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ORIGINAL PAPER

Fine mapping of *qSB-11*^{LE}, the QTL that confers partial resistance to rice sheath blight

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Abstract Sheath blight (SB), caused by *Rhizoctonia solani* kühn, is one of the most serious global rice diseases. No major resistance genes to SB have been identified so far. All discovered loci are quantitative resistance to rice SB. The qSB-11^{LE} resistance quantitative trait locus (QTL) has been previously reported on chromosome 11 of Lemont (LE). In this study, we report the precise location of *qSB-11*^{LE}. We developed a near isogenic line, NIL-qSB11^{TQ}, by marker-assisted selection that contains susceptible allele(s) from Teqing (TQ) at the qSB-11 locus in the LE genetic background. NIL-qSB11^{TQ} shows higher susceptibility to SB than LE in both field and greenhouse tests, suggesting that this region of LE contains a QTL contributing to SB resistance. In order to eliminate the genetic background effects and increase the accuracy of phenotypic evaluation, a total of 112 chromosome segment substitution lines (CSSLs) with the substituted segment specific to the *qSB-11*^{LE} region were produced as the fine mapping population. The genetic backgrounds and morphological characteristics of these CSSLs are similar to those of the recurrent parent LE. The donor TQ chromosomal segments in these CSSL lines contiguously overlap to bridge the *qSB-11*^{LE} region. Through artificial inoculation, all CSSLs were evaluated for resistance to SB in the field in 2005. For the recombinant lines, their phenotypes were evaluated in the field for another 3 years and during the final year were also evaluated in a controlled greenhouse environment, showing a consistent phenotype in SB resistance across years and conditions. After comparing the genotypic profile of each CSSL with its phenotype, we are able to localize *qSB-11*^{LE} to the region defined by two cleaved-amplified polymorphic sequence markers, Z22-27C and Z23-33C covering 78.871 kb, based on the rice reference genome. Eleven putative genes were annotated within this region and three of them were considered the most likely candidates. The results of this study will greatly facilitate the cloning of the genes responsible for *qSB-11*^{LE} and marker-assisted breeding to incorporate *qSB-11*^{LE} into other rice cultivars.

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Introduction

Sheath blight (SB) is one of the most serious global diseases of rice causing huge yield losses and low grain quality under severe disease conditions (Lee and Rush 1983). Its causal pathogen, *Rhizoctonia solani* kühn (*R. Solani*), is a soil borne fungus with strong saprophytism and broad host range. No rice germplasms, including

wild-rice species, are found to be completely resistant to this disease. However, variations in resistance to SB exist among different rice varieties (Li et al. 1995; Jia et al. 2009). Rice SB resistance has been reported to be a typical polygenic quantitative trait (Groth and Nowick 1992; Li et al. 1995; Sato et al. 2004; Pinson et al. 2005; Jia et al. 2009).

To date, around 50 SB resistance quantitative trait loci (SBR QTLs) have been detected on all 12 rice chromosomes in cultivated varieties, deep-water varieties and wild species (Jia et al. 2009; Zuo et al. 2010; Wang et al. 2012; Xu et al. 2011; Fu et al. 2011). Some of them were identified in multiple mapping populations and/or environments and not associated with either heading date (HD) or morphological traits, and they are believed to be real SBR QTLs (Han et al. 2003; Jia et al. 2009; Zuo et al. 2010; Pinson et al. 2005; Wang et al. 2012). However, none of the SBR QTLs detected so far were fine mapped: they are mapped in an interval of at least 800 kb, with an average of 3.644 Mb (Zuo et al. 2010). The greatest limiting step in fine mapping of the SBR QTLs lies in the disease phenotyping, which is confounded by many factors such as the environment, plant height (PH), canopy density, and fertility input. In attempts to control these factors, some researchers have resorted to experimentation in greenhouses and growth chambers for conducting phenotypic evaluation (Wamishe et al. 2007; Jia et al. 2007). In addition to improving the phenotypic evaluation method, employing a special mapping population, such as introgression lines (ILs) and chromosome segment substitution lines (CSSLs) is always a complementary and efficient approach to enhance the accuracy of phenotypic evaluation (Abiola et al. 2003; Ando et al. 2008). Also, due to relatively similar genetic backgrounds among the lines, the effects from plant morphological traits could be greatly reduced. A set of CSSLs from the cross of Teqing (TQ) and Lemont (LE) has been successfully used to fine map a major QTL controlling rice stripe disease (Wu et al. 2011). Through the use of ILs, the effects of SBR QTLs *qSB9-2* and *qSB12-1* were observed in both field and green house (Tan et al. 2005; Wang et al. 2012). Furthermore, the qSB9-2 was located on the bottom of chromosome 9 using the same ILs (Liu et al. 2009; Wang et al. 2012).

Although most SBR QTLs were detected in relatively resistant parents, a few were identified from relatively susceptible cultivars (Li et al. 1995; Zou et al. 2000, 2011; Han et al. 2002; Pinson et al. 2005; Xu et al. 2011). The resistant alleles from susceptible parents have also been reported in many studies of resistance to other diseases (Young 1996; Al-Chaarani et al. 2002; Chen et al. 2003; Perchepied et al. 2005). The $qSB\text{-}11^{LE}$ (named $qSB\text{-}11^{Le}$ in Zuo et al. 2007), from the susceptible LE, was first found by Li et al. (1995) to be linked to the molecular marker RG118 using a cross of TQ/LE. However, this QTL was not statistically confirmed in their second-year test due to a LOD value slightly smaller than 2.5 for supporting the existence of a QTL. Nevertheless, in the population generated from a Jasmine 85 and LE cross, the $qSB\text{-}11^{LE}$ was mapped within the interval of RG118/G44 in two subsequent years (Zou et al. 2000). Up to 31.2 % of the total phenotypic effects were attributed to $qSB\text{-}11^{LE}$ in this study. By aid of marker-assisted selection (MAS), the presence of $qSB\text{-}11^{LE}$ was further confirmed by an advanced backcross population and was found flanked by the simple sequence repeat (SSR) markers, RM167 and RM202 (Tan et al. 2005). According to the integrated genetic maps (McCouch et al. 2002), the interval of RG118/G44 almost overlaps with that of RM167/RM202. Using a BC_4F_2 population, we further mapped the $qSB\text{-}11^{LE}$ to a 1,041-kb region and found it rescued the yield loss by 10.71 % under severely diseased conditions (Zuo et al. 2007). Recently, the phenotypic effect of $qSB\text{-}11^{LE}$ was further observed to reduce lesion length development by approximately 4 cm (22 %) at adult stage (Zuo et al. 2011). In the same study, eight *indica* rice cultivars from distant areas were found to lack $qSB\text{-}11^{LE}$ and the introgression of $qSB\text{-}11^{LE}$ enhanced their resistance. Therefore, the $qSB\text{-}11^{LE}$ locus is a verified SBR QTL and is ready for employment for SB disease resistance by MAS.

So far, only a few quantitative disease resistance genes have been characterized (Poland et al. 2009; Kump et al. 2011). Based on the substantial progress on *R* gene-mediated resistance and disease resistance signaling, more and more researchers have hypothesized that quantitative resistance genes function as defense genes or components of defense signaling (Hu et al. 2008; Kump et al. 2011). Therefore, several high throughput assays have been employed to analyze the candidate genes underlying SBR QTLs. For example, via whole genome sequencing on 13 rice cultivars, Silva et al. (2012) obtained more than 200 candidates for SBR resistance genes through nsSNPs analysis and found ten of them located in the *qSB-9-2* region and likely to contribute to the *qSB-9-2* quantitative resistance. Using RL-SAGE and microarray, Venu et al. (2007) found that many genes responsive to SB fungal infection are located in the known SBR QTL regions. In the study by Zhao et al. (2008), many defense-related genes induced by the SB fungus were identified through suppression subtractive hybridization. However, whether these genes are associated with SBR QTLs is unclear. By joint analysis on phenotypic data from multiple years, SBR QTL *qSB-11-3* was identified in a 0.85-Mb chromosomal region, which contains 11 tandem repeats of genes encoding chitinases, well known to be involved in defense against fungi (Channamallikarjuna et al. 2010). In summary, these efforts have provided valuable information to the cloning

of candidate SBR QTLs and the elucidation of the mechanism of SB resistance.

