been mapped on all 12 rice chromosomes using different mapping populations (Li et al., 1995; Pan et al., 1999; Zou et al., 2000; Han et al., 2002; Kunihiro et al., 2002; Sato et al., 2004; Pinson et al., 2005; Tan et al., 2005; Sharma et al., 2009; Channamallikarjuna et al., 2010; Fu et al., 2011; Xu et al., 2011; Nelson et al., 2012; Eizenga et al., 2013; Liu et al., 2009, 2013; Taguchi-Shiobara et al., 2013). Furthermore, specific molecular markers have been found to be associated with rice ShB resistance (Xie et al., 2008; Eizenga et al., 2006, 2009; Li et al., 2009; Jia et al., 2012). However, only a few ShB-QTL have been fine-mapped, including *qSBR-11-1* and *qSB-11<sup>LE</sup>* (Channamallikarjuna et al., 2010; Zuo et al., 2013). The phenotypic effects of several ShB-QTL have also been evaluated (Wang et al., 2012b).

Two agronomic traits, plant height (PH) and heading date, were reported to be correlated with ShB resistance (Zou et al., 2000; Kunihiro et al., 2002; Sato et al., 2004; Li et al., 1995, 2009; Channamallikarjuna et al., 2010; Fu et al., 2011; Nelson et al., 2012; Eizenga et al., 2013), with some ShB-QTL co-localizing with either the PH-QTLs or the heading date-QTL (Li et al., 1995; Kunihiro et al., 2002; Fu et al., 2011).

The mechanisms responsible for resistance to rice ShB remain largely unknown. Two mechanisms contributing to ShB resistance were proposed by Srinivasachary et al. (2011), including physiological resistance (innate resistance) and disease escape. According to this hypothesis, ShB-QTL co-localized with the PH-QTL or that the heading date-QTL were responsible for disease escape, while other ShB-QTL contributed to physiological resistance.

An *indica* cultivar, Yangdao 4, has been reported to be the most resistant cultivar among 68 cultivars inoculated with the *R. solani* isolate RH-9 (Pan et al., 2001). To identify QTL that control ShB resistance in Yangdao 4, 2 F<sub>2</sub> and 1 F<sub>2,3</sub> populations derived by crossing Lemont with Yangdao 4 were used in this study. The mapping populations were grown in 3 environments to test the stability of the ShB-QTL. We developed an approach for quickly

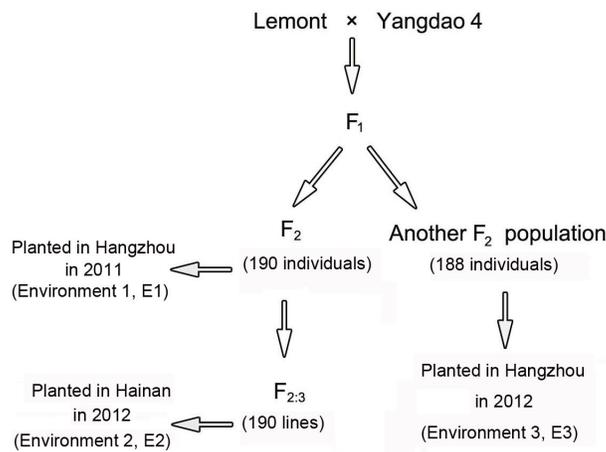
detecting and confirming ShB-QTL when permanent populations (recombinant inbred line or doubled haploid line populations) are unavailable.

## MATERIAL AND METHODS

### Plant materials and mapping populations

The mapping populations were derived by crossing Lemont with Yangdao 4. Lemont is an *US japonica* cultivar susceptible to *R. solani*. Yangdao 4 is a Chinese *indica* cultivar released from Jiangsu Province and has been reported to show remarkable resistance to ShB (Pan et al., 2001).

Three mapping populations were used in this study: 1) A Lemont/Yangdao 4  $F_2$  population, consisting of 190 individuals, was sown on May 27, 2011, on a farm at the China National Rice Research Institute in Fuyang, Hangzhou (119°95'E, 30°07'N) (Environment 1, E1). This population was used to construct the molecular marker linkage map and identify ShB-QTL. 2) A total of 190  $F_{2,3}$  family lines derived from the above population were planted on November 25, 2012, in Lingshui, Hainan (110°02'E, 18°48'N) (Environment 2, E2) for QTL analysis. 3) Another Lemont/Yangdao 4  $F_2$  population consisting of 188 individuals was planted on May 22, 2012, at the farm of China National Rice Research Institute in Hangzhou (119°95' E, 30°07'N) (Environment 3, E3). This population was used to confirm the QTL detected in E1 and E2 (Figure 1).



