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ORIGINAL PAPER

# Fine mapping of *qSTV11*$^{TQ}$, a major gene conferring resistance to rice stripe disease

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**Abstract** The indica rice cultivar, Teqing, shows a high level of resistance to rice stripe virus (RSV). It is believed that this resistance is controlled by the gene, *qSTV11*$^{TQ}$. For positional cloning of the resistance gene, a set of chromosome single segment substitution lines (CSSSLs) was constructed, all of which had the genetic background of the susceptible japonica cultivar, Lemont, with different single substituted segments of Teqing on chromosome 11. By identifying the resistance of the CSSSLs-2006 in a field within a heavily diseased area, the resistance gene *qSTV11*$^{TQ}$ was mapped between the markers Indel7 and RM229. Furthermore, in that region, six new markers were developed and 52 subregion CSSSLs (CSSSLs-2007) were constructed. The natural infection experiment was conducted again at different sites, with two replicates used in each site in order to identify the resistance phenotypes of the CSSSLs-2007 and resistant/susceptible controls in 2007. Through the results of 2007, *qSTV11*$^{TQ}$ was localized in a region defined by the markers, CAPs1 and Indel4. In order to further confirm the position of *qSTV11*$^{TQ}$, another set of subregion CSSSLs (CSSSLs-2009) was constructed. Finally, *qSTV11*$^{TQ}$ was localized to a 55.7 kb region containing nine annotated genes according to the genome sequence of *japonica* Nipponbare. The relationship between *qSTV11*$^{TQ}$ and *Stvb-i* (Hayano-Saito et al. in Theor Appl Genet 101:59–63, 2000) and the reliability of the markers used on both sides of *qSTV11*$^{TQ}$ for marker-assisted breeding of resistance to rice stripe disease are discussed.

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## Introduction

Rice stripe disease is one of the most severe rice diseases in the subtropical and temperate regions of the world. In China, the disease has caused serious grain yield losses of 5–10%, although the loss is usually 20–30% and can even be as high as 100%. Since 2001, severe disease outbreaks have occurred each year in the Yangtze River Delta. In the Jiangsu province alone, approximately 600,000 ha of rice per year were affected with rice stripe disease from 2000 to 2003 (Sun et al. 2007; Wang 2006).

Rice stripe disease is caused by the rice stripe virus (RSV), and this virus is transmitted mainly by the small brown planthopper (*Laodelphax striatellus* Fallen). Resistance to rice stripe disease can be divided into resistance or tolerance to the virus and resistance to the vector insect (Fraser 1992). Breeding of virus-resistant cultivars is the ideal method of controlling rice stripe disease (Ise et al. 2002).

Many studies were carried out to identify RSV resistance genes in the last decade. By using $F_2$ individuals from a cross between Koshihikari (susceptible) and Asanohikari (resistant) cultivars, a Japanese group mapped an RSV resistance gene, *Stvb-i*, to the long arm of rice chromosome 11 (Hayano-Saito et al. 2000). Ding et al. (2004) mapped

*qStv11* (from DV85) to the long arm of rice chromosome 11 between markers XNpb202 and C1172 by using 81 recombinant inbred lines (RILs) from a cross of Kinmaze (*japonica*) and DV85 (*indica*) cultivars; the genetic distance between these two markers is approximately 27.5 cM. The same laboratory mapped another gene resistant to RSV to the long arm of rice chromosome 11 between markers G257 and S2260 by using another mapping population, namely, backcross inbred lines (BILs) derived from a cross of Nipponbare/Kasalath//Nipponbare cultivars (Ding et al. 2005), and the genetic distance between these two markers is approximately 8.8 cM. Furthermore, a resistance gene was mapped near the RFLP marker, G257, on chromosome 11 with a distance of 1.0 cM (Maeda et al. 2004). Recently, two resistance genes ($qSTV11^b$ and $qSTV11^c$) from Dular were located between RM287 and RM209 and between RM209 and RM21 by using a set of an $F_2$ clonal population from a cross of Balilla/Dular cultivars (Wu et al. 2009).

We constructed a set of chromosome single segment substitution lines (CSSSLs) (Zuo et al. 2007); all CSSSLs had the genetic background of the susceptible japonica cultivar, Lemont, with different single substituted segments of Teqing (resistant cultivar) on the long arm of chromosome 11. The healthy plant ratio of these CSSSLs was determined by performing an experiment in a heavily infected zone under natural infection conditions. The proportion of viruliferous vector insects in the experimental field was as high as 39.6%, as determined by enzyme-linked immunosorbent assay (Qin et al. 1994). The CSSSLs were clustered into resistant or susceptible groups and the resistance gene locus was mapped. Next, two sets of subregion CSSSLs in the target region were developed for fine mapping of the gene.

