

Fine mapping of $qSTV11^{KAS}$, a major QTL for rice stripe disease resistance

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Received: 18 November 2009 / Accepted: 16 February 2011 / Published online: 8 March 2011
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Abstract Rice stripe disease, caused by rice stripe virus (RSV), is one of the most serious diseases in temperate rice-growing areas. In the present study, we performed quantitative trait locus (QTL) analysis for RSV resistance using 98 backcross inbred lines derived from the cross between the highly resistant variety, Kasalath, and the highly susceptible variety, Nipponbare. Under artificial inoculation in the greenhouse, two QTLs for RSV resistance, designated $qSTV7$ and $qSTV11^{KAS}$, were detected on chromosomes 7 and 11 respectively, whereas only one QTL was detected in the same location of chromosome 11 under natural inoculation in the field. The stability of $qSTV11^{KAS}$ was validated using 39 established chromosome segment substitution lines. Fine mapping of $qSTV11^{KAS}$ was carried out using 372 $BC_3F_{2;3}$ recombinants and 399 $BC_3F_{3;4}$ lines selected from 7,018 BC_3F_2

plants of the cross SL-234/Koshihikari. The $qSTV11^{KAS}$ was localized to a 39.2 kb region containing seven annotated genes. The most likely candidate gene, LOC_Os11g30910, is predicted to encode a sulfotransferase domain-containing protein. The predicted protein encoded by the Kasalath allele differs from Nipponbare by a single amino acid substitution and the deletion of two amino acids within the sulfotransferase domain. Marker-resistance association analysis revealed that the markers L104-155 bp and R48-194 bp were highly correlated with RSV resistance in the 148 landrace varieties. These results provide a basis for the cloning of $qSTV11^{KAS}$, and the markers may be used for molecular breeding of RSV resistant rice varieties.

Introduction

Rice stripe disease, one of the most serious viral diseases in the temperate regions of East Asia, is caused by rice stripe virus (RSV), which is transmitted by small brown plant hopper (SBPH, *Laodelphax striatellus* Fallen). The spread of this disease has resulted in severe losses in *japonica* rice production in the Middle and Lower Yangtze River Area of China since 2000 (Sōgawa 2005; Wang 2006). Currently, the most common control measure is the application of pesticides that target the SBPH vector, which is costly and harmful to the environment. Development of resistant varieties offers a more economical, efficient and environmentally safe way to control this disease.

Resistance to RSV has been studied in Japanese upland rice varieties and two loci, *Stv-a* and *Stv-b*, have been reported (Washio et al. 1968a). *Stv-a* and *Stv-b* are complementary dominant genes. The former was linked with the glutinous endosperm (*wx*) and *photosensitivity-1* (*Se-1*)

Communicated by T. Tai.

Y.-X. Zhang and Q. Wang contributed equally to this work.

Electronic supplementary material The online version of this article (doi:10.1007/s00122-011-1557-0) contains supplementary material, which is available to authorized users.

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loci on chromosome 6, and the latter was located on chromosome 11. Some *indica* paddy varieties, including the Pakistani *indica* paddy variety, Modan, have a different resistance allele *Stvb-i*, which is incompletely dominant and allelic with *Stv-b* on chromosome 11 (Washio et al. 1968b). In the 1960s, *Stvb-i* from Modan was introduced into many *japonica* varieties, including St. No. 1, Chugoku 31, Aichi 6 and Aichi 97 for the control of RSV (Toriyama et al. 1966; Maeda et al. 2006). Thereafter, the derivative varieties harboring *Stvb-i* were widely cultivated in Japan and in the Jiangsu province of China, and showed a stable resistance to RSV (Wang 2006). However, varieties with a single resistance gene are at an increased risk of their resistance being overcome by new strains of the virus. It is thus necessary to discover new genetic resources for resistance to RSV for use in rice breeding programs.

The *Stvb-i* gene in Modan was preliminarily localized to a 1.8-cM segment on chromosome 11 using restriction fragment length polymorphism (RFLP) markers (Hayano-Saito et al. 1998), and then finely mapped to a 286 kb region covered by two overlapping BAC clones (Hayano-Saito et al. 2000). In the Korean *indica* variety Milyang 23, one QTL for RSV resistance was detected in the interval between markers XNpb202 and C1172 on chromosome 11 (Maeda et al. 1999), which was reported to be allelic with *Stvb-i*. Subsequently, the same research group using both RFLP and SSR markers reported two QTLs in the Japanese upland rice variety, Kanto 72. The QTL on chromosome 11 corresponding to *Stv-b* exerted a greater effect than the other on chromosome 2 by reducing the infection rate of RSV (Maeda et al. 2004, 2006). In the *indica* variety, DV85, Ding et al. (2004) also detected two major QTLs for RSV resistance. One was mapped to the same chromosomal region as *Stvb-i*, and the other was mapped on chromosome 7. More recently, three QTLs were detected in the Indian landrace Dular, one on chromosome 3 and the other two in the RM287–RM209 and RM209–RM21 intervals on chromosome 11, respectively (Wu et al. 2009). All these studies indicate the presence of a single resistance gene or gene cluster against RSV on chromosome 11. In addition, most of previous studies on QTL detection for RSV resistance used RFLP molecular markers, which are tedious and costly, and difficult to use for marker-assisted selection (MAS). Recently, Wu et al. (2010) reported the fine mapping of *qSTV11^{TQ}* to a 55.7 kb interval, which based on pedigree and physical mapping appears to be *Stvb-i* or an allele.

The objectives of this study were to identify QTLs for RSV resistance from the resistant rice variety Kasalath and to develop PCR-based molecular markers for fine mapping and marker-assisted selection of any major QTLs detected.

Materials and methods

Plant materials

The varieties IR36 (*indica*) and Wuyujing #3 (*japonica*) were used as resistant and susceptible controls, respectively. A total of 314 rice accessions (299 landraces and 15 commercial varieties from Japan, Korea, the International Rice Research Institute (IRRI), and China) were screened for resistance to RSV. Of these, 148 landraces with different levels of resistance were selected for marker-trait association analysis (Supplementary Table 1).

Ninety-eight backcross inbred lines (BILs, BC₁F₈), derived by single seed descent from the cross Nipponbare/Kasalath//Nipponbare (Lin et al. 1998), were used to detect QTL for RSV resistance. These BILs were genotyped using 245 RFLP markers distributed along the 12 rice chromosomes. A panel of chromosome segment substitution lines (CSSLs) was generated in Koshihikari genetic background by first selecting 49 lines from BC₁F₃ plants of Koshihikari/Kasalath//Koshihikari in which a single large chromosome segment of Kasalath was introduced to the genetic background and then back-crossing with the recurrent parent Koshihikari to the BC₃ generation without selection. A whole-genome survey, based on 129 RFLP loci, was used to select 39 CSSLs, designated from SL-201 to SL-239. Except for a small region on the short arm of chromosome 8 and the long arm of chromosome 12, the whole Kasalath genome is represented within the CSSL set.