**Figure 1.** Mapping populations used in identifying ShB-QTLs in the three environments.

### Field evaluation of ShB resistance

The *R. solani* isolate ZJ03 deposited in this lab was used for inoculation. Truncated bamboo-toothpicks (2-2.5 cm long) were used as inoculums for pathogen infection based on the method described by Zou et al. (2000), with some modification: the toothpicks were incubated with the ZJ03 strain on potato dextrose agar medium for 7 days at 28°C, then inserted into the third leaf sheath, counting from the top during the late-tillering stage. The plant reac-

tions to ShB were recorded 30 days after inoculation.

For the  $F_2$  population, 3 tillers of an individual plant were inoculated. The maximum disease score among the 3 tillers was considered to be the final disease reaction for the specific individual.

For the  $F_{2:3}$  families, 18 individual plants within a family were grown. Two tillers of an individual plant were inoculated and the maximum disease score of the 2 tillers was considered to be the final disease reaction for the individual. The average disease score of the 18 individual plants was used as the disease reaction for the specific  $F_{2:3}$  family line.

Three parameters were recorded to assess ShB resistance for each inoculated individual plant: 1) Disease rating (DR): the 0-9 rating scale system was used, where 0 indicated no disease and 9 indicated dead or collapsed plants (Rush et al., 1976). 2) Lesion height (LH): ShB lesions extending along the stem; LH was measured from the lowest site to the highest site. 3) Percentage of lesion height (PLH):  $PLH = LH \div PH \times 100\%$ . PH was measured from the soil surface to the tip of the tallest panicle at maturity (Sharma et al., 2009).

### Molecular marker assays

A total of 507 simple sequence repeat (SSR) markers were screened for parental polymorphisms. Because the SSR marker polymorphisms were low between Lemont and Yangdao 4, we developed insertion-deletion (InDel) markers to augment the polymorphic markers (Zeng et al., 2013b). Using the  $F_2$  population planted in E1, a genetic linkage map with 180 markers was constructed, including 52 SSR markers and 128 InDel markers. SSR marker information can be downloaded from the Gramene database (<http://www.gramene.org/>). The forward and reverse sequences of the 128 InDel markers were previously described by Zeng et al. (2013b). Based on the ShB-QTL identified in E1 and E2, the  $F_2$  population grown in E3 was assayed using 41 markers. The 41 markers covered the regions of the QTL detected in E1 and E2. DNA extraction and the polymerase chain reaction protocol were described previously (Zeng et al., 2013a).

### Construction of marker linkage map and statistical analyses

The marker linkage map was constructed using the MAPMAKER/EXP 3.0 software (Lander et al., 1987). The Kosambi mapping function was used to transform recombination frequencies into genetic distances (cM). The calculation of correlation coefficient and analysis of variance were run in the SAS software (version 8.1). Genetic and physical maps of the rice chromosomes were drawn using the MapChart software (version 2.1) (Voorrips, 2002).

### QTL mapping for ShB resistance

Composite interval mapping was used to detect QTL for ShB resistance and PH using the Windows QTL Cartographer 2.5 software (Wang et al., 2012a). To compare the data with previously identified ShB-QTL, a limit of detection threshold of 3.0 was used to determine the presence of putative QTL. Two or more QTL with overlapping confidence intervals (limit of detection  $>3.0$ ) were defined as a QTL cluster.

## RESULTS

### ShB resistance of parental cultivars

Significant differences were found between Lemont and Yangdao 4 for DR, LH, and PLH when they were planted in E1 or E3 ( $P < 0.01$  or  $P < 0.05$ ). However, no significant differences were detected between Lemont and Yangdao 4 for DR, LH, and PLH when they were planted in E2. Yangdao 4 had lower DR, LH, and PLH compared with Lemont in all 3 environments (Table 1).

**Table 1.** Disease rating (DR), lesion height (LH), percentage of lesion height (PLH), and plant height (PH) of the two parents grown in the three environments.

