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REVIEW

Genetic Studies of Two QTLs for Rice Stripe Resistance Identified in Japanese Upland Rice Line, Kanto 72

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Abstract

Quantitative trait locus (QTL) analysis was performed to identify the chromosomal region controlling rice stripe virus (RSV) resistance in Japanese upland rice, Kanto 72. As a result, two QTLs were detected on chromosomes 2 and 11. Using near-isogenic lines possessing a single QTL (QTL-NILs) on chromosomes 2 and 11, the effects of two QTLs were evaluated. The target QTL regions were introduced in the genetic background of Koshihikari with marker-assisted selection. A combined QTL-NIL, possessing two QTLs, was developed from the cross between two QTL-NILs. Investigation of RSV resistance using three QTL-NILs revealed that the effects of the two QTLs clearly differed in the reaction to RSV. The QTL on chromosome 11 provided a major effect on reducing the infection rate of RSV. The QTL on chromosome 2 did not affect the infection rate, but made symptoms of diseased plants milder. The combined QTL-NIL showed high and stable resistance to RSV equivalent to upland rice, Kanto 72. From these results, RSV resistance in upland rice, Kanto 72, was controlled by the complementary effect of two QTLs. Major agricultural traits of the three QTL-NILs were not significantly different to those of Koshihikari, therefore, these QTL-NILs were thought to be useful in RSV-resistant rice breeding.

Discipline: Plant breeding
Additional key words: near-isogenic lines, quantitative trait loci, rice breeding

Introduction

The rice stripe virus (RSV) is transmitted by the small brown planthopper (SBPH), *Laodelphax striatellus* Fallen. This disease is one of the most serious virus diseases affecting rice (*Oryza sativa* L.) production in East Asia, especially in China, Korea and Japan. In Japan, this disease caused serious loss of rice production in the 1960s, and the estimated area of paddy fields damaged was over 500,000 hectares. Recently, this disease is getting serious in China and an RSV epidemic affected a large area in Jiangsu Province of China[13].

In the early 1960's, screening of RSV-resistant rice cultivars was initiated and many *indica* rice cultivars and Japanese upland rice cultivars showed high resistance[16]. An incompletely dominant resistance gene, *Stvb-i*, and two dominant complementary resistance genes, *Stva* and *Stvb*, have been identified by genetic analysis in *indica* cultivars and Japanese upland rice cultivars, respectively[17,18]. RSV-resistant rice breeding was started using the *Stvb-i* gene in the Pakistan *indica* cultivar, Modan. This gene was introduced by backcross breeding into the *japonica* paddy rice cultivar, Norin 8, and two novel resistant lines, St. No. 1 and Chugoku 31, were selected[15]. After that, many resistant cultivars were subsequently bred from St. No. 1 and they have been cultivated widely in Japan. Almost all the RSV-resistant paddy rice cultivars cultivated in Japan were introduced with the *Stvb-i* gene from the same donor cultivar, Modan. Cultivars with a single resistance gene may lose their resistance once the strain of virus changes virulence, for example, the grassy stunt virus resistance gene introduced from wild rice *O. nivara* Sharma et Shastry was ineffective against a new viral strain[2,6]. For that

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reason, it is necessary to exploit new genetic resources for resistance to RSV in rice breeding programs.

The resistance genes, *Stva* and *Stvb*, have been identified in Japanese upland rice cultivars[16]. These resistance genes were also introduced into three *japonica* paddy resistant lines, Chugoku 40, Chugoku 41 and Chugoku 42[19]. These lines showed high and stable resistance, similar to that of *Stvb-i*. Despite their stable resistance, undesirable inferior characteristics of grains and eating qualities were also introduced from the donor parent, upland rice Kanto 72 (URK 72), which was a *japonica* upland rice cultivar.

DNA marker technologies have facilitated the development of new breeding techniques such as marker-assisted selection (MAS). MAS of the *Stvb-i* gene has been performed in breeding programs for RSV resistance[4] and molecular cloning of this gene has been progressing by a map-based strategy[5]. This breeding method has also become a powerful tool to remove inferior characteristics that are linked to target genes in backcross breeding programs. Using this technique, we tried to introduce RSV resistance of URK 72 in rice breeding program.