The populations and lines used for fine mapping are shown in Fig. 1. For rough mapping of *qSTV11^{KAS}*, a small secondary F₂ population (BC₃F₂) consisting of 286 plants was obtained by self-pollinating the BC₃F₁ plants generated from the cross SL-234/Koshihikari and grown in a paddy field in Jiangsu Province, China, in the summer of 2006. Artificial inoculations of these 286 F_{2:3} lines were done in the greenhouse in September 2006. For the subsequent fine mapping, 5,518 BC₃F₂ plants (Population I) were grown in Lingshui County, Hainan Province, China in 2006 and another 1,500 BC₃F₂ plants (Population II) in Nanjing in 2007. All 7,018 plants were genotyped using two molecular markers flanking the *qSTV11^{KAS}* locus and the recombinants were selected for evaluation of RSV resistance. Three hundred and seventy-two BC₃F_{2:3} recombinant lines and their derived 372 BC₃F_{3:4} lines selected from Population I were used for evaluating and validating RSV resistance, respectively. In addition, 27 BC₃F_{3:4} recombinant families (each including ten lines) derived from Population II were used to further narrow the interval harboring *qSTV11^{KAS}*. For the allelism test between *qSTV11^{KAS}* and *Stvb-i*, an Aichi97/Kasalath F_{2:3} mapping population, consisting of 168 lines, was generated.

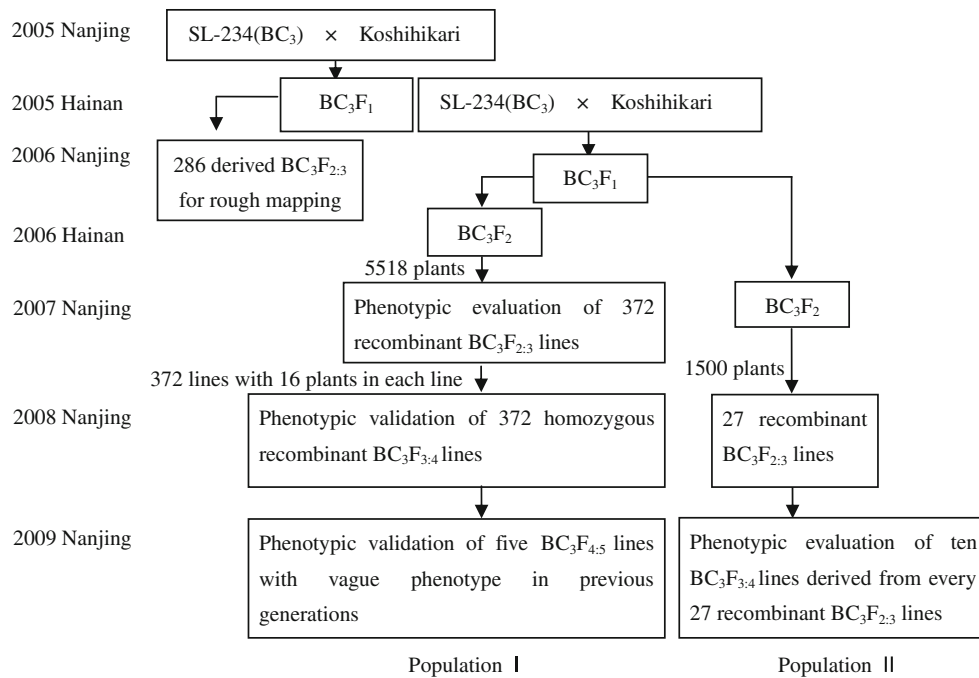


Fig. 1 Development of genetic populations for rough and fine mapping of *qSTV11^{KAS}*. MAS marker-assisted selection

Evaluation of RSV resistance in the field and greenhouse

Four hundred and twenty-two lines including 314 germplasm accessions and 98 Nipponbare/Kasalath//Nipponbare BILs were tested in the field under natural infection. The field trial was done at Louzhuang town of Jiangyan city, Jiangsu Province, China, where a serious RSV epidemic has taken place since 2000. The seeds were sown in early May 2004, and the weather was most favorable for natural infection when the incidence of rice stripe disease was evaluated at about 50 days after seed sowing. Field trials were conducted in randomized complete blocks with three replicates. Plots consisted of single 18 cm rows of 50 plants with 10 cm spacing between rows. No pesticide was used during the entire growth period. The percentage of virus-containing SBPH in the fields was estimated to be 39% in 2004, 27% in 2007 and 29% in 2008, respectively, as determined by the random sampling and ELISA analysis (Wang et al. 2004).

Phenotypic evaluation of BILs, CSSLs and populations for rough and fine mapping were conducted using artificial inoculation in greenhouse according to Sakurai et al. (1963) with some modifications. Thirty germinated seeds of each line were sown in plastic dishes filled with soil; the weak seedlings were eliminated at the one-leaf stage, and 25 healthy seedlings of each line were kept for inoculation. When the seedlings were at the one-and-half-leaf stage, SBPH nymphs of first to second instars were released into

the dish covered with plastic cylinder at a density of about five SBPHs per seedling. During inoculation period, the SBPHs in each dish were disturbed with a small soft brush every day to avoid aggregation. Three days later, all SBPH nymphs were killed with pesticide and seedlings were transferred to a greenhouse, where they grew for about a month until symptoms were observed. The experiments were performed with four replications. The percentage of virus-laden SBPH used in artificial inoculation was estimated to be 31% in 2006, 27% in 2007, 35% in 2008, and 41% in 2009, respectively.

Seedlings were evaluated and grouped into six classes (A, B, Bt, Cr, C and D) based on the severity of the RSV symptoms observed. The evaluation criteria of these six classes are shown in Supplementary Table 2. The disease-rating index (DRI) was calculated according to the disease severity as follows (Washio et al. 1968a):

Disease rating index

$$= \frac{100A + 80B + 60Bt + 40Cr + 20C + 5D}{\text{Number of seedlings examined}}$$

where A, B, Bt, Cr, C and D represent the number of seedlings in each class, respectively. The relative disease-rating index (RDRI = DRI × 100/the DRI value of susceptible control Wuyujing #3) was calculated and used for subsequent analysis. Lines with RDRI values less than 29, from 30 to 39, from 40 to 69, and over 70 were classified as highly resistant, resistant, susceptible, and highly susceptible, respectively. The infection rating (IR, the percentage

of plants with symptoms) of tested lines was also used for analyzing the resistance to RSV.

DNA extraction and molecular marker analysis

DNA was extracted from fresh leaves of each individual according to Dellaporta and Hicks (1983) with minor modifications. In brief, a small piece of rice leaf was ground in a 2.0 ml tube containing 400 μ l of 100 mM Tris/HCl, 1 M KCl, and 10 mM EDTA and incubated for 30 min at 65°C. Each mixture was centrifuged and 200 μ l of supernatant was transferred to a new 1.5 ml tube. Crude DNA in the supernatant was precipitated with an equal volume of isopropanol, rinsed with 70% ethanol and centrifuged again, and the pellet was re-dissolved in 100 μ l ddH₂O. The markers between G320 and C1172 in the *qSTV11^{KAS}* region were used for preliminary mapping. To obtain a high-density linkage map for fine mapping in the target region, new SSR, InDel, CAPS markers were developed according to the publicly available rice genome sequence (<http://rgp.dna.affrc.go.jp>) (Table 1).

SSR and InDel marker analysis was performed according to Chen et al. (1997) with some modifications. Each 10 μ l PCR reaction contained 10–20 ng of template DNA, 1 \times PCR buffer (TaKaRa, Dalian, China), 1.5 mM of MgCl₂, 50 μ M of dNTPs, 5 pmol of each primer and 0.5 U of *Taq* DNA polymerase (TaKaRa, Dalian, China). Amplification profiles consisted of 5 min of denaturation at 94°C, 35 cycles of 30 s denaturation at 94°C, 30 s annealing at 50–58°C (depending on primers), and 45 s

extension at 72°C, followed by a final 10 min extension at 72°C. Denatured amplified products were electrophoresed on 8% (w/v) native polyacrylamide gels at 220 V for 1 h, and visualized by silver staining following Sanguinetti et al. (1994). For CAPS analysis, the PCR products were cut with *EcoRV*, and then electrophoresed as described above.