In this study, we performed a fine mapping of *qSB-11*^{LE} by developing a set of 112 CSSLs. Through artificial inoculation, the phenotypes of these CSSLs were evaluated in field and in greenhouse under controlled temperature and humidity. These results will accelerate the cloning of the gene(s) responsible for *qSB-11*^{LE} and help to elucidate the mechanism underlying quantitative resistance to rice SB.

Materials and methods

Plant materials

Lemont, a *japonica* rice cultivar from Louisiana, USA, is a susceptible cultivar to rice SB; however, it possesses a resistance QTL (*qSB-11*^{LE}) (Li et al. 1995; Zou et al. 2000, 2007; Tan et al. 2005). In our previous study (Zuo et al. 2007), we developed a near isogenic line, NIL-qSB11^{TQ}, by marker-assisted selection that contains susceptible allele(s) from Teqing (TQ) at the qSB-11 locus in the LE genetic background. TQ is an *indica* rice cv. from Guangdong, China, which has been confirmed to possess susceptible allele(s) in the qSB-11 locus (Zou et al. 2000, 2011; Tan et al. 2005). In an attempt to fine map *qSB-11*^{LE}, LE was therefore used as a relatively resistant or less susceptible control in the present study.

A total of 112 CSSLs developed in this study were used as the fine mapping population.

Primer design and marker development

Sequence divergence within and around the target interval between the *indica* rice cv. 93-11 (http://www.btn.genomic.org.cn/rice) and the *japonica* rice cv. Nipponbare (http://rgp.dna.affrc.go.jp, the International Rice Genome Sequencing Project (IRGSP) Build5 Pseudomolecules.) were identified by a basic local alignment search tool (BLAST) search. The identified inserts and deletions (InDels) and single nucleotide polymorphisms (SNPs) embedded in restriction recognition sites were used for developing sequence-tagged site (STS) and cleaved-amplified polymorphic sequence (CAPS) markers, respectively. According to the primer criteria described by Ji et al. (2005), primers for candidate markers were designed on the flanking regions of the InDels and SNPs using software Primer Premier V5.0 (http://www.tucows.com/preview/205452).

Genomic DNA was extracted using the method described by Murray and Thompson (1980). Markers showing polymorphisms between parental lines were developed after polymerase chain reaction (PCR) and gel analysis according to the method described by Ji et al. (2005).

Method for development of NILs and CSSLs specific to the *qSB-11*^{LE} region

Modified MAS similar to that reported by Lecomte et al. (2004) was used in a backcrossing program to save time in constructing CSSLs (Fig. 1). This MAS selection resulted in one BC_4F_1 (the F_1 from four consecutive backcrossing) plant having a heterozygous *qSB-11*^{LE} region. By utilizing 112 polymorphic markers evenly distributed on the rice genome (Supplementary Fig. 1), it was revealed that this plant also shared an identical genetic background with LE. This individual was selected for complete self-pollination (BC_4F_2) for which each panicle was protected in an enclosed envelope during flowering. In the BC_4F_2 population, a plant with homozygous TQ segment from markers RM167 to Z58 covering the *qSB-11*^{LE} region was selected and its progeny was named NIL-qSB11^{TQ}, a near isogenic line to LE.

Based on the marker genotype, the BC_4F_2 individuals containing chromosomal cross-overs in the *qSB-11*^{LE} region were selected. These recombinant BC_4F_2 individuals were heterozygous for the markers either on the right

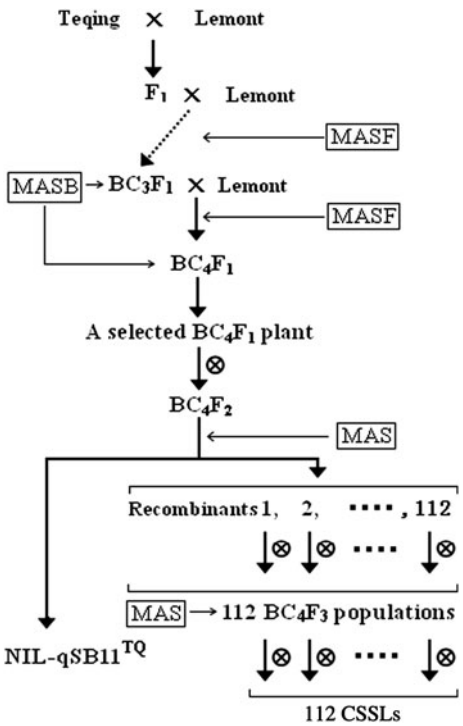

Fig. 1 Diagram of strategy for developing CSSLs specific to qSB-11. *MAS* marker-assisted selection; *MASF* MAS for foreground with the markers flanking the qSB-11 region; *MASB* MAS for genetic background with 112 polymorphic markers evenly distributed on the other rice genome regions (Supplementary Fig. 1). MASF was conducted from BC_1F_1, and the plant with the heterozygous genotype at the qSB-11 region was selected for backcrossing. MASB was conducted on the plants selected by MASF and from the BC_3F_1 generations

side or the left side of the cross-over point. This required further self-pollination of each BC_4F_2 individual to produce homozygous genotypes at all tested markers in BC_4F_3 generation. The progeny (BC_4F_4) of each homozygous plant was named as CSSL.

Investigation of morphological traits

To evaluate the morphological traits, the CSSLs, LE, and NIL-qSB11^{TQ} were planted in the experimental farm at the Agriculture College, Yangzhou University, China, in the summer of 2005. All materials were sown in the seedling nursery on May 10 and 30-day-old seedlings were transplanted into two-row plots. Ten plants were placed in each row. Plants were spaced 20 cm apart; rows were spaced 35 cm apart. The plots were arranged in a completely randomized block design with three replications. All test materials were measured for eight traits: heading date (HD), plant height (PH) and flag-leaf height (FLH), flag-leaf length (FLL, cm) and flag-leaf width (FLW, mm), panicle length (PL, cm), tiller number (TN), and tiller angle (TA). All traits, except HD were measured from seven plants in each plot. HD was recorded as days from sowing to panicle emergence of 50 % plants in a plot. PH and FLH were measured from the soil surface to the tips of the tallest panicles (PH) and flag leaves (FLH). FLL and FLW were measured as the length and width of the flag leaves of three main tillers of each plant before maturity. PL was measured after maturity. TN was counted as the number of tillers of each plant before maturity. TA was measured as the maximum degree among tillers in the plant using a protractor.

Data was processed with Microsoft Excel 2003. The variance in each trait between CSSLs and NIL-qSB11^{TQ} and LE was analyzed using ANOVA procedure packaged in SAS V9.1.

Fungal inoculation and symptom scoring

RH-9, a *R. solani* strain with strong pathogenicity, was obtained from the Plant Protection Institute of Jiangsu Academy of Agricultural Science, Nanjing, China. Truncated thin woody matchsticks (2–3 mm wide, 0.8–1.0 cm long, and 1 mm thick) colonized by RH-9 were used as the inoculums for pathogen infection (Zou et al. 2000). Inoculation was performed at the late tillering stage according to the method described in previous studies in both greenhouse and field (Li et al. 1995; Zou et al. 2000; Tan et al. 2005). Using forceps, the inoculums were inserted at the joint of the stem and the third sheath from the top of a plant, taking care to keep the sheath-stem holding status. In addition to the primary tiller, the other two largest tillers in each plant were also inoculated to ensure successful inoculation. In addition, to reduce the variation of fungal infection due to the time lag of inoculation, more than 40 pre-trained graduate and undergraduate students were employed to finish these large-scale field inoculations in a timely manner. Four pictures in Supplemental Fig. 2 present snapshots of the inoculation process in the field.