## Materials and methods

### Plant materials

Lemont, a japonica rice cultivar from Louisiana, USA, is susceptible to rice stripe disease. Teqing is an indica rice cultivar resistant to the disease from Guangdong, China.

### Methods

#### *Marker selection and PCR analysis*

*Selection of SSR marker* SSR markers evenly distributed in the $qSTV11^{TQ}$ region were selected from a public database on the basis of sequence differences between Nipponbare and 9311, and the polymorphism between parents was then tested using PCR. The polymorphic markers were used for fine mapping.

*Development of new markers* Since very few polymorphic SSR markers were found in the target region, the InDel and CAPS markers were designed using PRIMER0.5 software (http://www.genome.wi.mit.edu/ftp/pub/software/primer.0.5). The primers for candidate markers were first used to amplify parental lines, and those that gave the expected PCR products were used in further experiments. For the CAPS marker, the PCR product was digested with a corresponding restriction enzyme and then subjected to electrophoresis on either a 1% agarose gel or 6% polyacrylamide gel, depending on the size and difference between two polymorphic bands. Following electrophoresis, the agarose gels were stained with ethidium bromide, whereas polyacrylamide gels were silver-stained for visualization. All newly developed markers used for fine mapping contained polymorphisms between Lemont and Teqing. Primer information for the 14 markers is listed in Table 1.

#### *Construction of the fine-mapping population*

The primary mapping population was a set of CSSSLs with the Lemont genetic background (Zuo et al. 2007). In 14 CSSSLs (CSSSLs-2006), the targeted region of the long arm of rice chromosome 11 contained segments from Teqing.

Fifty-two recombinant plants were selected from the $BC_6F_2$ population on the basis of genotype in the target region (Indel7-RM229). Detection via marker continued in $BC_6F_3$; 52 subregion CSSSLs (CSSSLs-2007) with different substitute segments of the donor parent in the target region and the same genotype as the recurrent parent in the other chromosome regions/chromosomes were constructed in $BC_6F_4$. Subsequently, 21 subregion CSSSLs (CSSSLs-2009) were constructed from a cross between Lemont and the 49th line, which was a subregion CSSSL (comes from the CSSSLs-2007) with the smallest single target substituted segment from Teqing (Fig. 2).

#### *Evaluation of rice stripe disease incidence*

Resistance evaluation was conducted in the fields for 5 years from 2006 to 2010. During those years, the primary mapping population (CSSSLs-2006) was evaluated in 2006 and 2007, while the fine-mapping population (CSSSLs-2007 and CSSSLs-2009) was evaluated in 2007, 2008, 2009, and 2010. Each year, 96 plants were planted in four rows in each plot, and the plots were arranged in randomized blocks. In each block, seven resistant (Teqing) and seven susceptible (Lemont) control plots were randomly inserted.

A field within a disease-severe area was infected by natural means in order to identify plants with resistance to rice stripe disease (Li et al. 2008). The seedling bed was

**Table 1** Primer sequence of the markers used for fine mapping of the *qSTV11*$^{TQ}$ gene

| Marker | Primer sequence (5′–3′) | Product size (bp)[a] | Restriction enzyme[b] |
|---|---|---|---|
| Indel1 | Forward: GTGACTTGGTCTTGGTTGTT | 233 | – |
| | Reverse: TATAGAAGCCCTAACTGACT | | |
| Indel2 | Forward: GCTTGTTGTTTAGCCAGTA | 410 | – |
| | Reverse: AAATCTGACCATCCGTTAT | | |
| Indel3 | Forward: TCCAATGCCCAAAACCAAGA | 300 | – |
| | Reverse: CCAGGCCGTCAACTACCTCA | | |
| Indel4 | Forward: ACACGATGTAACTTGAGGCG | 374 | – |
| | Reverse: TCCATACAGGAACGTTGCAC | | |
| Indel5 | Forward: ATGTGCTAATGACGGCTTAA | 274 | – |
| | Reverse: TGCTGCGTGCTTTGTTTGAC | | |
| Indel6 | Forward: AGATGGTGCCCAAATGAAAT | 302 | – |
| | Reverse: AAGAAACGTACAAGAGGAGC | | |
| Indel7 | Forward: CATCCTCTGCTACCCAAAC | 172 | – |
| | Reverse: ATACCCTCGTGAACCTCTT | | |
| Indel8 | Forward: GGGCTTTCAACTCGTACTCTG | 337 | – |
| | Reverse:ATGAACTGCGGGTCCAATAA | | |
| Indel9 | Forward: CATTGTCGTTGTGGAGGATA | 417 | – |
| | Reverse: AAGCAGTAGGAGGGTGTGAG | | |
| Indel10 | Forward: TATGGCATTGCTACGACAA | 217 | – |
| | Reverse: TATCAGGAGCGACGGGAG | | |
| CAPs1 | Forward: AATGGAGGGAGCAACTAATC | 291 | *Sca*I |
| | Reverse: GAAGAAGGACAGACCAAACG | | |
| CAPs2 | Forward: GAGGGTGAGCGAGTGATTT | 223 | *Sac*I |
| | Reverse: ACACGCCATTTAGGATAGG | | |
| CAPs3 | Forward: GTGGGAATCAAGCACTAAAG | 593 | *Hin*dIII |
| | Reverse: ACTTCCGTATTGCTGAAATG | | |
| dCAPs1 | Forward: TCAGTGAGCACAGGAGTGA | 292 | *Fba*I |
| | Reverse: TCTTTTGGCTGCGTACATC | | |