QTL detection, gene mapping, and marker-trait association

QTL detection in Nipponbare/Kasalath//Nipponbare population was carried out using Windows QTL Cartographer 2.5 software with composite interval mapping method (Wang et al. 2007). A LOD threshold of 2.5 was employed for declaring the significance of a putative QTL. For CSSLs, the position of a QTL was determined by comparing phenotypes and genotypes of RFLP markers according to the method of Ebitani et al. (2005). A probability level of 0.001 was used as the threshold for the detection of a QTL. QTL nomenclatures followed the recommendations of McCouch and CGSNL (Committee on Gene Symbolization, Nomenclature and Linkage, Rice Genetics Cooperative) (2008). A molecular marker linkage map between G320 and C1172 was constructed using Mapmaker/EXP version 3.0 (Lander et al. 1987) to determine the order of the markers and the genetic distance in the target region. Association analysis of two markers (L104 and R48) flanking *qSTV11^{KAS}* and rice stripe virus resistance in 148 diverse landraces was conducted using

Table 1 Details of polymorphic markers used for fine mapping of *qSTV11^{KAS}*

Primer Id	Motif repeat	Forward primer sequence	Reverse primer sequence	Product size (bp) in Nipponbare
R5	InDel	5' TTGGCCTATACGGTTTAA 3'	5' CTGCATCTGAATCATGAGA 3'	90
R12	InDel	5' TTATGGAGAAATTTGGGT3'	5' ATCCTCTTATTGATGGGT 3'	173
R13	InDel	5' AACATCGGCTACCACCAAAA 3'	5' GAATGCTTCGTCGTTCTCAA 3'	272
R15	InDel	5' GGGCTTCAACTCGTACTCTG 3'	5'ATGAACTGCGGGTCCAATAA 3'	275
R21	InDel	5' CAACGTGGTTGATTGTTC 3'	5' TTGCCAGGTTAGTTGTAC 3'	128
R25	InDel	5' GTTAATAAAGCAATTGTTAGAC3'	5' TTTACTCTACGCCCTAAA 3'	182
R27	InDel	5' AAATGTCCACGTCGAAAC 3'	5' CCAAACAGGAGTAGGGTA 3'	145
R41	InDel	5' CAGAGCGTTTATTAGCATG 3'	5' CTGCGTTCGTTTATAGAGGT 3'	181
R53	InDel	5' GTTCACCGAACTGAATGA 3'	5' ACTGAGCTGCGAGGATAT 3'	257
R48	InDel	5' GCCTTCATCCGTAAATCCATAAGC 3'	5'GAGTACCACATGGCATTATGAGAGC 3'	166
L64	(AGAT) ₅	5' TTGTTATTGGGCTTGGGTTGC3'	5' ACGTTGGAGACAGGTTGGATATATGG3'	493
L72	(CT) ₆	5' CAGCCGATTATCCGACAAA 3'	5' TGATCCTCTGGCAGAACGC 3'	156
L104	(AT) ₂₂	5' TCAGATCCCAACTAGCACCTATCC3'	5' AAGACGATGCATGTCAACAAGG 3'	199
L126	(AT) ₂₂	5' TACTGCACATGATCTGAATCCACTGC 3'	5' CCCGTAAGGCACATACTGAACTTGG 3'	478
C1	CAPS/ <i>EcoRV</i>	5' GGGTTTCCTAGGTGGATTAGGATAT 3'	5' GCAACACCAGCACTCCTTAC 3'	182 + 2 ^a

^a The fragments of PCR products cut by *EcoRV*

Student's *t* test. According to the genotypes of L104 and R48, 148 landraces were classified into three and four groups, respectively.

Identification of putative genes in the target region and candidate gene sequence analysis

The DNA sequence in the QTL region was downloaded from the genome database of *Oryza sativa* subsp. *japonica* cv. Nipponbare (<http://www.ncbi.nlm.nih.gov>) and used for gene prediction. Gene models were obtained from the MSU Rice Genome Annotation Project Release 6.1 (<http://rice.plantbiology.msu.edu/cgi-bin/gbrowse/rice/>). The predicted genes were functionally annotated using the software BLASTp (bitscore ≥ 100 , *E* value ≤ -20). The protein domains were analyzed using the Pfam database of protein domains and HMMs (<http://pfam.jouy.inra.fr/>). Full-length cDNAs for rice (Rice FL-cDNA) database and Expressed Sequence Tags (EST) database from GenBank (<http://www.ncbi.nlm.nih.gov>) and KOME (Knowledge-based Oryza Molecular biological Encyclopedia, <http://cdna01.dna.affrc.go.jp/cDNA/>) were analysed of cDNA or EST of the annotated genes. Microarray Probes ('Affymetrix GeneChip Rice Genome Array' and 'Yale Rice Oligo Microarray') blasted from the Affymetrix and Yale (<http://bioinformatics.med.yale.edu/riceatlas/>) array mapped to the Rice Genome Annotation Project Pseudomolecules (Release 6.1) at 100% identity over 100% of their length. Based on gene annotation results and the sequence of BAC clone OSJNBa0025K21 (<http://www.ncbi.nlm.nih.gov>), we designed PCR primers (Y2.1-F: 5'TGAGCGACAA-TACTCCAGA3', Y2.1R-R: 5'AAGATAGATACTAGCTTGACACAT 3'; position in OSJNBa0025K21: 115,438–116,748; fragment length: 1,311 bp) for the amplification of the coding region of LOC Os11g30910 from Kasalath and Nipponbare. The PCR products were purified and sequenced by Shanghai Invitrogen Biotechnology Co., China. The sequence was analyzed by DNAMAN software (<http://www.lynonn.com>).

Results

Screening the rice lines for resistance to rice stripe disease

The tests in the field and greenhouse indicated that the resistant control IR36 showed no symptoms, with a DRI value of 0, whereas DRI values of the susceptible control Wuyujing #3 were 70.25 under natural inoculation in the field and 76.8 under artificial inoculation in the greenhouse. These results suggested the natural infection in the field was similar in effectiveness as artificial inoculation for

RSV resistance evaluation. Therefore, field inoculation was employed for germplasm screening. Among the 314 landraces and commercial varieties collected from different rice-growing regions, 68 were highly resistant to RSV, 25 were resistant, 93 were susceptible, and 128 were highly susceptible (Table 2). In the highly resistant lines, the *japonica* type subspecies accounted for 47.1%, and *indica* types were 52.9%. In the 128 highly susceptible lines, the *japonica* and *indica* subspecies accounted for 85.2 and 14.8%, respectively. Under artificial inoculation, an Indian landrace 'Kasalath' showed an RDRI value of 2.9, thus it was classified as highly resistant. In agreement with previous studies (Maeda et al. 2004, 2006), two Japanese elite *japonica* varieties Nipponbare and Koshihikari showed obvious symptoms with high RDRI values (72.0 and 76.7, respectively). Both were grouped into highly susceptible class. In contrast, the Japanese *japonica* variety 'Aichi 97' (harboring *Stvb-i*) exhibited an RDRI value of 2.4 and was therefore classified as highly resistant.