We recorded the SBR rating at 25 days after heading (DAH) by a rating scale (Table S1) modified from that reported by Rush et al. (1976), (Zuo et al. 2006). This modified rating scale is capable of measuring individual tillers on a plant with improved objectivity. For LE at this scoring time, most tillers in the disease-free plant had six leaves, where the sixth sheath from the top was the inoculated sheath. The modified rating scale started to score from the sixth sheath. Rating zero is defined as no disease, rating one as lesion restricted to sheath six. Higher ratings represent longer lesions extending up the tiller and sheaths. Ratings higher than seven represent lesion development on the flag leaf or affecting the viability of panicles. Since the CSSLs exhibit the same development and morphological traits, the sheath order is used as the uniform marker when rating.

Two disease criteria were assessed for each inoculated plant: (1) the SBR score, denoted by the most seriously diseased tiller in the plant; and (2) PTSS8, the percentage of the tillers with SBR scores of eight and higher in each plant. The first criterion represents the vertical spreading degree of the disease on a plant, while the second reflects the disease severity on the whole plant. The PTSS8 was calculated by the following formula: (the number of tillers with SBR scores of eight and higher in a plant) ÷ (the total number of tillers in a plant) × 100 %.

For inoculation in greenhouse, the plants were sowed in small pots with autoclaved soil and grown in a natural environment until inoculation at the late tillering stage. The methods for inoculum preparation and plant inoculation were same as those for field tests (Zou et al. 2000). Three days before inoculation, healthy plants were transferred into the greenhouse with controlled humidity (RH: 80–85 %) and temperature (30 °C/13 h, 26 °C/11 h). Lesion length was measured with a ruler at 14 days after inoculation (DAI). The experiment was repeated three times with 20 individuals per replication. The data was analyzed by ANOVA procedure (packaged in SAS V9.1).

Field arrangement and phenotype determination for CSSLs

In the summer of 2005, a two-replicate experiment was conducted in the field to evaluate the SB resistance of all CSSLs in the experimental farm of the Agriculture College, Yangzhou University. In the field, irrigating water was maintained at a height of 12 cm at all times. Each replicate

consisted of 118 plots; each plot represented an independent CSSL or a control and was arranged in three rows. Each row contained 12 plants, spaced 20 cm between plants. Rows were set 35 cm apart. Only the ten plants in the center of the middle row of each plot were inoculated with the fungus. The less susceptible (relatively resistant compared to NIL-qSB11^{TQ}) LE and the highly susceptible NIL-qSB11^{TQ} (as controls) each occupied three plots in a replicate; the remaining 112 plots were used for growing the CSSLs. During the summers of 2006 and 2007, some of the CSSLs were repeatedly evaluated with five replicates in field in a completely randomized block design. The planting criteria and field management were the same as those of 2005. Several key recombinant CSSLs were further inoculated in field, and in greenhouse with controlled humidity and temperature in the summer of 2009.

Both the SBR score and the PTSS8 score of each plant were collected. The phenotypic data of each CSSL were calculated by averaging the scores of all inoculated plants from two replicates. The few plants that suffered from pests and other diseases were removed from the data analysis. Based on the two disease-criterion data, the *K*-means dynamic clustering algorithm (Fast-clustering program in SAS V9.1) was used to preliminarily distinguish the phenotypes of the CSSLs into susceptible and highly susceptible. ANOVA procedure (packaged in SAS V9.1) was further applied to analyze the differences in SB disease data among the key recombinant CSSLs and the controls.

Results

Marker development

In previous independent studies (Li et al. 1995; Zou et al. 2000, 2007; Tan et al. 2005), a total of five markers were reported as associated with the *qSB-11^{LE}*. According to the rice reference genome (www.gramene.org), these five markers are aligned in the order of RM167-RG118-Z43.9-RM202-G44 on chromosome 11. Consequently, the longest interval of RM167/G44 is most likely to cover the qSB-11^{LE} locus.

In addition to the two publicly available SSR markers (RM202 and RM229), a total of 26 molecular markers, including 11 of them developed in our pervious study (Zuo et al. 2007), within and around the interval of RM167/G44 were used in this study (Table 1). Twenty-three markers including RM202 were located within the interval of RM167/RG44, while the remaining five markers including RM229 were located outside this region. In order to enhance efficiency in MAS, one STS marker (Z529) was used to replace the RFLP G44 marker at a similar position. In addition, since the STS marker Z405 was located near RM167, the interval of Z405/Z529 was believed to encompass the *qSB-11^{LE}* locus.

Establishment of NILs and CSSLs specific to the *qSB-11^{LE}* region

After monitoring the genetic backgrounds of approximately 40 BC_4F_1 lines with 112 polymorphic markers, which evenly cover all 12 chromosomes (Fig. S1), we developed a line (NIL-qSB11^{TQ}) that shares the same genetic background to LE but contains a TQ chromosomal segment over the region of Z405/Z529 (Fig. 1). Even though LE was a known susceptible control in previous studies, it was clearly less susceptible than NIL-qSB11^{TQ} after repeated tests in the field and greenhouse (Fig. 2). The NIL-qSB11^{TQ} was observed to have faster disease development and larger lesion area than LE, representing lower basal resistance to SB fungus infection. The phenotypic difference between the isogenic lines was most striking by visual observation at 35 DAH where nearly all leaves of the NIL-qSB11^{TQ} plants were dead and dry while many leaves of each LE plant remained green. However, the SBR score and the PTSS8 value showed the highest quantitative difference at 25 DAH in field. Thus, 25 DAH was used to measure phenotypic values for disease rating in field. In the greenhouse, lesion lengths were measured at 14 DAI and completed before the booting stage.

In order to narrow down the *qSB-11^{LE}* interval, we followed the method proposed by Abiola et al. (2003) to produce a sub-congenic set of CSSLs. We developed a set of 112 CSSLs (Fig. 3) through multiple rounds of MAS-based backcross for both genetic background and the region of *qSB-11^{LE}* (described in "Materials and methods"). The *qSB-11^{LE}* region between markers Z115 and RM229 is completely covered by contiguous, overlapping TQ segments of 112 CSSLs. Each CSSL had 1–3 cross-over points within the interval of Z115/Z84. CSSLs with cross-overs occurring in the same marker-interval were temporarily grouped in the same class. Accordingly, the CSSLs were grouped into 18 classes. The region between Z405 and Z529 was saturated with high-density markers with an average interval of 375.73 kb (Table 1), resulting in high resolution of the recombinant CSSLs. These homozygous CSSLs have a similar genetic background to LE because they were from a single parental BC_4F_1 plant whose genetic background was monitored by 108 molecular markers (Fig. S1). Due to the availability of CSSLs, multiple replications were conducted in multiple years to accurately determine the degree of resistance or susceptibility. These criteria made it possible to fine map the *qSB-11^{LE}* locus.