[a] The size of amplicon in Nipponbare

[b] Four markers CAPs1, CAPs2, CAPs3, and dCAPs1 displayed genetic polymorphism after digestion of amplified DNA fragments with an indicated restriction endonuclease

arranged near wheat fields where the vector insect density was adequate. The materials were sparsely sown on May 7 (about 3 weeks before wheat ripening and harvest) and the seedlings were transplanted on June 7 while they were in the 6- to 7-leaf stage. No pesticide was applied before the seedlings were fully sucked by the vector insect. However, pesticide was applied once or twice at the 6- to 7-leaf stage to prevent seedlings from being sucked excessively by insects, which could cause the seedlings to weaken or even die.

The basic seedling number was investigated 10 days after transplanting. The disease incidence was investigated in three different stages, viz., 10 days after transplanting, end of the effective tillering stage (20–25 days after transplanting), and during the heading stage. Plants with yellow and white stripe disease lesions and more severe symptoms were considered susceptible plants. In contrast, plants without typical disease lesions, or those that exhibited slight symptoms, which disappeared after heading, were considered resistant plants. The rate of healthy plants (the percentage of healthy plants in a given plot) represented the resistance level of a CSSSL against the disease.

*Data analysis*

Differences in healthy plant rates among experiment materials (CSSSLs and resistant/susceptible checks) were statistically analyzed using SPSS software on the basis of the variance analysis model of two-factor random blot experiment design. Dot graphs of healthy plant rates of the materials were prepared using Excel.

## Results

Reliability of the natural infection method used to identify resistance to the disease

Yangzhou is an ideal place to assess the resistance of cultivars to rice stripe virus under the conditions of naturally induced disease because there have been frequent

outbreaks of vector infestation, leading to disease outbreaks. The density of the insect population was about 1,700 per $m^2$ with about 23.4, 39.6, 13.1, 12.8, and 10.5% of them being viruliferous in 2006, 2007, 2008, 2009, and 2010, respectively.

The disease was investigated in three stages: 10 days after transplanting, late tillering stage, and heading stage. Among the plants with the susceptible parents (Lemont), the number of plants that showed symptoms of rice stripe disease sharply increased with time, while among plants with the resistant parents (Teqing), there was nearly no change in the number of plants that showed symptoms. Although most of the infected plants of Lemont did not die in the heading stage, they had many yellow and white stripe lesions on their leaves and sheaths. Nearly all plants of Teqing were healthy in the heading stage, although a few plants had slight symptoms in the earlier stages of growth. Therefore, the healthy plant rate at the heading stage was finally used as the criterion to estimate the severity of the disease for each CSSSL population.

Figure 1b shows the healthy plant rate in different plots of resistant/susceptible check parents in 2007. The healthy plant rate of Teqing from 14 plots in two locations was 98.7%, within a narrow range from 95.7 to 100% and a small coefficient of variation (CV) of 1.38%. In contrast, the healthy plant rate of Lemont was 40.4%, within a broad range from 21.8 to 55.2% and a large CV of 21.8%. This situation is recognized as normal in experiments of naturally occurring infection.

Furthermore, the distribution of the healthy plants of the resistant and susceptible checks was discontinuous, with a large gap from 60 to 95%. This phenomenon showed that it is reliable to divide the CSSSLs into resistant or susceptible groups on the basis of their phenotypes under the natural infection condition in heavily diseased areas. Therefore, we mapped the gene of resistance to the disease from Teqing.