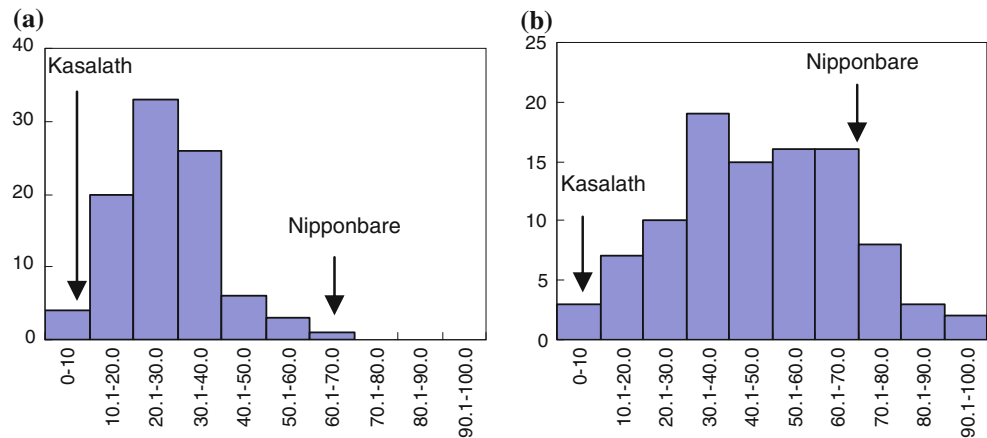
QTL analysis of RSV resistance using backcross inbred lines

A backcross inbred line (BIL) population derived from Nipponbare/Kasalath//Nipponbare was used to detect QTL for resistance to RSV. The continuous distributions of RDRI values ranging from 0 to 100 were observed by both natural and artificial inoculation (Fig. 2). Under natural

Table 2 Rice stripe virus resistance in 314 rice landraces and varieties

Origin	Classification								Total
	<i>Japonica</i> type				<i>Indica</i> type				
	HR	R	S	HS	HR	R	S	HS ^a	
Taihu Valley, China	4	1	26	61	–	–	6	3	101
Yunnan Province, China	11	2	20	33	8	3	9	9	95
Zhejiang, China	–	2	1	3	–	1	–	2	9
Jilin, China	2	–	–	3	–	–	–	–	5
Guangxi, China	1	–	–	–	2	1	2	–	6
Shandong, China	2	1	3	3	1	–	–	–	10
Anhui, China	–	1	–	–	3	2	2	1	9
Jiangxi, China	–	–	–	–	8	–	–	–	8
Fujian, China	–	–	1	2	4	1	–	–	8
Taiwan, China	4	–	1	1	–	2	2	–	10
Hunan, China	1	–	1	1	2	2	1	–	8
IRRI	–	–	–	–	6	4	7	1	18
Japan	2	–	2	–	–	–	–	–	4
Korean	1	–	1	–	–	–	–	–	2
Other	4	–	4	2	2	2	4	3	21
Total	32	7	60	109	36	18	33	19	314

Fig. 2 Frequency distribution of resistance to RSV based on the relative disease-rating index (RDRI) of the backcross inbred lines (BILs) **a** Natural field infection; **b** Artificial inoculation



inoculation in the field, only one QTL was detected on chromosome 11, which resided in the interval between G257 and S2260 and accounted for 22.5% of the phenotypic variance. The favorable allele to increase RSV resistance was contributed by Kasalath. By artificial inoculation in greenhouse, two QTLs for RSV resistance were detected on chromosomes 7 and 11, respectively. The locus on chromosome 7, designated as *qSTV7_{BIL}*, was located in the marker interval of R1440 and C1226 with a LOD score of 2.77 and explained 9.0% of the phenotypic variance (Fig. 3). The other locus, located in the same interval as the QTL detected in field conditions, had a LOD score of 8.58 and explained 31.8% of the phenotypic variance. This major QTL was designated as *qSTV11_{BIL}*. The positive alleles at both loci came from Kasalath. No gene interaction or epistatic effect was detected either in the field or in greenhouse tests.

Validation of *qSTV11_{BIL}*

The 39 CSSLs derived from introgression of Kasalath (highly resistant) into the Koshihikari (highly susceptible) genetic background, were phenotyped for RSV resistance by artificial inoculation. Of the 39 lines tested, only three lines (SL-233, 234, and 235) showed significant decrease in RDRI values ($P < 0.001$), indicative of high RSV resistance, compared with Koshihikari. The remaining CSSLs exhibited inferior or slightly superior resistance to Koshihikari (Fig. 4). Based on the RFLP genotypes of the three highly resistant CSSLs, a QTL (*qSTV11_{CSSL}*) was identified between G320 and C1172 (Fig. 5). The CSSL SL-234 showed stable resistance to RSV and was used for further studies. Based on the RFLP integrated map (McCouch et al. 2002), *qSTV11_{BIL}* and *qSTV11_{CSSL}* were located in the same region, suggesting that they were the same QTL (hereafter designated as *qSTV11^{KAS}*) derived from Kasalath. The *qSTV11^{KAS}* allele explained the largest trait variance and was independent of genetic background and

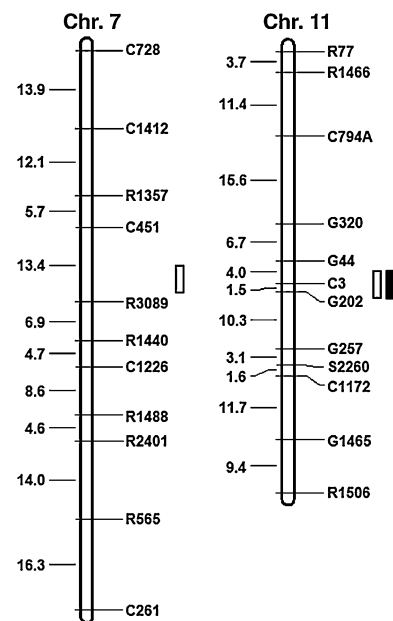


Fig. 3 QTLs identified for resistance to RSV in Nipponbare/Kasalath/Nipponbare backcross inbred line (BIL) population. *Hollow boxes* denote QTLs for resistance to RSV identified by artificial inoculation, and *solid box* denotes QTL identified by natural field infection

test method, and thus it can be considered as a stable major QTL.

A small $BC_3F_{2.3}$ population of 286 plants derived from the cross SL-234/Koshihikari was initially used to confirm the genetic effect of *qSTV11^{KAS}*. Eight SSRs and one InDel marker (i.e., R48) were developed around RFLP markers G320 and C1172, which flank the *qSTV11^{KAS}* locus. Composite interval mapping indicated that the locus resides in a 3.4 cM region between the markers L104 and R48 with a LOD value of 13.16 and explains 39.3% of the total phenotypic variance (Fig. 6a).