Table 1 Primer sequence and physical position of developed molecular markers

Primer	Physical position on chromosome 11 (bp)	Sequence (5′–3′)	
		Forward	Forward
Z115[d]	2517784[a]	TGTATGTTCCACTTTGCCACC	CACCTCCTCTATCACAGATTCCA
Z405[d] (RM167[b])	4079072	TACTTCCCGGTACTGAGAC	CTTTGGTTTAGGTGCTGTT
Z439[d] (RG118[b])	4396094	TGCGAGACGAATTTATTAAGCC	CAAAGGGTAGTGAAAGAAGGAGA
Z21-12	4570961	ACAAATCCACCCAACACAT	GGGACCAATCACTCACAAG
Z22-17	4655503	TACTTGAGGGGTGAACATC	TATCTTTCTGAGACTGGGA
Z22-27C (*Bam*H I[c])	4785044	CTGTTCGTACATCAGCAAGG	TGACAGGTTGATGTGATGGA
Z22-18	4855244	ATTTGCTTGTCAGGTTGCCC	CGGCTGTAGTGGAGTCGTCG
Z23-33C (S*ma* I[c])	4863915	TTCATCGCACCTGTCCTTAAA	TGACGAGCCAGGAAAGTTTTA
Z22-32C (A*at* II[c])	4865655	CAAGTTTCAATGCAGGACGT	GTGACAGAAAAGAAAGGAACA
Z23-3	4879527	AATCAAACTTATTAGCAGCAATC	CATCGTTGTTTAGAGTTGTATTG
Z23-4C (*Eco*R I[c])	4986788	CCTGGGTCATCCTGTTGTG	TGGGCGAAGACCTGTATTT
Z24	5141016	ACAATCAGTTGCTGGCTCT	AACGCCACATCAGACTTCA
Z286[d]	5463510	TTGCGACTGATTACTTTGA	CATTGCTCCATTGTTTGAC
Z316[d]	5919567	GTCCTTCCAAAGATCCCTA	GATGCCCTAAACCCAAATA
Z334[d]	6317790	TTACGGTGTATTCCCATTC	TAGTGACCAGCATCCAAAG
Z394	7098454	TAGTTGCGAATGTGCGATAA	AAAGGAAGAAAAGGAATGAG
Z455[d]	7604442	TGCGTAGTTGTGGGTATAGGG	GAAACAGGATTATTGGCATCA
Z454	7648744	AAGAAGGACCTGCTAGGCA	CAGGGATGGGTTGGTAAAT
Z456	7889059	CTTTCCTCTACTTCAACTCTTCC	TAGGATTTTACTGTCCGTTTTCA
Z453[d]	7922592	AACCAAAGCCGAGTAACAT	TTCCTGAGCCCAGAAACCC
Z48	8100987	GAGCTAGAGGGAGGAGGTGC	TTGACTGATAGCCGATTGGG
Z525	9446957	GCCCGTTAGTATGGTTCCC	TTCGCATCTGCTTAGTGGAG
Z529[d] (G44[b])	9734177	GGTAGTTTTGCCCTTGACGA	GCTCCTGCATGTACTCCTTTTT
Z58[d]	13481928	ATTCATGCTTCCTTTCAGTG	GATTACTGGTTTGCCATTTG
Z62[d]	15100909	CATCCTCTGCTACCCAAAC	ATACCCTCGTGAACCTCTT
Z84-11[d]	21972925	ATACTCCCTCTGTCCCATAA	TCAGTTCTGTGGAGGTGGC

[a] Physical position on the IRGSP Build5 Pseudomolecules of rice variety Nipponbare

[b] The molecular marker located in the similar position to the corresponding STS marker developed in the present study

[c] Restriction endonuclease used in the corresponding CAPS marker analyses

[d] The markers developed in our previous study (Zuo et al. 2007)

Morphological characteristics of CSSLs

Seven morphological traits plus HD were recorded for each CSSL and the controls LE and NIL-qSB11^{TQ}. LE and NIL-qSB11^{TQ} differ only in the HD trait by 3.3 days among the examined traits (Table 2). Furthermore, this HD difference was also observed among CSSLs that appear as a single-QTL segregation mode because only two HD phenotypes were observed (data not shown). In summary, all CSSLs showed a uniform morphological phenotype in field. This minimized the effects of genetic variation between lines and from the local environmental variations, making the contribution of *qSB-11^{LE}* towards resistance easily observable.

Evaluation of CSSLs in resistance to rice SB and preliminary mapping of *qSB-11^{LE}*

For the 2005 experiment, only 2 out of 112 CSSLs were excluded because of their large variations in disease data between the two replicates. As a result, 110 CSSLs and 2 controls (six replicates for each control) were used in data analysis. High correlations were found between the two replicates on the SBR data ($r = 0.75$**) and on the PTSS8 data ($r = 0.72$**). No correlation existed between HD data and SBR data ($r = -0.11$) and PTSS8 data ($r = -0.07$), indicating that the modest HD difference had no effects on disease development.

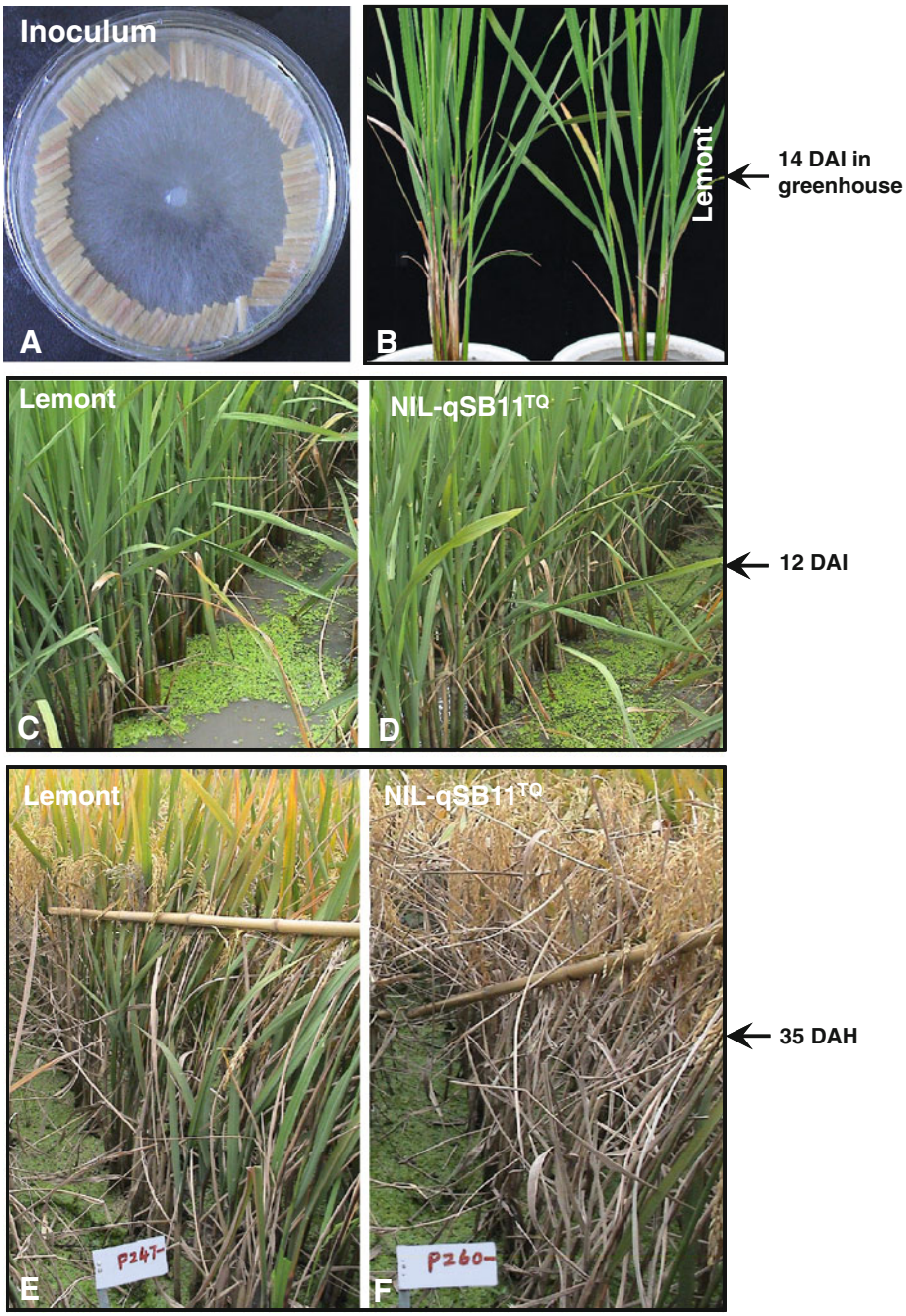

Fig. 2 Sheath blight (SB) fungus inoculum and disease severity of the near isogenic lines in field and in green house. **a** The inoculum used in the present study. **b** Lemont (LE) and its near isogenic line (NIL) inoculated in greenhouse and the picture was taken at 14 days after inoculation (DAI). **c**, **d** LE and its NIL inoculated in field and the pictures were taken at 12 DAI, respectively. **e**, **f** LE and its NIL inoculated in field and the pictures were taken at 35 days after heading

Because the contribution of *qSB-11*^{LE} is relatively small, the CSSL disease data needed to be closely examined. We compared the two disease criteria data of all lines in 2005 using a clustering strategy, and obtained two distinct classes: one class contained 50 CSSLs and the LE control and the other consisted of 60 CSSLs and the highly susceptible NIL-qSB11^{TQ} control. Consistent with this result, two distinct classes are observed on the graph in Fig. 4, where SBR was plotted against PTSS8 for the CSSLs and controls. From Fig. 4, we observed that combining both disease criteria presents a better overall phenotypic determination than relying on either one. As a result, the 50 CSSLs grouped with LE were preliminarily considered less susceptible lines and the 60 CSSLs grouped with NIL-qSB11^{TQ} considered highly susceptible ones. The less susceptible CSSLs had an average SBR score of 7.4 and a PTSS8 score of 23 % while the highly susceptible CSSLs had respective scores of 8.3 and 52 %. By comparison, the PTSS8 scores showed a larger difference than the SBR score between the less susceptible and highly susceptible groups.