(Environment) Year/location	Trait	Range		Mean $\pm$ SD	
		Lemont	Yangdao 4	Lemont	Yangdao 4
(E1) 2011/Hangzhou	DR	7-7.5	1.5-2.5	7.2 $\pm$ 0.3**	2.0 $\pm$ 0.5**
	LH (cm)	26.2-52.3	2.4-22.5	39.1 $\pm$ 9.4**	12.4 $\pm$ 8.2**
	PLH (%)	34.7-69.3	2.0-18.4	51.8 $\pm$ 12.5**	10.2 $\pm$ 6.7**
	PH (cm)	72.3-79.8	114.3-129.2	75.5 $\pm$ 3.9**	122.3 $\pm$ 7.5**
(E2) 2012/Hainan	DR	6-7	4-6	6.7 $\pm$ 0.6	5.2 $\pm$ 1.0
	LH (cm)	22.2-39.1	18.9-31.9	32.6 $\pm$ 6.7	26.1 $\pm$ 5.2
	PLH (%)	25.6-45.1	22.3-37.6	37.6 $\pm$ 7.8	30.8 $\pm$ 6.1
	PH (cm)	83.9-90.6	84.1-85.5	86.7 $\pm$ 3.5	84.8 $\pm$ 0.7
(E3) 2012/Hangzhou	DR	3-7	0.5-1.5	5.5 $\pm$ 2.18*	1 $\pm$ 0.5*
	LH (cm)	24.3-53.9	4.5-28.3	31.1 $\pm$ 9.7**	15.9 $\pm$ 9.1**
	PLH (%)	30.8-68.4	3.7-23.2	39.4 $\pm$ 12.3**	13.0 $\pm$ 7.5**
	PH (cm)	77.4-79.5	111.0-128.1	78.8 $\pm$ 1.2**	122.0 $\pm$ 9.6**

\* $P < 0.05$ ; \*\* $P < 0.01$ .

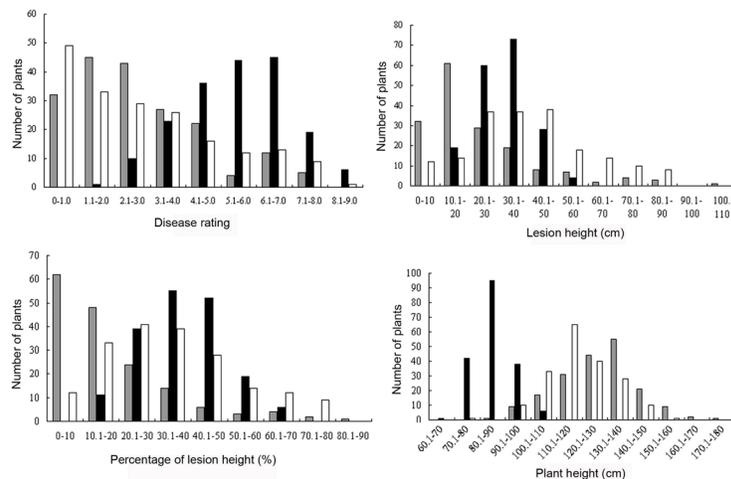
Analysis of variance was conducted to test the influence of the environment on ShB resistance of the 2 parents. The results showed that the DR, LH, and PLH of Yangdao 4 were significantly different when grown in Hangzhou (E1 or E3) than in Hainan (E2), but the DR and LH of Lemont were not significantly different among the 3 environments (Table 2). This suggests that compared to Lemont, the ShB resistance of Yangdao 4 was more easily influenced by the environment.

**Table 2.** Effect of environment on ShB resistance and plant height of Lemont and Yangdao 4 as demonstrated by ANOVA ( $P < 0.05$  were listed).

Cultivar	Trait	Comparison	F value	P value
Lemont	PLH	2011Hangzhou (E1) vs 2012Hainan (E2)	5.62	0.039
	PH	2011Hangzhou (E1) vs 2012Hainan (E2)	13.75	0.021
	PH	2012Hangzhou (E3) vs 2012Hainan (E2)	13.72	0.021
Yangdao 4	DR	2011Hangzhou (E1) vs 2012Hainan (E2)	22.56	0.009
	DR	2012Hangzhou (E3) vs 2012Hainan (E2)	39.06	0.003
	LH	2011Hangzhou (E1) vs 2012Hainan (E2)	12.02	0.006
	LH	2012Hangzhou (E3) vs 2012Hainan (E2)	6.03	0.030
	PLH	2011Hangzhou (E1) vs 2012Hainan (E2)	31.17	0.000
	PLH	2012Hangzhou (E3) vs 2012Hainan (E2)	22.54	0.001
	PH	2011Hangzhou (E1) vs 2012Hainan (E2)	74.14	0.001
	PH	2012Hangzhou (E3) vs 2012Hainan (E2)	45.16	0.003

### Distribution of ShB resistance in the 3 mapping populations

The DR, LH, and PLH in the 3 mapping populations were continuously distributed over wide ranges. The distributions of DR, LH, and PLH in the F<sub>2</sub> population planted in E1 and the distribution of DR in the F<sub>2</sub> population planted in E3 were not normally distributed (Figure 2).