Association analysis of marker and RSV resistance indicated that the allele *L104-155 bp* at SSR locus L104

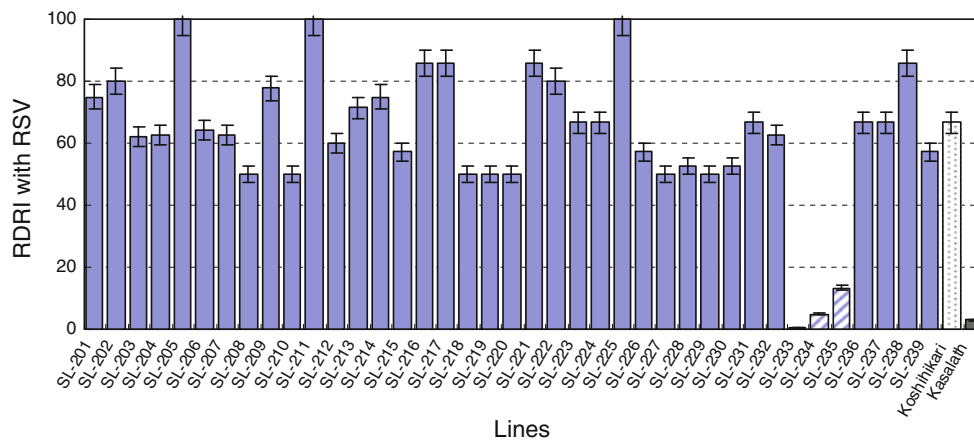


Fig. 4 The ratio for the relative disease-rating index (RDRI) of chromosome segment substitution lines (CSSLs) and their parents. In each line, bars denote the mean value with standard deviation ($n = 4$). Shaded bars represent the susceptible (RDRI ranged from

40 to 69) and highly susceptible (RDRI over 70.0) lines; hatched bars indicate the highly resistant (RDRI less than 29.0) lines; dotted bar indicates the recipient parent, Koshihikari; black bar indicate donor parent, Kasalath

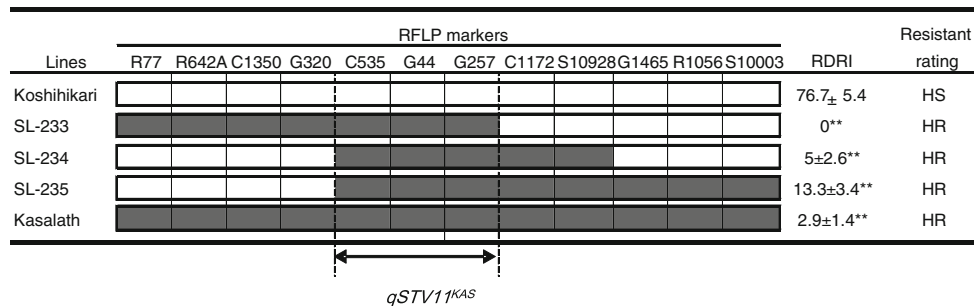


Fig. 5 The identification of $qSTV1^{KAS}$ using chromosome segment substitution lines (CSSLs). The stripe-resistance phenotypes and the graphical genotypes of three CSSLs harboring $qSTV1^{KAS}$ locus. The dashed lines represent the positions of RFLP markers. Black and white bars indicate chromosome regions derived from donor parent

(Kasalath) and background parent (Koshihikari), respectively. The RDRI of each line was denoted as mean and standard derivation ($n = 4$). **Indicates significant difference ($P < 0.001$) between CSSLs and Koshihikari (Student's t test). HS highly susceptible, HR highly resistant

was significantly associated with RSV resistance in 148 landraces/varieties. The allele $R48-194 bp$ at the R48 locus was also significantly associated with RSV resistance (Table 3, Supplementary Table 1). These results indicated a major QTL linked with SSR marker L104 on chromosome 11.

Allelic test of $qSTV1^{KAS}$ and *Stvb-i*

To clarify the relationship between $qSTV1$ and *Stvb-i* (a well-known RSV resistance gene on chromosome 11 from the variety Modan), Kasalath was crossed with Aichi97 (*Stvb-i*) to construct an $F_{2:3}$ mapping population with 168 lines. The RSV incidence distribution among $F_{2:3}$ lines was unimodal with RDRI ranging from 0 to 18.33 (Fig. 7), indicating a high level of resistance to RSV in all the lines. Plants with disease symptoms in $F_{2:3}$ lines were mostly classified as mild damage types, such as Bt, Cr, C, and D, whereas the serious damage types (A and B) were rarely

observed. This result indicated that *Stvb-i* and $qSTV1^{KAS}$ are either allelic or tightly linked.

Fine mapping of $qSTV1^{KAS}$

In order to fine map $qSTV1^{KAS}$, a larger BC_3F_2 population with 5,518 plants was genotyped using markers L104 and R48. Three hundred and seventy-two recombinants in the target region were obtained and their phenotypes were determined by testing $BC_3F_{2:3}$ lines for RSV resistance using artificial inoculation. Due to the incomplete dominance of $qSTV1^{KAS}$ (the RDRI of Koshihikari/SL-234 F_1 plants was 34.7 ± 8.7), the phenotypes of heterozygotes and homozygotes were easily confused. Therefore, the homozygous recombinants were necessary for precise evaluation of RSV resistance. All 372 recombinant $BC_3F_{2:3}$ lines (16 plants per line) were grown in the field and one homozygous recombinant $BC_3F_{3:4}$ line was selected from every $BC_3F_{2:3}$ line using markers L104 and R48. Then

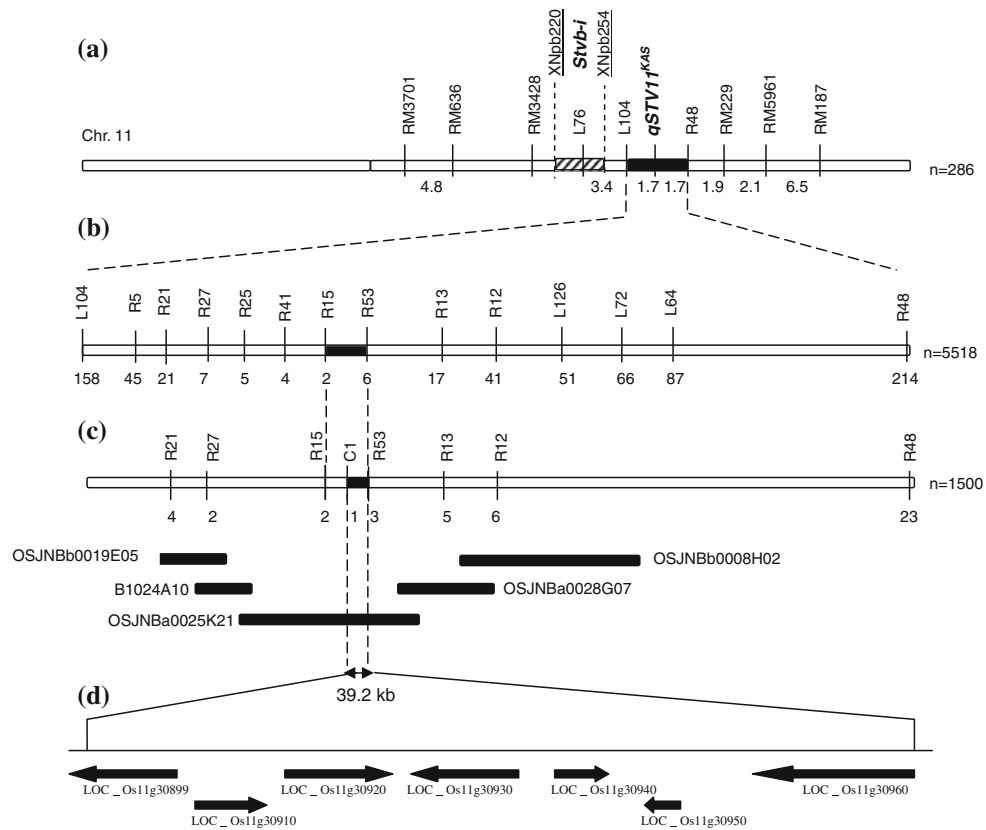


Fig. 6 Genetic and physical maps of the $qSTV11^{KAS}$ gene and candidate gene analysis. **a** Linkage map of chromosome 11 constructed using 286 BC_3F_2 plants. The $qSTV11^{KAS}$ locus was mapped between markers L104 and R48. Numbers show genetic distance between adjacent markers. The location of *Stvb-i* between two RFLP markers XNpb220 and XNpb254 (*underlined*) is shown with diagonally hatched box. **b** Fine mapping of $qSTV11^{KAS}$. The

locus was mapped between markers R15 and R53. The number of recombinants between the markers and $qSTV11^{KAS}$ is indicated under the map. **c** High-resolution genetic map of $qSTV11^{KAS}$. The locus was narrowed down to a 39.2 kb region on the BAC clone OSJNBa0025K21 (GenBank no. AC136009) between the markers C1 and R53. **d** Rice gene models from Release 6.1 of the MSU Rice Genome Annotation Project (<http://rice/plantbiology.msu.edu/>)