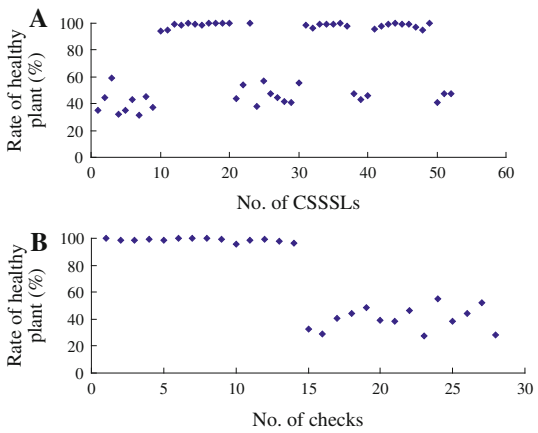


**Fig. 1** The rate of healthy plant distribution of 52 CSSSLs (CSSSLs-2007) and controls in 2007. **a** The rate of healthy plant distribution of CSSSLs-2007 at two sites, each with two replicates. **b** The healthy plant rate in different plots of resistant/susceptible checks in 2007 (shown as *dots*)

In addition, in the experiments in each year, the number of vector insects in each experiment plot was investigated several times before transplant. We found that there were no significant differences in the number of insects that were present among different CSSSLs or checks (data not shown), which suggests that the insect does not have sucking preferences among CSSSLs with the same genetic background. We also found that no plant showed disease symptoms before transplanting; the symptoms appeared mainly after transplanting.

## Mapping the $qSTV11^{TQ}$ gene

In 2006, a set of CSSSLs (CSSSLs-2006) with the genetic background of Lemont were planted. After transplanting, distinct differences in resistance to the disease emerged among the CSSSLs-2006. Combined with their (CSSSLs-2006) genotypes, it was found that there was only one chromosome interval among the substitution segments of Teqing related to resistance/susceptibility, while in the other regions, there were no substitution segments related to the resistant/susceptible phenotype. On the basis of these 14 CSSSLs-2006, the resistant gene ($qSTV11^{TQ}$) could be narrowed down to between two markers, Indel7 and RM229.

To validate the result obtained in 2006, the CSSSLs-2006 were planted again in 2007. We found that there were large phenotypic differences among the CSSSLs-2006, and it was clear which ones had the resistant or susceptible phenotype. The results were identical to those obtained in 2006. Of the 14 CSSSLs-2006, 9 were resistant to the disease and the healthy plant rate ranged from 96.3 to 100%, and the healthy plant rate for those that were susceptible ranged from 12.9 to 49.8%. Therefore, the $qSTV11^{TQ}$ gene was located between the markers Indel7 and RM229 based on the phenotype of each CSSSL (Table 2). The genetic distance between these two markers was about 16.0 cM.

## Determination of the phenotypes of subregion CSSSLs

On the basis of the results obtained in 2006, 52 subregion CSSSLs (CSSSLs-2007) were developed. All CSSSLs-2007 and check parents were then planted at two sites, each with two replicates. The results of analysis of variances of the data on healthy plant rates are shown in Table 3. The differences in blot items within a site were not significant, suggesting that the number of insects distributed at each site did not differ. However, the differences among the CSSSLs in terms of resistance were highly significant. Although the differences among site items were significant,

**Table 2** The genotype and resistant/susceptible phenotype of CSSSLs (CSSSLs-2006) in 2006 and 2007

| No. of CSSSLs | PCR markers and genotype | | | | | | Phenotype [a] | Rate of health plant (%)[b] |
|---|---|---|---|---|---|---|---|---|
| | RM 525 | RM 529 | RM 3428 | Indel 7 | RM 229 | RM 21 | | |
| 51429 | 1 | 1 | 1 | 1 | 3 | 3 | S | 44.1 |
| 51436 | 3 | 3 | 3 | 3 | 1 | 1 | R | 96.3 |
| 51437 | 3 | 3 | 3 | 3 | 1 | 1 | R | 100 |
| 51438 | 3 | 3 | 3 | 3 | 1 | 1 | S | 44.8 |
| 51447 | 1 | 1 | 1 | 1 | 3 | 3 | R | 100 |
| 51495 | 3 | 3 | 3 | 3 | 1 | 1 | R | 100 |
| 51500 | 1 | 1 | 1 | 1 | 3 | 3 | R | 100 |
| 51539 | 3 | 3 | 3 | 3 | 1 | 1 | R | 98.6 |
| 51540 | 3 | 3 | 3 | 3 | 1 | 1 | R | 100 |
| 51541 | 3 | 3 | 3 | 3 | 1 | 1 | R | 100 |
| 51542 | 3 | 3 | 3 | 3 | 1 | 1 | R | 100 |
| 51545 | 1 | 1 | 1 | 1 | 3 | 3 | S | 12.9 |
| 51546 | 1 | 1 | 1 | 1 | 3 | 3 | S | 36.7 |
| 51547 | 1 | 1 | 1 | 1 | 3 | 3 | S | 49.8 |
| Lemont | 1 | 1 | 1 | 1 | 1 | 1 | S | 40.4 |
| TQ | 3 | 3 | 3 | 3 | 3 | 3 | R | 98.7 |

*Note* "1" stands for the Lemont genotype, and "3" stands for the Teqing genotype