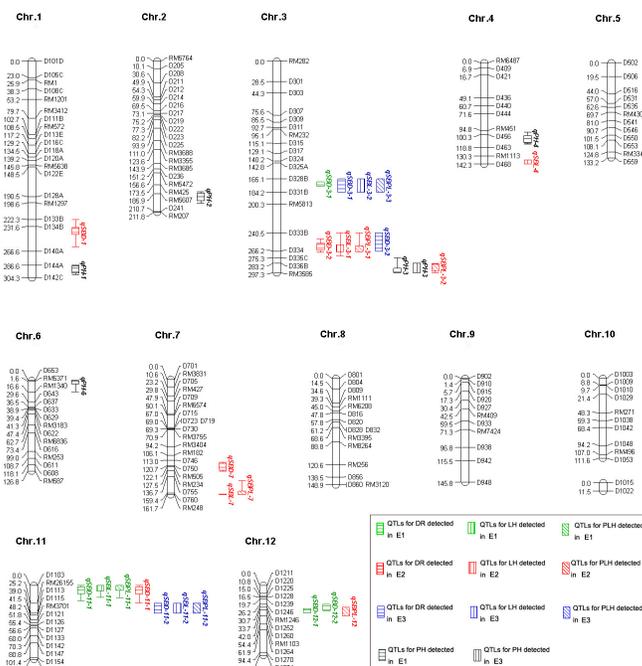
**Figure 2.** Distribution of the disease rating (DR), lesion height (LH), percentage of lesion height (PLH), and plant height (PH) in the three mapping populations. Gray, black, and white columns indicate the three mapping populations growing in environment 1 (E1), environment 2 (E2) and environment 3 (E3), respectively.

### Construction of genetic linkage maps

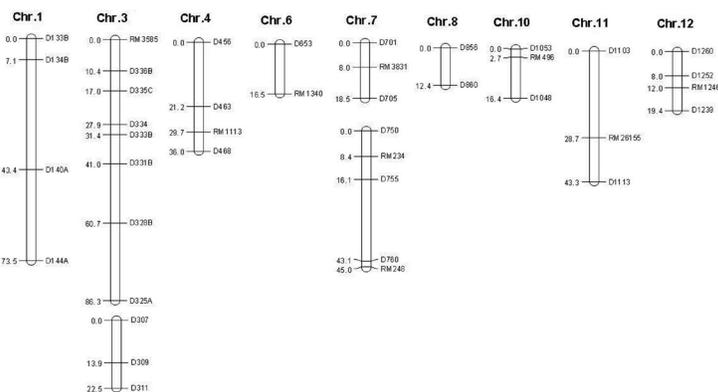
Two genetic linkage maps were constructed. 1) The first map consisting of 180 markers was constructed using 190 F<sub>2</sub> individuals planted in E1. This map represents a total of 1996.2 cM of the genetic distance at an average of 11.8 cM between linked marker loci. The 180 markers were grouped into 13 linkage groups (Figure 3). 2) The second map with 41 markers was constructed using 188 F<sub>2</sub> individuals grown in E3. This map represents a total of 389.8 cM with an average of 13.3 cM between adjacent markers. The 41 markers were grouped into 11 linkage groups (Figure 4).

### QTLs for ShB resistance

A total of 8 QTL for DR were detected in the 3 mapping populations, located on chromosomes 1, 3, 7, 11, and 12 (Figure 3). The resistance alleles *qSBD-1*, *qSBD-11-1*, and *qSBD-11-2* from Lemont individually explained 1.56-15.19% of the total phenotypic variation, and the alleles *qSBD-3-1*, *qSBD-3-2*, *qSBD-7*, *qSBD-12-1*, and *qSBD-12-2* from Yangdao 4 explained 1.12-14.66% of the total phenotypic variation. The DR-QTL *qSBD-3-1* was identified on chromosome 3 in both E1 and E3. The DR-QTL *qSBD-3-2* was mapped onto chromosome 3 in both E2 and E3. The DR-QTL *qSBD-11-1*, located on chromosome 11 and flanked by markers D1103 and RM26155, were detected in both E1 and E2. These DR-QTL detected in 2 environments were more stable than those detected in 1 environment.



**Figure 3.** Chromosomal locations of the ShB-QTLs identified in the three environments. Numbers at the left of the chromosome bars indicate genetic position (cM) of the corresponding markers. QTL for plant height (PH) was not detected in E2.



**Figure 4.** Genetic linkage map constructed using 188 F<sub>2</sub> individuals grown in E3.

Six QTLs for LH (*qSBL-3-1*, *qSBL-3-2*, *qSBL-4*, *qSBL-7*, *qSBL-11-1*, and *qSBL-11-2*) were detected in 3 environments. The resistance alleles *qSBL-11-1* and *qSBL-11-2* from Lemont accounted for 2.28 and 12.58% of the total phenotypic variation, respectively. The alleles *qSBL-3-1*, *qSBL-3-2*, *qSBL-4*, and *qSBL-7* from Yangdao 4 accounted for 4.15-31.53% of the phenotypic variation. Although all 6 LH-QTL were detected in only 1 environment, some were co-localized with the DR-QTL, including *qSBL-3-2* and *qSBD-3-1*, *qSBL-3-1* and *qSBD-3-2*, *qSBL-11-1* and *qSBD-11-1*, and *qSBL-11-2* and *qSBD-11-2* (Figure 3 and Table 3).