Table 3 Association analysis of two markers tightly linked with $qSTV11^{KAS}$ and rice stripe virus resistance in 148 landraces

Marker	Allele	Number of landraces/varieties	Means of RDRI ^a	Standard deviation	Range
L104	L104-199 bp	44	92.3A	13.8	5.8–165.0
	L104-243 bp	27	77.6A	10.3	5.1–143.2
	L104-155 bp	77	39.4B	10.5	0–140.7
R48	R48-152 bp	2	94.5a	10.5	87.0–101.9
	R48-166 bp	84	65.6a	15.3	0–143.8
	R48-208 bp	23	63.7a	13.0	0–150.5
	R48-194 bp	39	41.5ab	12.0	0–165.0

^a Numbers followed by the same letter are not significantly different at $P < 0.05$ (with lowercase letter) and $P < 0.01$ (with capital letter), respectively

RSV resistance evaluation of these $BC_3F_{3;4}$ lines was carried out by artificial inoculation. In addition, five $BC_3F_{3;4}$ lines with vague phenotypes were advanced to the $BC_3F_{4;5}$ generation, then five $BC_3F_{4;5}$ individual lines from each $BC_3F_{3;4}$ line were chosen randomly and tested repeatedly to obtain highly consistent phenotypes against

RSV. In the interval of L104 and R48, 12 markers were selected from newly developed markers and used in further fine mapping of $qSTV11^{KAS}$ (Table 1). The locus was narrowed down to a 79 kb region defined by the markers R15 and R53 (Fig. 6b) according to the Build 5 pseudomolecules of the Nipponbare genome released by the

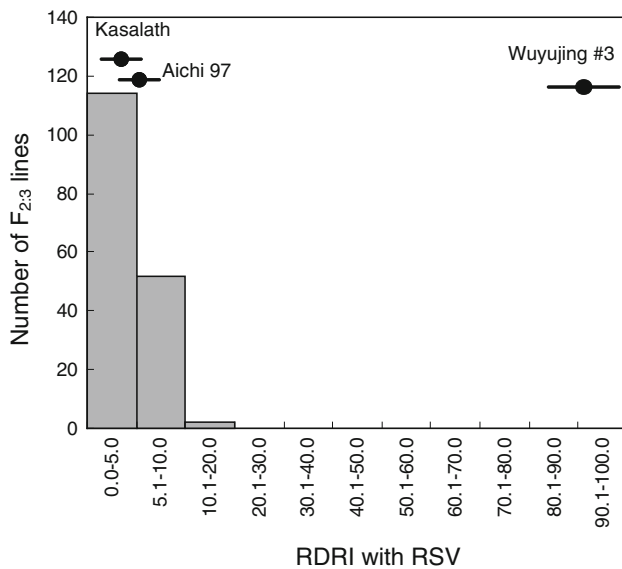


Fig. 7 Frequency distribution of $F_{2:3}$ lines ($n = 168$) with different ratio for the relative disease rating index (RDRI), derived from the cross Aichi97/Kasalath. Means and ranges of the parents and the highly susceptible check Wuyujing #3 ($n = 4$) are indicated as *black dots* with *short bars*

International Rice Genome Sequencing Project (IRGSP) in 2008 (<http://rgp.dna.affrc.go.jp/E/IRGSP/Build5/build5.html>). To further delimit the $qSTV11^{KAS}$ interval, 27 additional recombinants were identified by scanning 1,500 more plants of the same BC_3F_2 population with the markers R21 and R48. Ten $BC_3F_{3:4}$ lines derived from each $BC_3F_{2:3}$ line were tested for RSV resistance by artificial inoculation, and the lines with extremely resistant or susceptible phenotypes were selected for further fine mapping. The additional 27 recombinant $BC_3F_{3:4}$ line families enabled us to precisely map $qSTV11^{KAS}$ to a 39.2 kb interval defined by markers C1 and R53 (Fig. 6c, Supplementary Fig. 1).

Candidate genes for $qSTV11^{KAS}$

Rice genome annotation (<http://rice.plantbiology.msu.edu/cgi-bin/gbrowse/rice/>; released on June 3, 2009) indicates that there are seven genes in the 39.2 kb target region (Fig. 6d, Table 4, Supplementary Fig. 2). Expression data consisting of either full-length cDNA, expressed sequence tag (EST), or microarray analysis from Affymetrix and Yale University (<http://bioinformatics.med.yale.edu/riceatlas/>) were available for five of these genes (LOC_Os11g30910, LOC_Os11g30930, LOC_Os11g30940, LOC_Os11g30950, and LOC_Os11g30960). Four of these five putative genes (LOC_Os11g30930, LOC_Os11g30940, LOC_Os11g30950, and LOC_Os11g30960) are classified as encoding hypothetical or retrotransposon proteins (Table 4). LOC_Os11g30910 is predicted to

encode a sulfotransferase domain-containing protein with one unique exon and a transcript length of 1,131 bp. This is supported by the existence of a full-length cDNA (no. AK061003) in the Knowledge-based Oryza Molecular biological Encyclopedia (<http://cdna01.dna.affrc.go.jp/cDNA/>; Kikuchi et al. 2003). As sulfotransferases have been shown to be differentially expressed in response to avirulent and virulent pathogens (Lacomme and Roby 1996), LOC_Os11g30910 appears to be the most likely candidate for $qSTV11^{KAS}$. To investigate this possibility, we amplified the LOC_Os11g30910 coding region from Kasalath and Nipponbare. Analysis of the sequence of the gene LOC_Os11g30910 revealed two polymorphisms that affect the coding region of Kasalath relative to Nipponbare. One results in an amino acid substitution (serine to threonine at position 214) and the other involves the deletion of tandem alanines (positions 224 and 225) in the Kasalath protein. Both changes are located in the sulfotransferase domain (Fig. 8). The identification of differences between the Kasalath and Nipponbare alleles of LOC_Os11g30910 is consistent with this gene being the most likely candidate for $qSTV11^{KAS}$ and definitive functional complementation experiments are in progress.