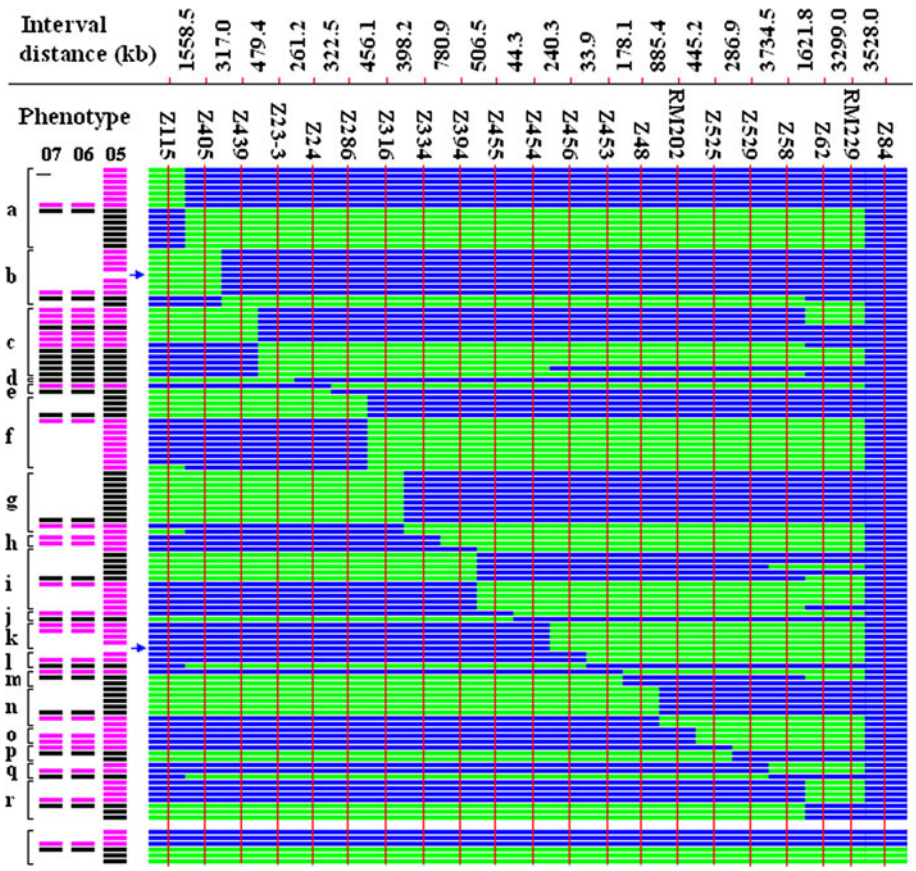

Fig. 3 Description of genotype and resistance phenotype of each CSSL and the controls. Letters *'a–r'* represent different types of CSSLs classified on the basis of the position of cross-over points between neighboring markers. *'07*, *06*, *05'* represent the years 2007, 2006, and 2005. *Mauve and black bars* under *'07*, *06*, *05'* indicate less susceptible (or can see resistant) and highly susceptible phenotypes of each CSSL in different years, respectively. *Blue* and *green horizontal thick lines* represent the Lemont (LE) and Teqing (TQ) chromosome segments, respectively. The *lowest six lines* include three lines representing the less susceptible control of LE (*mauve bars*) and the other three representing highly susceptible NIL-qSB11^{TQ} (*black bars*). *Vertical lines* represent the marker positions, whereas the map distance between adjacent markers do not represent their physical interval distance that is shown on the *upper panel* of the figure. *Cross-over points* within the same marker intervals among CSSLs are depicted at the same positions although they may not be at the same site. Two CSSLs denoted by the *arrows* were eliminated in fine mapping process of 2005 due to large variance in SBR data between the two replicates (color figure online)

Table 2 Morphological traits of Lemont and its near isogenic line NIL-qSB11^{TQ}

Traits	Lemont	NIL-qSB11^{TQ}	*F* value	*P* value
HD (days)	98.25 ± 0.71	95.0 ± 0.76	78.87	0.00
PH (cm)	76.25 ± 1.54	76.65 ± 1.62	0.19	0.67
PL (cm)	21.7 ± 1.63	21.25 ± 1.69	0.22	0.65
FLH (cm)	93.02 ± 1.97	93.25 ± 1.55	0.05	0.83
FLL (cm)	30.45 ± 1.13	30.27 ± 1.28	0.07	0.80
FLW (cm)	2.08 ± 0.11	2.05 ± 0.14	0.25	0.63
TN (number)	5.36 ± 1.25	5.41 ± 1.18	0.02	0.90
TA (°C)	27.95 ± 5.04	27.5 ± 5.51	0.08	0.78

The values are expressed as average ± standard deviation

HD heading date, *PH* plant height, *PL* panicle length, *FLH* flag-leaf height, *FLL* flag-leaf length, *FLW* flag-leaf width, *TN* tiller number, *TA* tiller angle

The genotypes and phenotypes are summarized in Fig. 3, where blue represents LE genotype and green stands for TQ genotype. Comparing the phenotype and genotype of each line, we observed that all CSSLs containing the TQ chromosomal segment over the Z439/Z23-3 region are highly susceptible, while those harboring the LE chromosomal segment in this region are less susceptible. Importantly, the CSSLs with cross-over points occurred in the Z439/Z23-3 region (class C of CSSLs) carried both less susceptible and highly susceptible phenotypes. We therefore conclude that *qSB-11^{LE}* is located in the Z439/Z23-3 region. This conclusion is consistent with our preliminary QTL mapping results based on a temporary, non-homozygous BC_4F_2 population (Zuo et al. 2007).

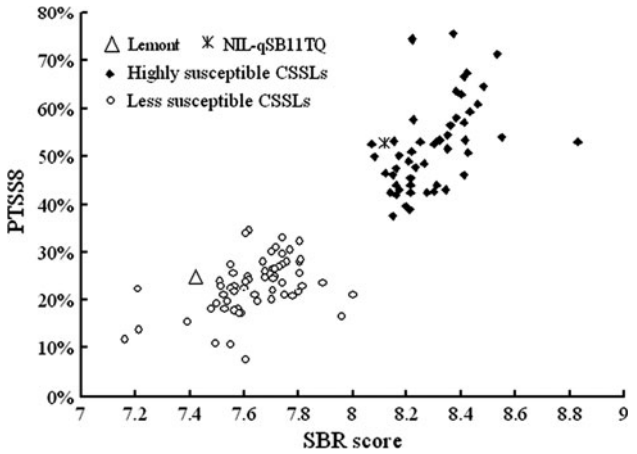

Fig. 4 Distribution of two kinds of disease data of 110 CSSLs and the controls in 2005. The PTSS8 is plotted against the SBR. Two groups are formed among the 110 CSSLs. The *empty triangle* and *asterisk* indicate the less susceptible control and highly susceptible control, respectively

Phenotype confirmation of recombinant lines and fine mapping of $qSB\text{-}11^{LE}$

In 2006 and 2007 tests, we repeatedly evaluated the 12 candidate recombinant lines from class C that contained cross-over points within the Z439/Z23-3 region and 34 CSSLs randomly selected from the other 17 classes (two lines per class) and the two lines excluded in the 2005 data analysis. Each year, 50 lines including two controls were evaluated in field with five replicates (plots) per line. After clustering analysis of the phenotypes, the results are completely consistent with those of 2005 (Fig. 3). Visual observation at 35 DAH (data not shown) confirmed the clustering results.