[a] The phenotypes were detected in 2006

[b] Results were analyzed in 2007

**Table 3** Variance analysis of the data of healthy plant rate of 52 subregion CSSSLs (CSSSLs-2007) and 28 check parents in 2007

| Variation source | *df* | SS | MS | *F* | *P* |
|---|---|---|---|---|---|
| Locations within the block | 2 | 0.04059 | 0.02029 | 1.75127 | 0.17691 |
| Place | 1 | 0.07526 | 0.07526 | 6.49413 | 0.01178 |
| Variety | 79 | 25.93482 | 0.32829 | 28.32896 | 0.00000 |
| Variety × place | 79 | 1.11606 | 0.01413 | 1.21909 | 0.14766 |
| Test error | 158 | 1.83098 | 0.01159 | | |
| Total variance | 319 | 28.99771 | | | |

differences among interaction items, line × site, were not significant. These results suggest that the experiment of naturally introducing the disease was reliable.

A multiple comparison of the differences in healthy plant rates of all plots of Teqing or Lemont showed that different CSSSLs could be clustered into resistant or susceptible groups (Fig. 1a).

## Fine mapping of *qSTV11*$^{TQ}$

The genotypes and phenotypes of CSSSLs-2007 and checks are illustrated in Fig. 2. On the basis of the results for these lines, the resistant gene locus was narrowed to the region between the markers Indel1 and Indel5. Nine CSSSLs, including two resistant CSSSLs (32nd and 33rd) and seven susceptible CSSSLs (the 24th, 25th, 26th, 27th, 28th, 29th, and 30th) provided information on the left exchange limit, while one resistant CSSSL (the 31st) provided information on the right exchange limit.

In the region between the markers Indel1 and Indel5, six new markers were developed and used to detect ten exchange lines in order to distinguish the size and location of their substituted segments. From among the ten exchange lines, two lines (32nd and 33rd) provided information on the right limit of exchange and one resistant line (31st) provided information on the left limit. The resistance gene was finely mapped between the markers CAPs1 and Indel4, and four other markers between the two markers co-segregated with the phenotype.

In 2008, ten recombinant lines were planted again to validate the results obtained in 2007 (Table 4), and the phenotypes of all ten lines were consistent with those obtained in 2007, suggesting that the results of gene mapping in 2007 were correct.

In order to narrow down the location of the gene into a smaller region, a new set of subregion CSSSLs (CSSSLs-2009) was generated by crossing Lemont with the 49th line. The genotypes and phenotypes of the CSSSLs-2009 and checks are listed in Fig. 3. Analysis with the CSSSLs-2009 revealed that the resistant gene locus could be narrowed to the region between the markers CAPs3 and CAPs2. Subsequently, the CSSSLs-2009 were planted