Discussion

In spite of low yield and poor agronomic traits, landraces often have high resistance to various biotic stresses. Natural variation arising from early landraces has played a vital role in the breeding of resistance to biotic and abiotic stresses. For example, the rice *Bph-1* gene originated from a Sri Lanka landrace, Mudgo, which subsequently was used as a parent of many of the commercial resistant varieties (Sōgawa 1982). Here we identified 68 highly resistant accessions by screening landraces collected from different regions, thus providing germplasm for RSV resistance breeding. We found that most of the *indica* types were highly resistant while *japonica* accessions tended to be susceptible, consistent with findings of previous studies (Washio et al. 1968a). Among the resistant accessions, the Indian landrace Kasalath was highly resistant and found to harbor a major QTL for RSV resistance, $qSTV11^{KAS}$. The association between markers tightly linked with $qSTV11^{KAS}$ and RSV resistance in diverse landraces (Table 3) suggests gene introgression among different landraces during evolution and domestication to withstand a common stress.

$qSTV11^{KAS}$ resides in a resistance gene cluster on chromosome 11

The QTLs detected in this study showed some difference under the natural and artificial inoculation. Similar results

Table 4 Annotated genes in the *qSTV11^{KAS}* mapped region on chromosome 11

	Gene ID ^a	Physical location ^b		Gene function	PFAM hits ^c	EST/cDNA ^d support	^e Microarray probe detection
1	LOC_Os11g30899	17517805	17516502	Hypothetical protein	Not found	Not found	Not found
2	LOC_Os11g30910	17518869	17520056	Sulfotransferase domain-containing protein, expressed	sulfotransferase domain	FL_cDNA and EST	Unique and far-ranging probe
3	LOC_Os11g30920	17525043	17527706	Transposon protein, putative, Mariner subclass	Not found	Not found	Not found
4	LOC_Os11g30930	17530542	17528108	Conserved hypothetical protein	Not found	Not found	Unique and far-ranging probe
5	LOC_Os11g30940	17532190	17533682	Retrotransposon protein, putative, unclassified	Not found	Not found	Unique probe
6	LOC_Os11g30950	17536401	17535913	Conserved hypothetical protein	Not found	Not found	Unique and far-ranging probe
7	LOC_Os11g30960	17550237	17545143	Retrotransposon protein, putative, unclassified	Reverse transcriptase (RNA-dependent DNA polymerase)	Not found	Far-ranging probe

^a MSU Rice Genome Annotation Project Locus ID

^b Pseudomolecule release 6.1

^{c,d} NCBI BLAST search

^e MSU Rice Genome Annotation Project Pseudomolecules (Release 6.1) ('Affymetrix GeneChip Rice Genome Array' and 'Yale Rice Oligo Microarray'). Those microarray probes that are uniquely aligned to the rice pseudomolecules are indicated as Unique probes. Those aligned to more than one location are indicated as Far-ranging probes

were observed in previous studies in rice. Maeda et al. (2004, 2006) identified only one RSV resistance QTL on chromosome 11 with a net-house inoculation method and two on chromosomes 2 and 11 with an artificial inoculation test. The detection of distinct QTL using different inoculation methods might reflect the fact that artificial inoculation caused more disease symptoms after RSV infection, thus resulting in more QTLs detected. In the case of *qSTV11^{KAS}*, the locus was detected in two populations (BILs and CSSLs), suggesting it is a key point in RSV resistance.

Many studies have shown that the region near the XNpb202–C1172 interval on the long arm of chromosome 11 harbors resistance genes/QTLs against RSV and other pathogens. For example, major QTL for RSV resistance were detected in G257 adjacent region of chromosome 11 across different populations (Ding et al. 2004; Kottapalli et al. 2006; Maeda et al. 1999; Maeda et al. 2004; Sun et al. 2006; Wu et al. 2009). In addition, the QTL for resistance to sap-sucking pests including brown planthopper (Su 2003), green leafhopper, green rice leafhopper (Yasui and Yoshimura 1999), small brown plant hopper (Duan et al. 2007, 2009) have been identified around XNpb202–C1172 interval. Three bacterial blight resistance genes (*Xa-3*, *Xa-4*, and *Xa-21*) and three blast resistance genes (*Pi-18*, *Pi-1*, and *Pi-7*) have also been mapped to the same region

of chromosome 11 (Lin et al. 1995; Mew et al. 1994; Wang et al. 1994; Ahn et al. 1996).

The clustering of many resistance genes on chromosome 11 might be due to the process of tandem duplication and subsequent divergence under the selective pressure of rice pathogens. In addition, the sequencing of rice chromosome 11 has confirmed that many disease resistance-like and defense-response genes including leucine zipper, nucleotide binding site - leucine rich repeat (NBS-LRR), and transmembrane proteins are located in tandem (The Rice Chromosomes 11 and 12 Sequencing Consortia 2005). It will be interesting to dissect this resistance region by high-resolution mapping and clarify the molecular mechanism of resistance by gene cloning and functional characterization.

qSTV11^{KAS} and *Stvb-i* are not allelic but tightly linked

Though previous studies have mapped RSV resistance QTLs on chromosome 11, the precise relationship between these QTLs has not been confirmed because of differences in both the marker types and resistance donors used. Based on our allelism test using a small F₂ population, *Stvb-i* and *qSTV11^{KAS}* appear to be either allelic or tightly linked. *Stvb-i*, present in most Japanese *japonica* varieties with