The difference of disease data among the controls and 12 candidate recombinant CSSLs were further analyzed by ANOVA (Table 3). By comparing the ANOVA result of each year independently on both disease criteria, these CSSLs can be easily separated into less susceptible or highly susceptible phenotypes with high consistency across 4 years. These results were identical to the results analyzed by combining data pertaining 3 years (2006, 2007, 2009) (Table S2). Only one line (#51436) had a deviating PTSS8 value in one (2007) out of 4 years of experimentation, which could have been due to a recording error. Based on the disease data of the other 3 years, this line should be considered less susceptible. As described above, the present PTSS8 scores showed more obvious difference than the SBR scores. We concluded that the phenotypes of the CSSLs are reliable and the rating in field is consistent across 4 years.

In the summer of 2009, the 12 candidate recombinant CSSLs were further evaluated in the greenhouse with controlled humidity and temperature. Lesion lengths were measured at 14 DAI. The ANOVA results showed that the lesion lengths of the less susceptible recombinant lines identified in field were all significantly shorter than those of highly susceptible lines (Table 3). The difference between less susceptible and highly susceptible controls was around 5.08 cm, and was around 5.16 cm between the less susceptible CSSLs and highly susceptible CSSLs. In summary, we confirmed the phenotypes of these 12 recombinant CSSLs in field tests for 4 years and in a controlled environment for 1 year.

These 12 recombinant CSSLs were further used to narrow down the $qSB\text{-}11^{LE}$ to a smaller region. Based on their genotypes and phenotypes, the cross-over points of 9 out of 12 CSSLs were placed on the left side of $qSB\text{-}11^{LE}$ and 3 on the right side (Table 3). Five new markers were subsequently developed to resolve these 12 CSSLs. In Table 3, we found four CSSLs that had cross-over points located in between the neighboring CAPS markers Z22-27C and Z23-33C. One of them (51439) showed highly susceptible phenotype. This line had highly susceptible genotypes (T) on the Z22-27C and its left three markers loci, while had less susceptible genotypes (L) on the Z23-33C and its right two markers loci. Therefore, we concluded that Z23-33C located at the right side of $qSB\text{-}11^{LE}$. Two of the rest three CSSLs (51442, 51444) also supported this conclusion that Z23-33C was the proximal marker on the right side of $qSB\text{-}11^{LE}$. Similarly, we identified Z22-27C as the proximal marker on the left side of $qSB\text{-}11^{LE}$ using one (51441) out of four CSSLs that contained cross-over points between the markers Z22-27C and Z23-33C. Ultimately, two CAPS markers Z22-27C and Z23-33C were determined as the proximal markers with one and three recombinants identified, respectively, after comparing with phenotypes and genotypes of the four CSSLs.

By aligning marker sequences on the IRGSP Build5 Pseudomolecules of Nipponbare on GRAMENE (http://www.gramene.org/Multi/blastview), we found that the physical distance of the region Z22-27C/Z23-33C is 78.871 kb (Fig. 5).

Putative genes in the 78.871 kb region

In the rice genome annotation database (Ouyang et al. 2007) (http://rice.plantbiology.msu.edu/), 12 putative genes were annotated in this 78.871 kb region (Fig. 5; Table 4). Six of these genes encode proteins with predicted functions: putative lipase (LOC_Os11g09010), trans-membrane amino acid transporter (LOC_Os11g09020), ATP-dependent RNA helicase (LOC_Os11g09060), receptor-like protein kinase 5 precursor (LOC_Os11g09110), mutator-like transposase (LOC_Os11g09120), and receptor-like protein kinase 5

Table 3 Genotypes of the 12 recombinant CSSLs and their resistance phenotypes to rice sheath blight evaluated in field and in greenhouse

Controls and no. of CSSL	Phen-otype	Phenotype data in field								Lesion length (cm) in greenhouse at 14 DAI	Genotypes on eight molecular markers						
		2005		2006		2007		2009			Z439	Z21-12	Z22-17	Z22-27C	Z23-33C	Z23-32C	Z23-3
		SBR	PTSS8 (%)	SBR	PTSS8 (%)	SBR	PTSS8 (%)	SBR	PTSS8 (%)								
NIL-qSB11^{TQ}	HS[a]	8.12	53.13	8.27 a[c]	38.45 a	8.48 a	60.42 A[d]	8.34 A	54.98 bc	17.10 ab	T[e]	T	T	T	T	T	T
51443	HS	8.06	50.08	8.28 a	37.38 ab	8.48 a	63.91 A	8.42 A	51.62 bc	16.93 ab	L[f]	L	T	T	T	T	T
51439	HS	8.21	39.83	8.24 a	44.79 a	8.22 abc	57.73 A	8.29 A	67.21 a	16.29 ab	T	T	T	T	L	L	L
51446	HS	8.22	42.39	8.27 a	43.65 a	8.40 a	62.67 A	8.37 A	59.54 ab	17.49 ab	L	L	L	T	T	T	T
51447	HS	8.06	52.59	8.26 a	43.01 a	8.23 ab	55.12 A	8.13 A	47.45 c	18.09 a	L	L	L	T	T	T	T
51445	HS	8.17	47.42	8.15 a	42.52 a	8.23 ab	58.29 A	8.19 A	55.89abc	15.70 b	L	L	T	T	T	T	T
51437	LS[b]	7.75	20.91	7.83 b	25.90 c	7.84 cd	29.37 B	7.56 B	26.43 de	11.26 c	T	T	T	L	L	L	L
51440	LS	7.62	25.01	7.84 b	24.68 c	7.52 de	30.11 B	7.49 B	29.72 de	12.63 c	T	T	L	L	L	L	L
51436	LS	7.76	28.88	7.66 bc	23.43 c	7.66 de	57.80 A	7.61 B	28.76 de	11.00 c	T	T	T	L	L	L	L
51438	LS	7.58	17.12	7.52 c	22.16 c	7.34 e	19.50 B	7.42 B	33.98 d	12.68 c	T	T	T	L	L	L	L
51441	LS	7.74	33.16	7.76 bc	27.08 bc	7.49 de	24.95 B	7.71 B	30.91 d	11.13 c	T	T	T	T	L	L	L
51442	LS	7.33	19.67	7.79 bc	23.19 c	7.52 de	23.61 B	7.37 B	19.38 e	12.53 c	L	L	L	L	T	T	T
51444	LS	7.50	19.67	7.79 bc	23.19 c	7.82 cd	31.70 B	7.54 B	32.73 d	10.71 c	L	L	L	L	T	T	T
Lemont	LS	7.42	25.34	7.71 bc	20.66 c	7.76 cde	30.03 B	7.65 B	23.87 de	12.02 c	L	L	L	L	L	L	L
F value/*P* value		–	–	21.54/ 0.0	18.67/ 0.00	17.97/ 0.00	50.83/ 0.00	16.92/ 0.00	14.62/ 0.00	12.77/0.00	–	–	–	–	–	–	–

ANOVA was not performed for the 2005 data because only two replicates were available for each CSSL. The genotype on the other markers of the 12 CSSLs is shown in Fig. 3