**Table 3.** QTLs for disease rating (DR), lesion height (LH), and percentage of lesion height (PLH) identified in the three environments using composite interval mapping.

(Environment) Year/location	Trait	QTL	Chr.	LOD	Marker interval (Physical distance)	Nearest marker	LOD peak position (cM)	R <sup>2</sup> (%)	Additive effect	DPE
(E1) 2011/Hangzhou	DR	<i>qSBD-3-1</i>	3	3.09	D328B-D331B (2842 kb)	D328B	172.1	10.51	0.76	L
	DR	<i>qSBD-11-1</i>	11	4.97	D1103-RM26155 (2891 kb)	D1103	7.0	2.13	-0.77	Y
	DR	<i>qSBD-12-1</i>	12	3.12	D1239-D1246 (2696 kb)	D1246	23.7	10.49	0.56	L
	DR	<i>qSBD-12-2</i>	12	3.74	RM1246-D1252 (874 kb)	RM1246	31.7	11.95	0.56	L
	LH	<i>qSBL-11-1</i>	11	4.48	D1103-RM26155 (2891 kb)	D1103	3.0	2.28	-8.26	Y
	PLH	<i>qSBPL-11-1</i>	11	4.89	D1103-RM26155 (2891 kb)	D1103	2.0	2.82	-0.07	Y
(E2) 2012/Hainan	DR	<i>qSBD-1</i>	1	4.23	D134B-D140A (5521 kb)	D134B	238.6	5.53	-0.66	Y
	DR	<i>qSBD-3-2</i>	3	3.44	D333B-D334 (581 kb)	D334	260.5	9.84	0.68	L
	DR	<i>qSBD-7</i>	7	3.91	RM505-RM234 (946 kb)	RM505	122.1	1.12	0.15	L
	DR	<i>qSBD-11-1</i>	11	3.71	D1103-RM26155 (2891 kb)	D1103	7.0	1.56	-0.57	Y
	LH	<i>qSBL-3-1</i>	3	5.34	D333B-D334 (581 kb)	D334	262.5	7.93	4.58	L
	LH	<i>qSBL-4</i>	4	3.35	RM1113-D468 (1175 kb)	D468	142.3	4.15	3.73	L
	LH	<i>qSBL-7</i>	7	3.12	D760-RM248 (77 kb)	RM248	161.7	4.82	3.91	L
	PLH	<i>qSBPL-3-1</i>	3	5.70	D333B-D334 (581 kb)	D334	264.5	8.70	8.63	L
	PLH	<i>qSBPL-3-2</i>	3	4.04	D336B-RM3585 (919 kb)	D336B	290.2	15.90	-7.12	Y
	PLH	<i>qSBPL-7</i>	7	5.07	D760-RM248 (77 kb)	RM248	161.7	6.97	5.65	L
(E3) 2012/Hangzhou	DR	<i>qSBD-3-1</i>	3	4.45	D328B-D331B (2842 kb)	D331B	NP	14.66	1.06	L
	DR	<i>qSBD-3-2</i>	3	3.85	D333B-D334 (581 kb)	D334	NP	11.25	0.79	L
	DR	<i>qSBD-11-2</i>	11	6.19	RM26155-D1113 (1831 kb)	RM26155	NP	15.19	-1.25	Y
	LH	<i>qSBL-3-2</i>	3	7.57	D328B-D331B (2842 kb)	D328B	NP	31.53	12.56	L
	LH	<i>qSBL-11-2</i>	11	4.57	RM26155-D1113 (1831 kb)	D1113	NP	12.58	-9.59	Y
	PLH	<i>qSBPL-3-3</i>	3	7.00	D328B-D331B (2842 kb)	D328B	NP	29.81	10.76	L
	PLH	<i>qSBPL-11-2</i>	11	3.70	RM26155-D1113 (1831 kb)	RM26155	NP	12.27	-6.79	Y

Physical distances based on Nipponbare reference sequence. NP, not presented since these QTLs were detected using a different genetic linkage map. DPE, direction of phenotypic effect. L and Y denote Lemont and Yangdao 4 alleles increasing the phenotypic values, respectively.