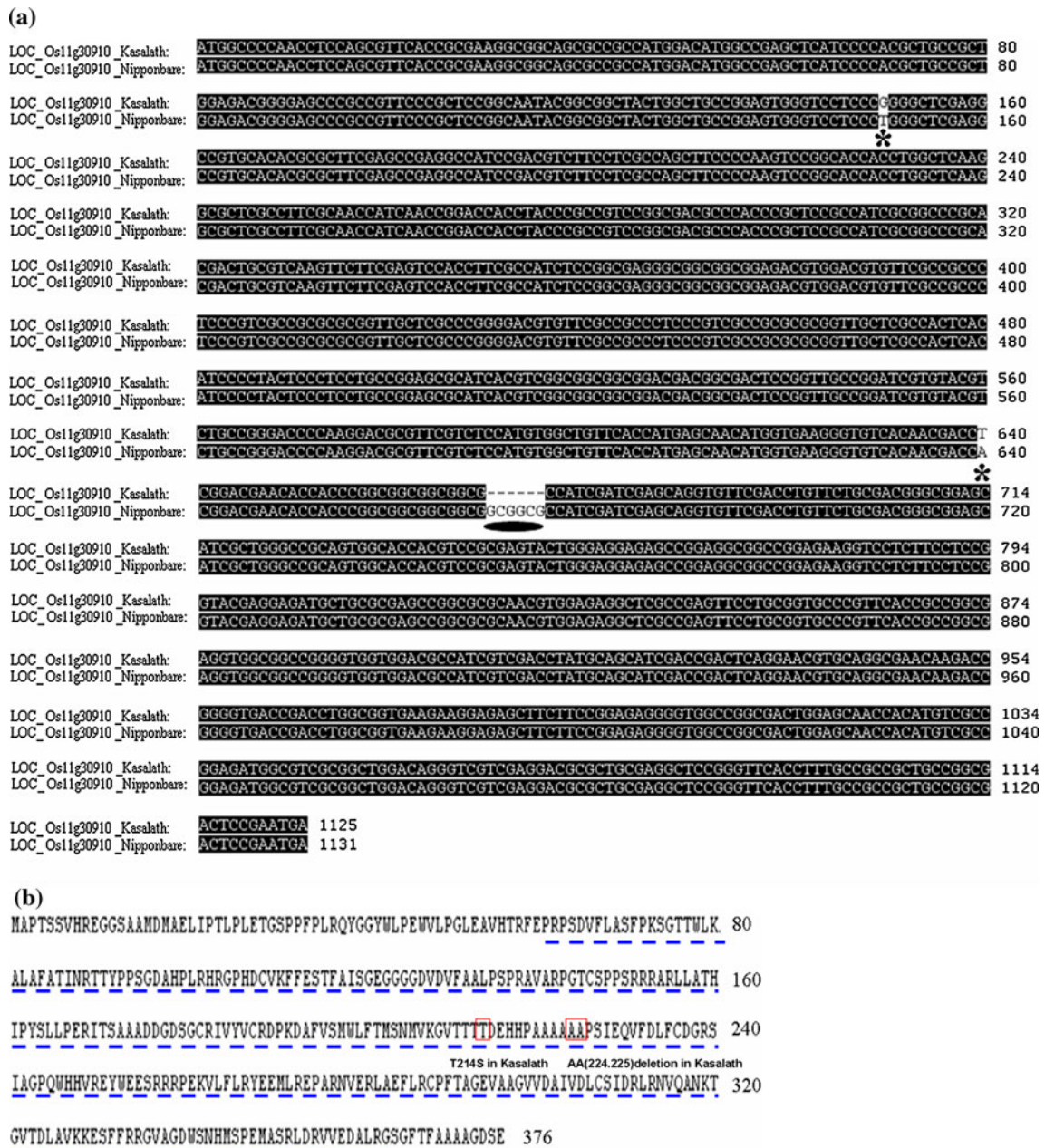


Fig. 8 cDNA and predicted amino acid sequence of LOC_Os11g30910 protein from the Kasalath and Nipponbare alleles. **a** Alignment of the cDNA sequence of LOC_Os11g30910 from the Kasalath and Nipponbare. Asterisks indicate single-base substitutions between the Kasalath and Nipponbare. Ellipsoid represents deletion in

Kasalath. **b** Predicted amino acid sequence of LOC_Os11g30910 protein. Small rectangular boxes indicate amino acid substitution or deletions between the Kasalath and Nipponbare. Dashed line indicates sulfotransferase domain-containing region of LOC_Os11g30910 protein

RSV resistance, was previously finely mapped between two RFLP markers XNpb220 and XNpb254 within 286 kb using a BAC contig (Hayano-Saito et al. 2000). Here we delimited the $qSTV11^{KAS}$ to a nearby 39.2 kb region. According to the probe sequences published in GRAMENE (<http://www.gramene.org/db/markers/>) and the integrated map between RFLP and SSR markers (McCouch et al. 2002), *Stvb-i* resides between our two markers L104 and

RM3428, which are adjacent to $qSTV11^{KAS}$ (Fig. 6a). Recently, Wu et al. (2010) reported the fine mapping of $qSTV11^{TQ}$ to a 55.7 kb interval, which based on pedigree and physical mapping appears to be *Stvb-i* or an allele. Likewise, the $qSTV11^{TQ}$ interval was adjacent to the $qSTV11^{KAS}$ region. This indicates that *Stvb-i* and $qSTV11^{KAS}$ are non-allelic although the relationship of these loci should ultimately be clarified via gene cloning.

Candidate genes of *qSTV11^{KAS}*

Using the available sequence annotation database (Nipponbare from <http://www.tigr.org/> and Kasalath from <http://www.rgp.dna.affrc.go.jp/>), the candidate genes for *qSTV11^{KAS}* were identified. There are seven predicted genes in the target region according to the genome sequence of Nipponbare. Of these putative genes, six are predicted to encode either hypothetical or retrotransposon proteins and had no EST data to support their expression, making them unlikely candidates for *qSTV11^{KAS}*. The remaining open reading frame, LOC_Os11g30910, is predicted to encode a sulfotransferase domain-containing protein and a full-length cDNA has been reported.

Sulfotransferases (SULTs) catalyze the transfer of a sulfonyl group (SO_2^-) from the universal donor 3'-phosphoadenosine 5'-phosphosulfate (PAPS) to a hydroxyl group of various substrates in a process called sulfonation. Acceptor molecules include a wide array of substances, from endogenous molecules, such as hormones, neurotransmitters, and peptides, to exogenous chemicals such as drugs, xenobiotics, and toxins. Sulfonation of small molecules can either activate or inactivate biological responses. There are two classes of SULTs: cytosolic and membrane-associated SULTs (see review by Hernández-Sebastià et al. 2008). In *Arabidopsis thaliana*, functional genomic approaches led to the characterization of brassinosteroid, hydroxyjasmonate, desulfoglucosinolate and flavonoid SULTs (Blanchard et al. 2004).

The biological functions of SULTs are still largely unknown, but plant sulfotransferase complexes and their resulting reactive products are believed to play a role in response to pathogens and the pathogen-related signals, such as methyl jasmonate and salicylic acid (Lacomme and Roby 1996). In some plants, sulfotransferase genes could be induced by pathogen-challenge or herbivore-attack (Yang et al. 2007; Reymond et al. 2004). Recently, Baek et al. (2010) reported the *Arabidopsis* sulfotransferase AtSOT12 (At2g03760), presumably through sulfonation of salicylic acid, enhances plant response to pathogen infection and contributes to long distance signaling in systemic acquired resistance. In rice, the sulfonation of the tyrosine residues in phytosulfokine- α through a membrane-bound SULT was important for plant cell proliferation (Yang et al. 1999), which is consistent with a possible role in the stunting and withering phenotypes observed after infecting with RSV in this study.

To date, the structures of some SULTs have been solved. They share a common globular structure and contain a single α/β fold with a central four- or five-stranded parallel β sheet surrounded by α -helices. The SULT structures are similar to those of nucleotide kinases suggesting a common ancestral protein (see review by

Hernández-Sebastià et al. 2008). The SULTs catalytic activity and substrate specificity were affected by PAPS binding site, substrate binding region, dimerization motif, and so on. A great deal of information on the catalytic mechanism of SULTs has been derived from the solved crystal structures and from the results of numerous site-directed mutagenesis studies (Marsolais and Varin 1998; Chapman et al. 2004). Even a conservative replacement of the lysine with arginine in the sulfotransferase coding region led to a significant reduction in catalytic efficiency (Marsolais and Varin 1995). Analysis of the amino acids of the proteins predicted to be encoded by LOC_Os11g30910 in Nipponbare (susceptible) and Kasalath (resistant) revealed that the Kasalath protein contains one amino acid substitution (serine to threonine) and two amino acid deletions (alanine-alanine) in the sulfotransferase domain-containing region (Fig. 8). The amino acid differences predicted by the Kasalath coding region of LOC_Os11g30910 may affect sulfotransferase structure resulting in functional differences that account for the difference in response to RSV between Kasalath and Nipponbare.

Because the identification of *qSTV11^{KAS}* candidate genes was based on annotation of the RSV susceptible Nipponbare, it is possible that there are other genes at the locus in the resistant cultivar Kasalath and sequencing of the Kasalath locus is being performed. To definitively identify the gene underlying *qSTV11^{KAS}*, functional complementation tests with LOC_Os11g30910 from Kasalath and the other candidates at the *qSTV11^{KAS}* locus are currently underway.

Acknowledgments This research was supported by the 863 Program of China (2007AA10Z191, 2009AA101101), National Key Transform Program (2008ZX08001-02, 2008ZX08001-01), the earmarked fund for Modern Agro-industry Technology Research System, Jiangsu Science and Technology Development Program (BE2008352, BE2009301-3) and the Education Department of Jiangsu (200910).

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