[a] Highly susceptible

[b] Less susceptible

[c] 5 % significance level

[d] 1 % significance level

[e] Teqing genotype

[f] Lemont genotype

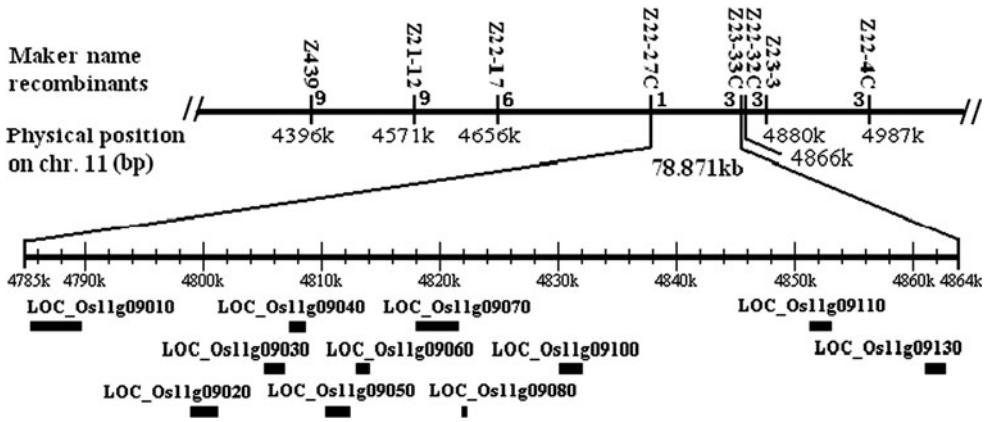

Fig. 5 Physical map of the *qSB-11*^{LE}. The number of recombinants was listed between the molecular markers. The physical distance of the fine-mapped region is 78.871 kb on the reference Nipponbare genome, cobering positions from 4785044 bp (physical position of Z22-27C) to 4863915 bp (physical position of Z23-33C) on chromosome 11. Twelve putative genes (Table 4) were annotated in rice genome annotation database (http://rice.plantbiology.msu.edu/), and one of them (LOC_Os11g09120) encoding a transposon protein was not labeled in the figure, because its sequence was confirmed not present in the genomes of Lemont (LE) and Teqing (TQ) used in this study

Table 4 Gene annotation in the fine mapping region of *qSB-11*^{LE}

ID	Locus identifier	5′end on Chr.11 (bp)	3′end on Chr.11 (bp)	Putative function
1	LOC_Os11g09010[a]	4785128[b]	4789394	Lipase
2	LOC_Os11g09020	4799114	4801313	Amino acid transporter
3	LOC_Os11g09030	4806780	4805208	Unknown protein
4	LOC_Os11g09040	4808617	4807400	Unknown protein
5	LOC_Os11g09050	4812233	4810271	Unknown protein
6	LOC_Os11g09060	4813046	4814007	ATP-dependent RNA helicase
7	LOC_Os11g09070	4821584	4818175	Unknown protein
8	LOC_Os11g09080	4821962	4822331	Hypothetical protein
9	LOC_Os11g09100	4832066	4830197	Unknown protein
10	LOC_Os11g09110	4851055	4852938	Receptor-like protein kinase 5 precursor
11	LOC_Os11g09120	4858813	4855687	Transposon protein
12	LOC_Os11g09130	4860885	4862816	Receptor-like protein kinase 5 precursor

[a] Locus number downloaded from the RGAP (Rice Genome Annotation Project) database (http://rice.plantbiology.msu.edu/)

[b] Physical position on the IRGSP Build5 Pseudomolecules of rice variety Nipponbare

precursor (LOC_Os11g09130). The remaining predicted genes encode either unknown or hypothetical proteins.

Among these predicted genes, the LOC_Os11g09120 sequence was not found in the corresponding region of the sequenced *indica* cultivar 93-11 genome by BLAST analysis. A pair of primers (Z22-18) were designed on the flanking sequences of LOC_Os11g09120 (4034 bp). The PCR results using specific primers confirmed that this sequence is also absent in the genomes of LE and TQ (data not shown). The presence of the remaining 11 genes was confirmed by either sequencing or RT-PCR (data not shown) and their physical locations are labeled in Fig. 5 and listed in Table 4.

Discussion

Different environments and SB strains may have contributed to the difficulty in detecting *qSB-11*^{LE} by other researchers

Many disease resistance QTLs have been discovered to be conditioned by environment, strains or isolates, developmental stage and genetic background (Young 1996; Perchepied et al. 2005; Poland et al. 2009). In rice SB resistance, one QTL named *qSB-9-2* was identified in many independent studies and different environments (Tan et al. 2005; Zuo et al. 2008; Liu et al. 2009; Wang

et al. 2012) and has recently been accepted as a development-independent QTL whose resistance can be observed at both seedling and adult stages (Wang et al. 2012). However, most SBR QTLs mapped so far do not behave consistently in different environments and studies. Fu et al. (2011) adopted two environments to map SBR QTLs and found only a few QTLs could be detected in both environments. The same phenomenon was observed by Channamallikarjuna et al. (2010) who employed three different locations in India to map SBR QTLs.

The *qSB-11*^{LE} locus was first detected by Li et al. (1995) in the environment of Texas, USA, but was not confirmed in their second-year test. Also, it was not detected in another study conducted by Pinson et al. (2005) using the same bi-parental cross in the same environment. However, in the environment of Yangzhou, China, the *qSB-11*^{LE} locus was stably detected in two independent mapping populations (Zou et al. 2000; Pan et al. 2005) across different years of tests and was confirmed by an advanced backcrossing population (Tan et al. 2005). Furthermore, a visually observable difference was obtained using a pair of near isogenic lines in both field and greenhouse (Zuo et al. 2011). More importantly, the current study has clearly shown the presence of *qSB-11*^{LE}. Many factors may have contributed to the failure to detect *qSB-11*^{LE} by other researchers. First, there are differences in the environments of Texas and Yangzhou. Second, different isolates or strains used in the above studies could have resulted in the different conclusions. Many reports have proposed that disease-resistance QTLs can be divided into three types: strain or isolate-specific QTLs, pathogen-specific QTLs, and non-host-specific QTLs (Young 1996). It is likely that the *qSB-11*^{LE} locus confers resistance only to particular isolates. Taken together, we hypothesize that the *qSB-11*^{LE} locus is conditioned by environments and/or strains. Using the pair of near isogenic lines specific to the *qSB-11*^{LE} locus, this hypothesis can be tested in future.

Interactions among QTLs are widely present. The genetic background which possesses the fewest number of SBR QTLs would be beneficial in evaluating individual SBR QTLs of interest and accelerate its cloning. Although the LE cultivar has been used as a susceptible control to SB disease in previous studies, it contained *qSB-11*^{LE}. When this resistant QTL was replaced by *qSB-11*^{TQ}, the new rice line NIL-qSB11^{TQ} evidently and consistently appeared more susceptible than LE in both field and greenhouse (Fig. 2; Table 3). Consequently, this NIL-qSB11^{TQ} line reveals a valuable resource for SBR QTL analysis.

By improving the mapping population, the fungal inoculation method in the field, and the SBR data analysis, the presence and location of *qSB-11*^{LE} was clearly established

The rate-limiting step to fine mapping and cloning of disease resistance QTL (dr-QTL) is the phenotypic evaluation. Most disease-resistance QTLs only confer small effects on resistance, which are generally confounded by environment, plant morphological and developmental stage, and fertility input. In the studies of SB disease resistance, large variance on SBR scores in the field was often observed. However, we were able to acquire consistent results in detecting the QTLs with a 0.5 or greater difference in SBR score on the modified 0–9 scoring system in field (Zuo et al. 2006). Recently, in the Wang et al. (2012) study, they also observed a phenotype of reducing SBR score by 0.67 for *qSB9-2*^{TQ} (also in 0–9 rating scale) in field. The *qSB-11*^{LE} locus reduced the SBR score by more than 0.70 (Zuo et al. 2007, 2011) and the PTSS8 score by around 25 %, making its effects readily detectable in our studies. This was accomplished by creating the CSSLs and by employing the precise inoculation method and data analysis strategy.

The use of the CSSLs minimized the effects of PH, TN, TA, and HD on SB resistance (Table 2). This kind of population has been considered to be an effective approach to identification of QTLs, especially for QTLs involved in complex traits (Eshed and Zamir 1995; Glazier et al. 2002; Ando et al. 2008; Kump et al. 2011). For minimizing the variations among the CSSLs due to inoculation, we employed more than 40 pre-trained assistants each time, which allowed us to accomplish the inoculation of all plants in field within 3 h (Supplemental Fig. 2). Thus, all plants were inoculated with similar quantity of the fungus in a short time. All these factors together resulted in the desired consistency on fungal infection and subsequent disease development on each plant, even though the test was conducted in the field. In the disease rating process, we scored each mature tiller, excluding small tillers in a plant. The SBR score in each replication was calculated by averaging a large number of tillers: 40 tillers (10 plants) per line or 120 tillers (30 plants) each for selected lines. Thus, high levels of reliability were achieved for the phenotypic data. We found highly significant consistency among replications on the SBR and PTSS8 data.