Materials and methods

1. Evaluation of resistance to RSV

(1) The net-house test method

The net-house method[12] was employed for QTL analysis. Twenty seeds for each cultivar were sown in a row in seedling boxes and grown to the 2.5-leaf stage. Inoculation was carried out in a net house into which several thousand viruliferous small brown planthoppers (SBPH) were released. After inoculation for three days, seedlings were cultivated in a green house for about one month until symptoms were observed. Resistance to RSV of each cultivar was judged based on the infection rate (IR). The IR was evaluated twice and the average was used for QTL analysis.

(2) The seedling test method

Thirty seeds for each cultivar were sown in a Petri dish filled with soil and grown to the 1.5-leaf stage. The Petri dish was covered with a glass cylinder fixed in place using vinyl tape. Then the top of the cylinder was covered with tetron gossamer. Two hundred nymphs of the viruliferous SBPH were released into each cylinder for two days. The inoculated seedlings were transplanted to plastic nursery boxes (25 × 33 × 11 cm) and grown to the 7- to 8-leaf stage. Diseased plants were classified into 6 classes (A, B, Bt, Cr, C, and D) at 30 days after inoculation based on the symptoms. A disease rating index was calculated by giving each of the 6 classes a different weighted value according to the severity of symptoms as follows[16]:

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

This disease rating index was influenced by the condition of viruliferous SBPH, therefore, the susceptible check cultivar, To-to, was used to test each population. Next, the ratio of the disease rating index (RDRI) for each line tested to that for To-to was calculated.

2. QTL analysis

A susceptible *japonica* paddy rice cultivar, Nipponbare, and a resistant Japanese upland rice line, Kanto 72 (URK 72), were used. One hundred and twenty F_2 plants were produced from a cross between Nipponbare and URK 72 for QTL analysis. The F_3 seeds of each F_2 plant were used for the investigation of RSV resistance. Total DNA was extracted from bulked young seedling leaves of 20 F_3 plants. A total of 328 RFLP markers, selected from a high-density linkage map constructed by the Rice Genome Research Program of Japan[5], and 221 SSR markers[14] were used for the analysis. Linkage mapping was performed using the software MAPMAKER/EXT 3.0[7] based on F_2 segregation data. To identify putative loci associated with RSV resistance, QTL analyses were done using MAPMAKER/QTL 1.1[8].

3. Selection of near-isogenic lines

Two near-isogenic lines with a single introduced target QTL (QTL-NILs) detected on chromosomes 2 and 11 were selected from the backcrossed progenies of the BC_5F_2 population derived from the cross between the susceptible *japonica* paddy rice, Koshihikari, and the resistant paddy line, Chugoku 40. Chugoku 40 was introduced RSV resistance from URK 72. For MAS of QTL-NILs, SSR markers located on chromosomes 2 and 11 were used to determine the genotype of the target QTLs. Graphical genotypes of three selected QTL-NILs were investigated using 528 SSR markers distributed on 12 chromosomes[11,13]. Two selected QTL-NILs were denoted as NIL-STV2 and NIL-STV11, respectively. A combined QTL-NIL (NIL-STV2/STV11), which possessed two QTLs, was also selected from the F_2 population derived from the cross between NIL-STV2 and NIL-STV11. Using the seedling test method, RSV resistances of three QTL-NILs were investigated.

4. Investigation of agronomic characters of three QTL-NILs

Major agronomic characters of three QTL-NILs were investigated. Three QTL-NILs and Koshihikari were transplanted to a paddy field in 2005. Heading date,

maturing date and 1,000-grain weight were investigated in three replicated plots, and culm length and panicle length were measured in ten plants from each plot.