A total of 7 QTLs for PLH (*qSBPL-3-1*, *qSBPL-3-2*, *qSBPL-3-3*, *qSBPL-7*, *qSBPL-11-1*, *qSBPL-11-2*, and *qSBPL-12*) were identified. The alleles *qSBPL-3-2*, *qSBPL-11-1*, and *qSBPL-11-2* from Lemont explained 2.82-15.90% of the phenotypic variation, while the alleles *qSBPL-3-1*, *qSBPL-3-3*, *qSBPL-7*, and *qSBPL-12* from Yangdao 4 explained 6.97-29.81% of the phenotypic variation. All 7 PLH-QTLs were detected in 1 environment, some of which

co-localized with the DR-QTL or LH-QTL.

By comparing the locations of the ShB-QTL detected in 3 environments, we found 6 ShB-QTL clusters consisting of 16 co-localized ShB-QTL: 2 clusters on chromosome 3 (D328B-D331B and D333B-D334 intervals), 1 on chromosome 7 (D760-RM248 interval), 2 on chromosome 11 (D1103-RM26155 and RM26155-D1113 intervals) and 1 on chromosome 12 (RM1246-D1260 interval). Of the 6 clusters, 2 clusters (D760-RM248 and RM26155-D1113 intervals) showed ShB-QTL in only 1 environment, while the other 4 clusters showed ShB-QTL in 2 environments.

The physical distance between the markers D333B and D334 was 581 kb according to the Nipponbare sequences. Three ShB-QTLs (*qSBD-3-2*, *qSBL-3-1*, and *qSBPL-3-1*) were identified in this 581-kb region while *qSBD-3-2* was detected in 2 environments.

The DR-QTL *qSBD-12-2*, detected in E1, was delimited to an 874-kb region defined by markers RM1246 and D1252 on chromosome 12. The region of *qSBPL-12* overlapped with that of *qSBD-12-2*.

### Correlation between ShB resistance and plant height

As shown in Table 4, the correlations were significant ( $P < 0.01$  or  $P < 0.0001$ ) between PH and DR, with correlation coefficients of -0.327, -0.410, and -0.256 in the 3 environments, respectively. The correlations between PH and LH were weak, and a significant correlation was detected only in E1 (correlation coefficient = -0.232). Highly significant negative correlations ( $P < 0.0001$ ) were detected between PH and PLH, with correlation coefficients of -0.352, -0.403, and -0.336 in the 3 populations, respectively.

As expected, highly significant ( $P < 0.0001$ ) positive correlations were detected among DR, LH, and PLH in the 3 environments (Table 4).

**Table 4.** Correlation analysis among disease rating (DR), lesion height (LH), percentage of lesion height (PLH), and plant height (PH) in the three mapping populations.

Environment	Item	DR	LH	PLH	PH
E1	DR	1			
	LH	0.810**	1		
	PLH	0.824**	0.987**	1	
	PH	-0.327**	-0.232*	-0.352**	1
E2	DR	1			
	LH	0.919**	1		
	PLH	0.966**	0.955**	1	
	PH	-0.410**	-0.129	-0.403**	1
E3	DR	1			
	LH	0.803**	1		
	PLH	0.818**	0.972**	1	
	PH	-0.256*	-0.131	-0.336**	1

\*\* $P < 0.0001$ ; \* $P < 0.01$

### QTL for plant height

In order to explore the relationship between ShB resistance and PH, we mapped the QTL for PH in the 3 environments. Five QTL were detected in E1. No QTL was identified in E2. Only 1 QTL, *qPH-3*, was detected in E3. The *qPH-3* was detected in both E1 and E3 (Table 5). It was found that 1 PH-QTL, *qPH-3*, co-localized with the ShB-QTL, *qSBPL-3-2*,

at the D336B-RM3585 interval on chromosome 3. The limit of detection peak for *qPH-3* was similar to marker RM3585, but the peak of *qSBPL-3-2* was closer to marker D336B. The positions of the other PH-QTLs were different from the ShB-QTL.

**Table 5.** QTLs for plant height detected using composite interval mapping.

Environment	QTL	Chr.	LOD	Marker interval (Physical distance)	Nearest marker	LOD peak position (cM)	R <sup>2</sup> effect	Additive	DPE
E1	<i>qPH-1</i>	1	3.50	D144A-D142C (1315 kb)	D144A	292.6	11.22	5.94	L
	<i>qPH-2</i>	2	3.67	RM5607-D241 (2149 kb)	RM5607	190.9	11.22	-4.49	Y
	<i>qPH-3</i>	3	16.41	D336B-RM3585 (919 kb)	RM3585	296.2	8.89	0.26	L
	<i>qPH-4</i>	4	4.12	D456-D463 (3475 kb)	D456	107.3	0.97	0.83	L
	<i>qPH-6</i>	6	5.22	D653-RM5371 (154 kb)	D653	0	16.23	-5.64	Y
	E3	<i>qPH-3</i>	3	3.76	D336B-RM3585 (919 kb)	RM3585	NP	2.18	0.29

Physical distances based on Nipponbare reference sequence. NP, not presented. DPE, direction of phenotypic effect. L and Y denote Lemont and Yangdao 4 alleles increasing the phenotypic values, respectively.