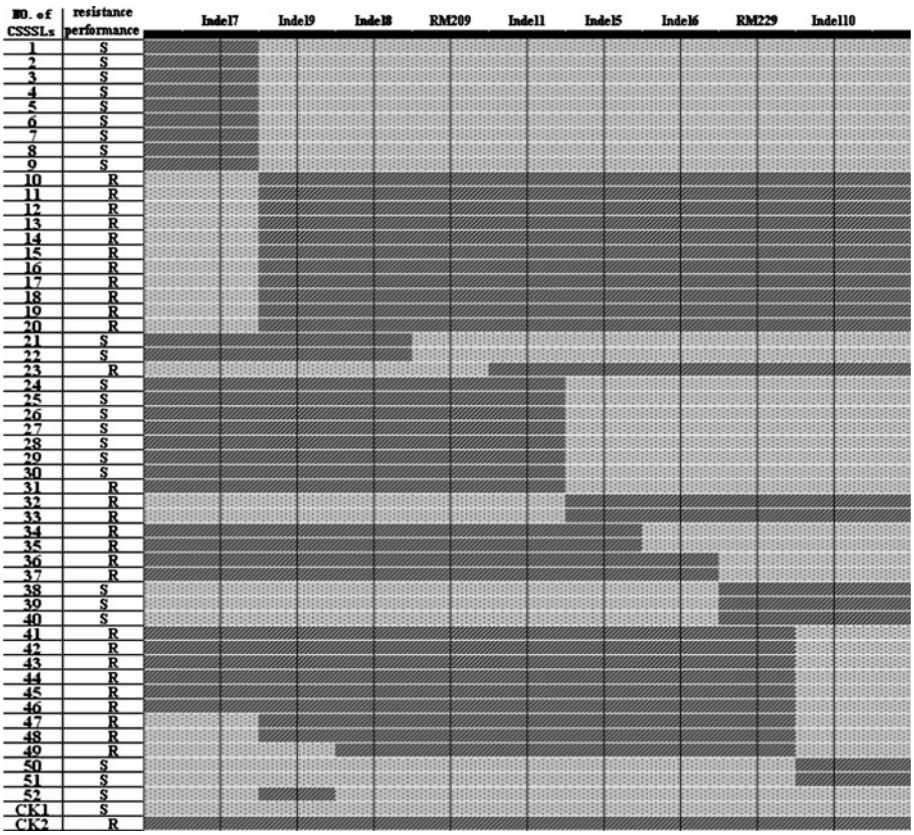


**Fig. 2** Description of genotype and resistant performance of the CSSSLs-2007 and controls. Indel7, Indel9, Indel8, RM209, Indel1, Indel5, RM229, and Indel10 are the markers used. *Slash-lines grid* denotes the resistant parent Teqing segment and *dots grid* denotes the susceptible parent segment. *S* denotes that susceptible lines and *R* denotes resistant lines. *CK1* means Lemont and *CK2* means the Teqing segment. Crossing-over points within the same marker intervals among CSSSLs are depicted at the same positions, although they were most likely not at the same site

**Table 4** Genotypes of 12 recombinant CSSSLs (CSSSLs-2007) and their performances in terms of resistance to rice stripe virus

| No. of CSSSLs | Phenotype | Rate of healthy plant of CSSSLs in 2-year test (%) | | Genotype on nine molecular markers | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 2007 | 2008 | Indel1 | CAPs1 | Indel2 | Indel3 | CAPs2 | dCAPs1 | Indel4 | Indel5 | Indel6 |
| 32 | R | 100.0 | 100.0 | 1 | 1 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |
| 33 | R | 100.0 | 97.6 | 1 | 1 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |
| 31 | R | 100.0 | 98.3 | 3 | 3 | 3 | 3 | 3 | 3 | 1 | 1 | 1 |
| 25 | S | 55.2 | 22.8 | 3 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| 26 | S | 47.4 | 41.6 | 3 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| 27 | S | 47.4 | 38.6 | 3 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| 28 | S | 43.3 | 43.5 | 3 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| 29 | S | 46.2 | 13.9 | 3 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| 30 | S | 47.1 | 25.7 | 3 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| 24 | S | 44.3 | 27.4 | 3 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| Lemont | S | 40.4 | 52.4 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| TQ | R | 98.7 | 99.2 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |

*R* resistant to RSV; *S* susceptible to RSV; "1" stands for the Lemont segment, "3" stands for the Teqing segment

again in 2010 to confirm the results of 2009, and the results were the same as those of 2009.

By aligning marker sequences with the complete genome sequence of the japonica cultivar, Nipponbare, on Gramene (http://www.gramene.org/Multi/blastview), the physical distance of the interval between the markers was 55.7 kb and was related to one BAC clone, OSJNBa0062E06 (AC136491) (Fig. 4). On the basis of the annotation of the Institute for Genomic Research (TIGR, http://rice.tigr.org), analysis of the 55.7-kb sequence harboring the $qSTV11^{TQ}$ locus indicated that this region contained nine candidate genes (Table 5). Given

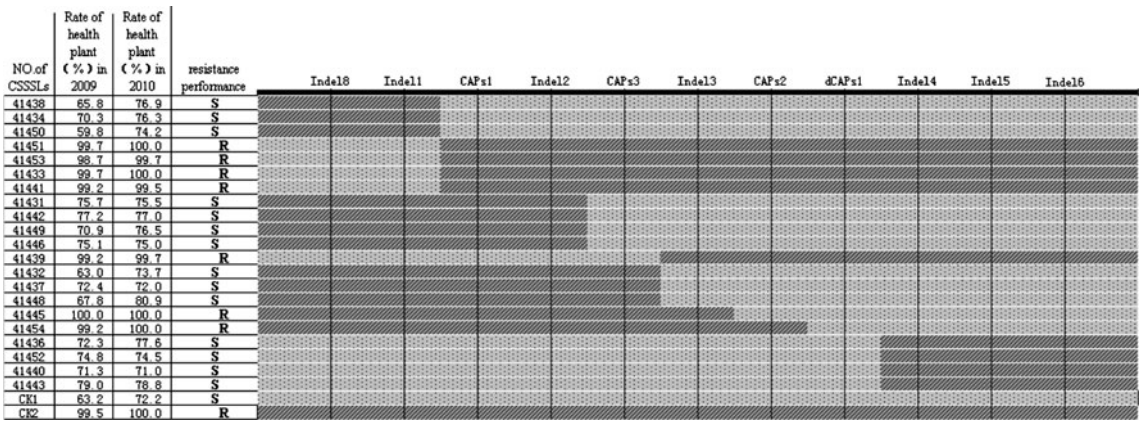


**Fig. 3** Description of genotype and resistant performance of CSSSLs-2009 and controls in 2009 and 2010. Indel8, Indel1, CAPs1, Indel2, CAPs3, Indel3, CAPs2, dCAPs1, Indel4, Indel5 and Indel6 are the markers used. *Slash-lines grid* denotes the resistant parent Teqing segment and *dots grid* denotes the susceptible parent segment. *S* denotes the line is a susceptible one and *R* that it is resistant. *CK1* means Lemont and *CK2* means the Teqing segment. *Vertical lines* represent marker positions, whereas the map distances between adjacent markers do not represent their practical distances on the chromosome. Crossing-over points within the same marker intervals among CSSSLs were depicted at the same positions, although they were most likely not at the same site