Results

1. QTL analysis for RSV resistance

Out of 328 RFLP and 221 SSR markers distributed across the 12 chromosomes surveyed, 76 RFLP and 38 SSR markers showed polymorphisms between Nipponbare and URK 72, respectively. Using these 114 markers, a linkage map of 17 linkage groups covering a genetic distance of 715.3 cM was constructed with MAPMAKER/EXP. Interval mapping was performed with MAPMAKER/QTL to identify QTLs associated with RSV resistance based on the IR of 120 F_3 lines evaluated using the net-house test. From the QTL analysis, one QTL was found on chromosome 11 (Fig. 1). The QTL detected in the marker interval of G257 and R728 on the long arm of chromosome 11 (LOD = 19.1) explained 61.4% of total phenotypic variation in F_3 lines and had an additive effect of 25.7 to IR. On the other hand, two QTLs on chromosomes 2 and 11 were detected based on the RDRI of the seedling test method (Fig. 1). On chromosome 11, the QTL (LOD = 11.3) was found in the same interval of QTL detected using the IR from the net-house test. This QTL explained about 35.7% of the total phenotypic variation in F_3 lines and had an additive effect of 21.0 RDRI. The QTL on the long arm of chromosome 2 (LOD = 6.6) was detected near RFLP marker C601 and explained about 22.5% of variation (additive effect was 16.2 RDRI) [9].

2. Characterization of two QTLs controlling RSV resistance

Three QTL-NILs (NIL-STV2, NIL-STV11 and NIL-STV2/STV11) were selected using SSR markers near the two QTL regions on chromosomes 2 and 11. Out of 528 SSR markers distributed on 12 chromosomes surveyed, 99 markers showed polymorphisms between Koshihikari and Chugoku 40. Using these markers, the

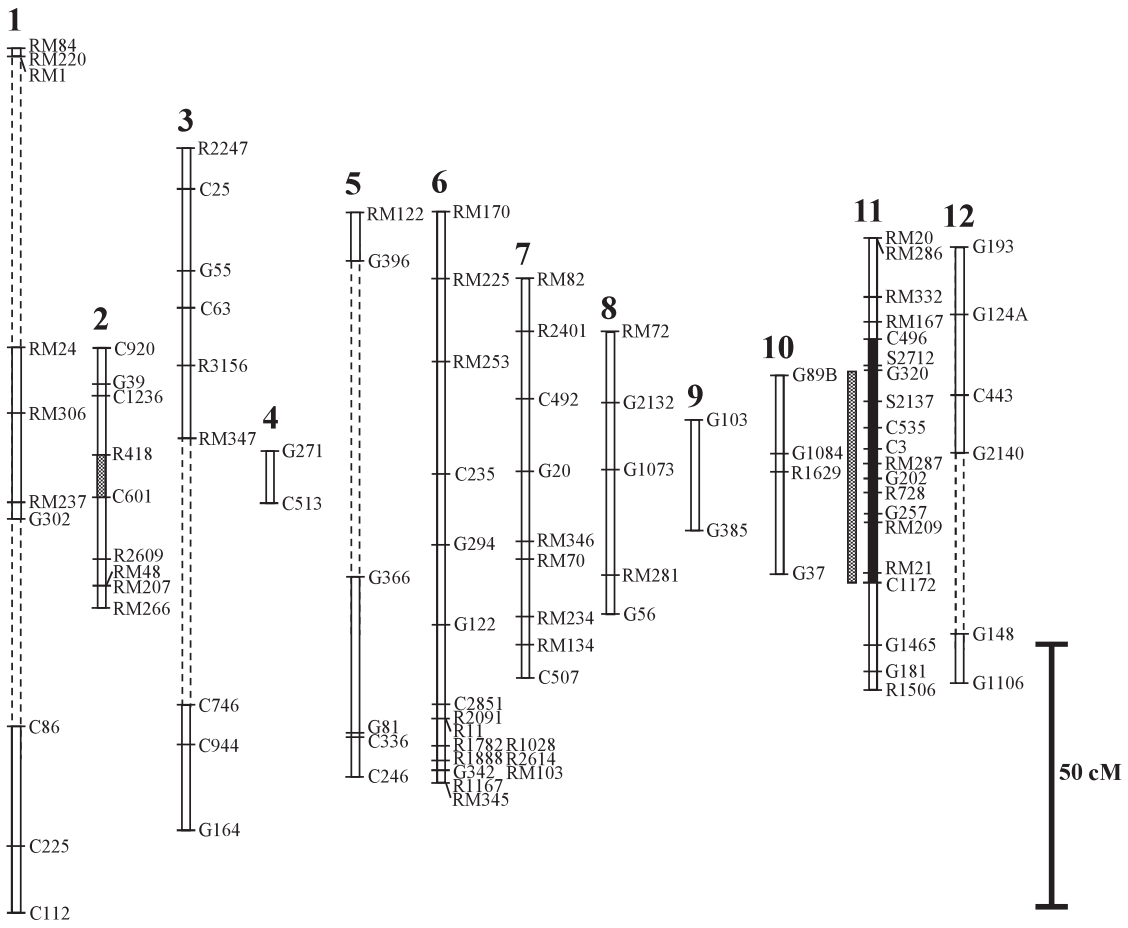

Fig. 1. Linkage map and positions of QTLs for rice stripe virus resistance in rice

Gray and black bars represent the putative region of QTL with LOD scores higher than 3.0.