## DISCUSSION

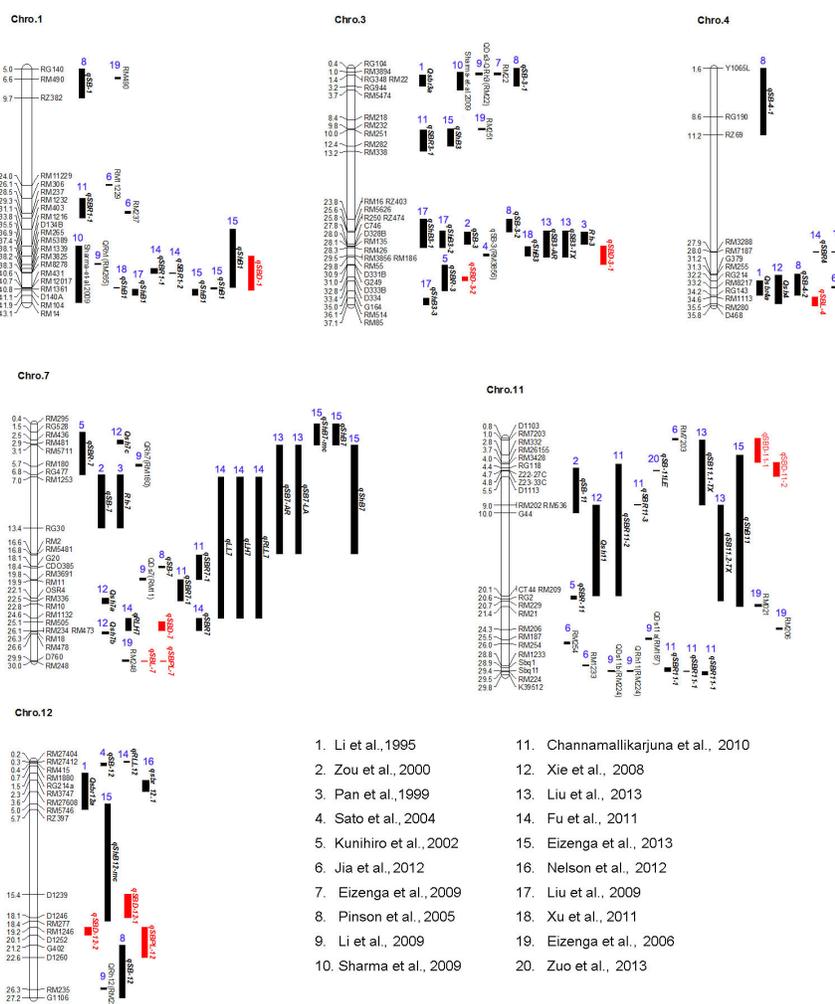
### A fast approach for detecting ShB-QTL

The disadvantage of using the F<sub>2</sub> population for QTL mapping studies is that the replication of accurate phenotypic values is unavailable. The recombinant inbred line or double-haploid populations may be used to overcome this problem, but it takes longer time to develop recombinant inbred line populations and gamete selection in developing double-haploid populations by anther cultures can affect the final mapping results (Zou et al., 2000). In order to quickly and accurately identify ShB-QTL in Yangdao 4, the strategy used in this study combined the F<sub>2</sub> and F<sub>2:3</sub> populations to detect QTL in multiple environments. 1) A genetic linkage map was constructed using the F<sub>2</sub> population planted in E1. QTL analysis was conducted in E1 (F<sub>2</sub> population) and E2 (F<sub>2:3</sub> population). 2) Based on the QTL analysis results identified in E1 and E2, another F<sub>2</sub> population planted in E3 was used to confirm the QTL identified in E1 and E2. 3) To reduce disease escape because of the limitation of single plants in the F<sub>2</sub> population, we inoculated 3 tillers in a single F<sub>2</sub> individual to increase the accuracy of the phenotypic data. Figure 3 shows that some ShB-QTL were detected in 2 environments, demonstrating that these ShB-QTL were stable across different environments and the feasibility of our QTL-mapping approach. These stable ShB-QTL co-localized to form 4 clusters: the D328B-D331B and D333B-D334 intervals on chromosome 3, the D1103-RM26155 interval on chromosome 11, and the RM1246-D1260 interval on chromosome 12 (Figure 3). These stable ShB-QTL loci have the potential for use in breeding ShB-resistant cultivars.