However, even with this carefully implemented system, a variation of SBR score less than 0.5 still existed among replications. This small variance rendered it difficult to set a subjective threshold to definitively assign the phenotype as less susceptible or highly susceptible. To resolve this issue, we employed the clustering method, in which the

position of each line is determined by comparing to all other CSSLs and controls on both SBR and PTSS8 scores. Based on the clustering results, we were able to assign each line to either the less susceptible group or the highly susceptible one. This allowed us to achieve the preliminary fine mapping of *qSB-11*^{LE}. We then focused on selected CSSLs to increase inoculation scale for verifying their phenotypes during the next 3 years. Furthermore, we were able to detect the effect of *qSB-11*^{LE} at the tillering stage in the greenhouse (Zuo et al. 2011) and the results confirmed our fine mapping conclusions made from field tests (Table 3).

Lastly, we would like to point out that all CSSLs possess the same genetic background as LE and thus should present a similar level of susceptibility as LE or higher because some of them carry the TQ susceptible allele(s) at the *qSB-11*^{LE} locus. The SBR scores of LE are usually around 7.5 (Zuo et al. 2011; Liu et al. 2009). Therefore, individuals in the CSSLs population gave SBR scores lower than five, which most likely resulted from failed inoculations or unsuccessful subsequent disease development. These individuals were removed from data analysis, because they would greatly increase the variance on disease scores of a few CSSLs and obscure gene mapping. However, this incident was very rare in our study.

LOD peak in QTL preliminary mapping is of high value in identifying of small effect QTLs

In the review by Price (2006), it was found that the positions of the fine mapped and characterized QTLs at that time were all under or close to the LOD peaks in their previous QTL mapping experiments. Therefore, Price suggested that, for those QTLs with small effect, one can use their LOD peak positions to perform candidate gene analysis and cloning. As an example, Ishimaru et al. (2004) accelerated the identification of a quantitative gene controlling PH by LOD peak information. We integrated the

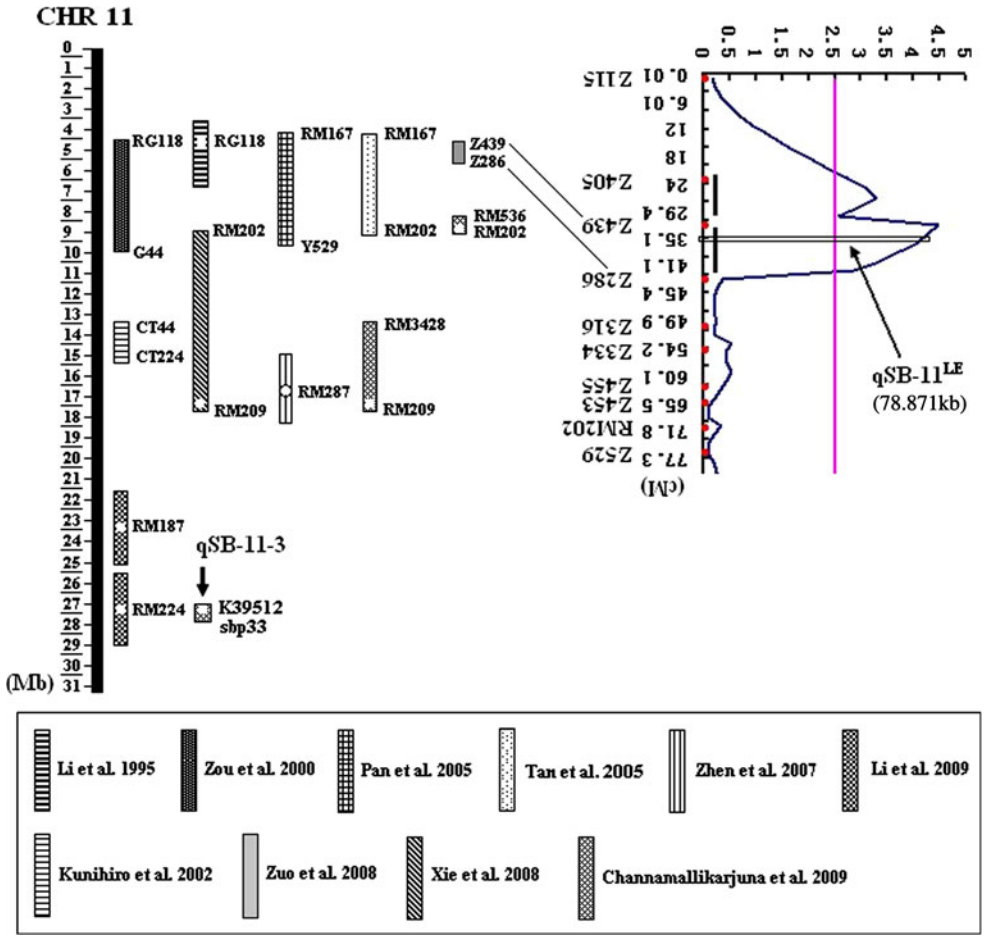

Fig. 6 Integrated map of SBR QTLs mapped so far on rice chromosome 11. For those SBR QTLs with clear marker interval information, their positions were considered between the markers on the reference Nipponbare genome, while for those SBR QTLs having only one associated marker information, their positions were temporally labeled on this marker and their interval length were estimated as the average interval length of all SBR QTLs mapped so far (calculated by Zuo et al. 2010). The curve of LOD value of *qSB-11*^{LE} in QTL mapping was cited from Zuo et al. 2007

SBR QTLs mapped so far on chromosome 11 onto the physical map of the rice reference genome by their associated or flanking markers (Fig. 6). From the integrated map, we clearly see that three regions containing SBR QTLs were detected in at least two independent studies. One of them, *qSB-11-3* on the long arm has been narrowed down to a region of 0.85 Mb by Channamallikarjuna et al. (2010), who fine mapped this QTL based on its LOD peak information. In the present study, we narrowed down another QTL ($qSB\text{-}11^{LE}$) to a 78.871-kb region by map-based cloning strategy. After comparison, we found this 78.871 kb region to be very close to the LOD peak position that we mapped before using a BC_4F_2 population (Fig. 6). Consequently, we consider using the common LOD peak information obtained from different years or locations for cloning the QTLs with small effects a good strategy compared to the time-consuming fine mapping process we had gone through in the present study.

$qSB\text{-}11^{LE}$ candidates

Twelve candidate genes are predicted within the 78.871 kb region in the Nipponbare reference genome and only 11 are present in the LE genome. Three of the 11 predicted genes are considered the most likely candidates for $qSB\text{-}11^{LE}$ and have been cloned for functional verification through gene silencing and overexpression. They encode lipase-like gene (LOC_Os11g09010), receptor-like protein kinase 5 precursor (LOC_Os11g09110), and putative receptor-like protein kinase 5 precursor (LOC_Os11g09130), respectively. In the *Arabidopsis* genome, we found that LOC_Os11g09010 is most homologous to AT3G52430, which encodes a lipase-like protein important for salicylic acid signaling, functioning in *R* gene-mediated resistance, and in plant basal resistance (Ng et al. 2011). In phenotypic evaluation, we observed that the NIL-qSB11^{TQ} lines appeared to have low basal resistance to SB fungus as they showed faster disease development and larger disease area than LE. LOC_Os11g09110 and LOC_Os11g09130 both are most homologous to AT1G34420, which encodes an LRR receptor-like kinase responsive to the infection of gemini virus in *Arabidopsis*. Receptor kinase and receptor-like proteins are known to be involved in disease resistance, governing immunity in rice (Chen and Ronald 2011). The remaining eight genes were annotated either as hypothetical or putative unknown proteins, except for one as a putative amino acid transporter protein and another as an ATP-dependent RNA helicase. These eight genes are of lower priority and were not selected for functional verification at the present time. However, due to the complex nature of quantitative resistance and the wide diversity of gene functions represented by the few characterized quantitative disease resistant genes (Li et al. 1999; Wang et al. 2001; Talukder, et al. 2004; Kump et al. 2011), we cannot exclude possible contributions of these genes to $qSB\text{-}11^{LE}$ at this stage.

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