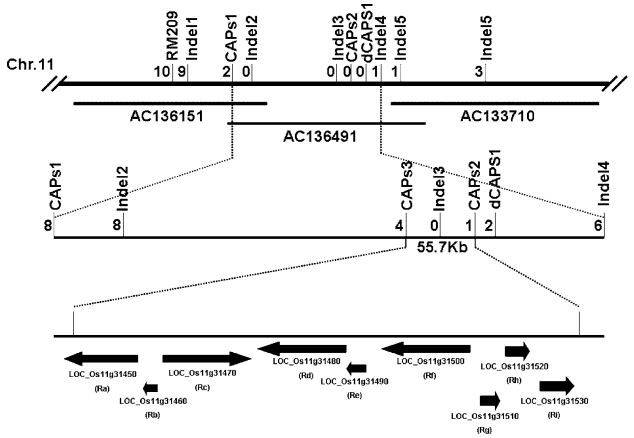


**Fig. 4** High-resolution genetic and physical maps of $qSTV11^{TQ}$ and gene prediction in the critical region of chromosome 11. The figure shows the number of the recombinant individuals corresponding to a marker. At the *top*, the position of markers used is indicated, and the *numbers* stand for the number of recombinant plants. The number on the *first line* denotes the CSSSL constructed in 2007 (CSSSLs-2007), and the number below denotes the CSSSL constructed in 2009 (CSSSLs-2009). The short *horizontal lines* represent bacterial artificial chromosome (BAC) clones of cv. Nipponbare along with their accession numbers (http://www.gramene.org/)

that Nipponbare is susceptible to RSV, the resistance gene may not be among the nine candidate genes. In order to find the resistance gene, a region on TQ was sequenced (the data have not been published). Sequence comparison revealed that this region on TQ was almost the same as that on Nipponbare, except for differences in some nucleotides. Therefore, the resistance gene must be among the nine candidate genes. Further gene complementation tests with candidate genes are underway for the final cloning of $qSTV11^{TQ}$.

## Discussion

Artificial inoculation and natural infection are two methods widely used to identify rice resistance to viruses (Ding et al. 2005; Li et al. 2008). Artificial inoculation involves inoculating plants with viruliferous insects in vivo at the rice seedling stage. However, it is difficult to apply the method for the large-scale resistance assessment of some genetic populations such as RILs since it is labor-intensive if sufficient insects are to be inoculated. Furthermore, a deviation can be caused by differences in the preference of the insect for sucking on rice with different genotypes when the experiment is conducted under a large insects' cover.

The natural infection method can be used in heavily infected areas. The advantage of this method is that it is easy to execute, but its limitation is that one cannot guarantee an even distribution of viruliferous vector insects and appearance of disease symptoms in all susceptible individuals. This may make it difficult to distinguish plants with the susceptible phenotype from those with the resistant phenotype. Therefore, this method is only applicable in heavily diseased areas. Even so, a deviation would appear because of food-ingesting preferences of the insect for rice genotypes.

By using a set of CSSSLs, we could systematically assess the reliability of the natural inoculation method in heavily infected areas. In recent years, rice cultivars of the Jiangsu province have been heavily infected by rice stripe disease because of a large density of vector insects and a high rate of viruliferous insects (Liu et al. 2007; Zhou et al. 2007). Differences in insect preference for different CSSSLs were not considered because plants of a consistent

**Table 5** Gene prediction analysis in the 55.7-kb delimitation region

| ID | Locus identifier | 5′ end | 3′ end | Putative function |
|---|---|---|---|---|
| 3 | LOC_Os11g31450(Ra) | 33858 | 39949 | Expressed protein |
| 4 | LOC_Os11g31460(Rb) | 41273 | 42462 | Retrotransposon protein, putative, Ty3-gypsy subclass |
| 5 | LOC_Os11g31470(Rc) | 43117 | 51549 | Expressed protein |
| 6 | LOC_Os11g31480(Rd) | 52577 | 60602 | ATP binding protein, putative, expressed |
| 7 | LOC_Os11g31490(Re) | 61500 | 63926 | Retrotransposon protein, putative, unclassified |
| 8 | LOC_Os11g31500(Rf) | 70833 | 77639 | ATP binding protein, putative |
| 9 | LOC_Os11g31510(Rg) | 78143 | 79372 | Transposon protein, putative, Pong subclass |
| 10 | LOC_Os11g31520(Rh) | 80121 | 81604 | Transposon protein, putative, Pong subclass, expressed |
| 11 | LOC_Os11g31530(Ri) | 87316 | 89290 | Brassinosteroid insensitive 1-associated receptor kinase 1 precursor, putative |