To accelerate QTL mapping process, we constructed 2 marker linkage maps: the first map contained 180 markers, while the second had only 41 markers. We used a small number of markers in the second linkage map to quickly confirm the ShB-QTL. These 41 markers covered the genomic regions of the QTL detected in E1 and E2. Consequently, some of the ShB-QTL may not be identified in E3 because of limitations in marker numbers in the second linkage map.

### Comparison of the ShB-QTL in present and previous studies

To compare the ShB-QTL identified in the present study with those detected in previous studies (Pan et al., 1999; Zou et al., 2000; Kunihiro et al., 2002; Sato et al., 2004; Pinson et al., 2005; Xie et al., 2008; Li et al., 1995, 2009; Sharma et al., 2009; Channamal-likarjuna et al., 2010; Fu et al., 2011; Xu et al., 2011; Jia et al., 2012; Nelson et al., 2012; Eizenga et al., 2006, 2009, 2013; Liu et al., 2009, 2013; Zuo et al., 2013), a physical map was drawn based on the alignment of published markers in relation to the Nipponbare sequences in GenBank (Figure 5).



*qSBD-1*, which was detected in this study, was co-located (or located in similar positions) to *qShB1* (Eizenga et al., 2013), *qSBR1-1* (Fu et al., 2011), *qSBR1-2* (Fu et al., 2011), *qShB1* (Liu et al., 2009; Xu et al., 2011), and a QTL detected by Sharma et al. (2009) (Figure 5). *qSBD-3-2*, which was detected on chromosome 3, coincided with *qSBR-3* (Kunihiro et al., 2002). According to Kunihiro et al. (2002), *qSBR-3* co-located with the culm length QTL *qCL-3*. In this study, we did not observe the co-localization of PH-QTL with *qSBD-3-2*, but a PH QTL, *qPH-3*, was detected nearby. We were unable to determine whether *qSBD-3-2* and *qSBR-3* were the same locus based on the available data. The location of *qSBL-4* overlapped with that of *Qsh4*, which was detected by Xie et al. (2008). *qSBD-7*, which was detected on chromosome 7, co-localized with *qSBR7* and *qRLH7* as identified by Fu et al. (2011) (Figure 5). *qSBD-11-2* was delimited to the RM26155-D1113 interval on chromosome 11, and a QTL *qSB-11<sup>LE</sup>* that was fine-mapped by Zuo et al. (2013) was also located in this region. Because the resistance alleles *qSBD-11-2* and *qSB-11<sup>LE</sup>* were both from Lemont, they are likely the same locus.

### Novel ShB-QTLs detected in this study

We identified 2 QTLs at the marker D760-RM248 interval on chromosome 7, where ShB-QTLs had not been mapped in previous studies (Figure 5). However, the marker RM248 was reported to be associated with ShB resistance by Eizenga et al. (2006).

*qSBD-12-2*, detected at the RM1246-D1252 interval on chromosome 12, is a novel ShB-QTL because ShB-QTL has not been previously observed in this region (Figure 5). The physical distance from RM1246 to D1252 is 875 kb based on the Nipponbare sequence. The QTL *qSBD-12-2* detected in E1 overlapped with *qSBPL-12* detected in E2. *qSBD-12-2* was detected in only 1 environment, and its stability requires further investigation.

### Relationship between ShB resistance and plant height

Several prior studies reported a negative correlation between ShB resistance and PH and co-localization of ShB-QTL and PH-QTL (Li et al., 1995; Zou et al., 2000; Kunihiro et al., 2002; Sato et al., 2004). In this study, a significant negative correlation was detected between PH and DR (or PLH) in all 3 environments and between PH and LH in E1. Co-localization of QTLs was also found: Of the 5 PH-QTLs detected, 1 of which, *qPH-3*, was co-localized with the ShB-QTL, *qSBPL-3-2*. The ShB-QTL and PH-QTL co-localized in the same genomic location because ShB resistance and PH are closely related: ShB disease severity generally decreases in individuals with taller PH, assuming that the disease spreads at the same rate on short and tall plants, as our scoring system for ShB resistance was based on the infected proportion of the vegetative parts of the plant (Li et al., 1995). Therefore, PH will inevitably affect ShB disease score using the current disease rating system, and some PH-QTL may be mistaken as 'ShB-QTL', indicating a pleiotropic effect of these PH-QTL. Caution should be used in evaluating 'ShB-QTL' that co-localize with PH-QTL. Only the ShB-QTL irrelevant to PH-QTL will be useful for developing ShB-resistant cultivars.

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