genetic background were used. We used a large population to assess resistance to the disease and the health plant rate was used as the criterion at the heading stage. The results showed that the variation (CV%) in the health plant rate among different lines with the resistant genotype was small, whereas that among lines with the susceptible genotype was large. A large gap exists between the health plant rate distributions of resistant and susceptible lines, which prevented misclassification of the phenotypes of the CSSSLs. Therefore, fine mapping of the resistance gene against the disease was possible by identifying the resistance of the CSSSLs by the natural inoculation method in heavily diseased areas.

When our results were compared to those of others through the integration of linked maps (Fig. 5), we found common markers such as C1172, XNpb179, and RM209. The results showed that the resistance QTLs detected in different studies are located in a connective in the same region. Therefore, one could surmise that all of the mapped genes (QTLs) are *Stvb-i* or its multi-alleles, or there is a cluster of resistance genes against the disease in the 55.7-kb region flanked by markers CAPs3 and CAPs2 on chromosome 11.

Markers RM209 and RM229 were used in a MAS program of backcross breeding to transform the resistance gene from Zhengdao 88 (a *japonica* rice) into Wuyujing 3, a famous commercial *japonica* rice cultivar with excellent eating quality and yield potential but high susceptibility to the disease (Zhou et al. 2007). Eighty-six homozygous individuals with the resistance allele on this locus were selected from $BC_3F_4$. After 2 years of consecutive selection under naturally occurring infection of the progeny lines of the 86 plants, all plants exhibited excellent resistance to the disease and the concordance rate between the resistance phenotype and marker genotype was 100% (data not shown). From among them, a new cultivar with the best-integrated traits, excellent taste quality, and resistance to the disease was bred and named as "Wulingjing 1" (Pan et al. 2009). On the basis of the pedigree analysis for Zhengdao 88, its resistance to the disease could be traced back to a resistance variety, Modan, which contains the resistance gene *Stvb-i* (Pan et al. 2005), and it is widely used for rice stripe disease resistance breeding. RM209 and RM229 were used as bilateral molecular markers of the resistance gene from both Zhengdao 88 and Teqing, suggesting that the resistance gene $qSTV11^{TQ}$ is *Stvb-i* or its allele.

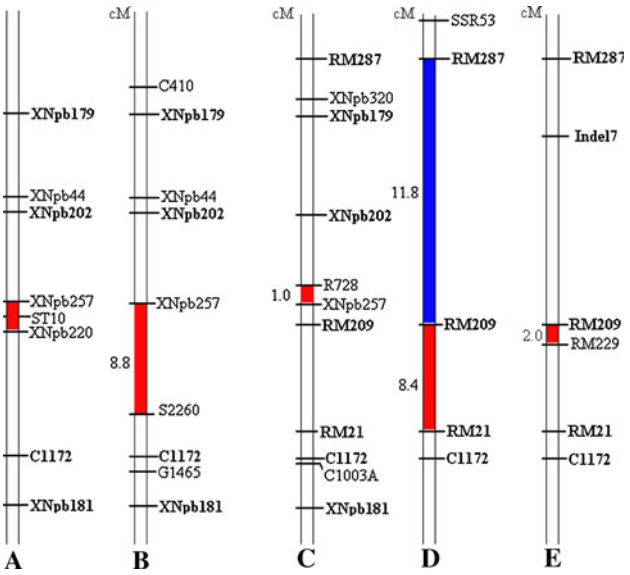


**Fig. 5** Comparison map of the location of rice stripe disease resistance gene(s) that was (were) mapped to chromosome 11. **a** Co-segregation of *Stv-b*$^{i}$ and ST10 (Hayano-Saito et al. 2000). **b** *qStv11* is located in the region between XNpb202 and C1172 (Ding et al. 2005). **c** A *qStv* locus is present near XNpb257 (Maeda et al. 2004). **d** Two linked QTLs are located in two adjacent regions, RM287–RM209 and RM209–RM21 (Wu et al. 2009). **e** $qSTV11^{TQ}$ is located in the region RM209–RM229

In this study, we delimited the $qSTV11^{TQ}$ gene to a 55.7-kb region flanked by markers CAPs3 and CAPs2 (Fig. 4). Nine predicted genes encoding putative resistance proteins, designated by Ra, Rb, Rc, Rd, Re, Rf, Rg, Rh, and Ri, were identified after sequencing clone AC136491 in this region from the genomic library of the resistant rice Teqing (Fig. 4). In order to determine as to which gene is $qSTV11^{TQ}$, we are now performing complementation tests on the candidates.

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