: QTL detected using the IR from the net-house test

: QTLs detected using the RDRI from the seedling test

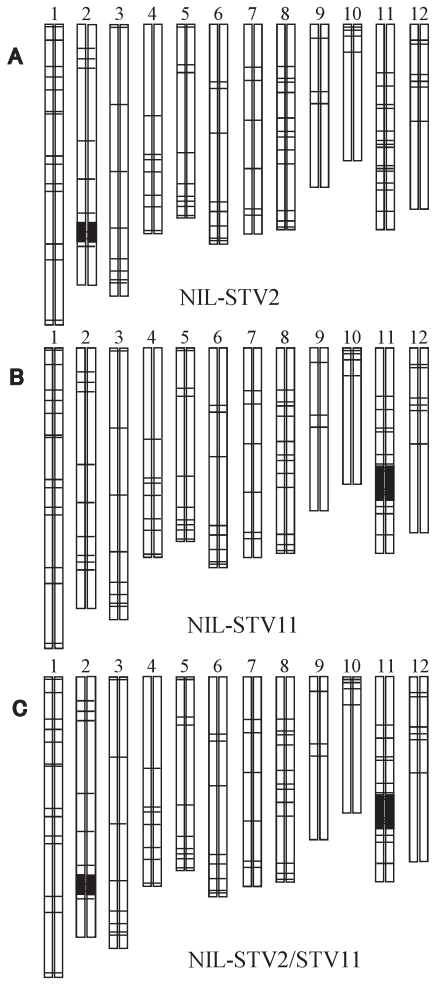

Fig. 2. Graphical genotypes of the three QTL-NILs
(A) NIL-STV2, (B) NIL-STV11 and (C) NIL-STV2/STV11.
The 12 pairs of bars indicate the chromosomes.
The horizontal lines show the positions of the SSR markers used in marker-assisted selection (MAS).
Hatched bars and solid bars denote the chromosome regions derived from Koshihikari and Chugoku 40, respectively.

graphical genotypes of three QTL-NILs were investigated (Fig. 2). Introgressed segments of the two QTL regions on chromosomes 2 and 11 are shown in Figure 3.

The respective RSV resistances of three QTL-NILs and check cultivars in the seedling test method are shown in Table 1. The averaged RDRI of the susceptible parent, Koshihikari, was 71.2 and that of the resistant parent, Chugoku 40, was 1.2. Three QTL-NILs showed lower RDRI than that of Koshihikari. The averaged RDRI of NIL-STV11 was 14.4, which was classified as "resistant". The averaged RDRI of NIL-STV2 was 39.8, which was classified as "moderately resistant". NIL-STV2/STV11 showed 1.3, which was the same as those of resistant lines Chugoku 40 and URK 72.

The infection rate of NIL-STV2 was not significantly different from that of Koshihikari. However, NIL-STV11 and NIL-STV2/STV11 showed low infection rates of 14.4% and 8.9%, respectively. These results indicated that the QTL on chromosome 11 exerted a major effect on decreasing the infection rate. In this seedling test method, diseased plants were classified into six classes from A (dead) to D (slight symptoms). Most diseased plants of the susceptible cultivar, Koshihikari, were classified as seriously damaged types from A to Bt, whereas diseased plants of resistant line, Chugoku 40, were classified into slight symptom types, such as C or D. NIL-STV11 showed few diseased plants. However, those diseased plants were classified into seriously damaged types of A to Bt. In contrast, diseased plants of NIL-STV2 were distributed from A to D. Especially, seriously damaged plants in classes A and B were few and those in medium damaged classes of Bt and Cr increased in comparison to those of Koshihikari. NIL-STV2/STV11 showed high resistance to RSV in the infection rate and diseased plant types[10].

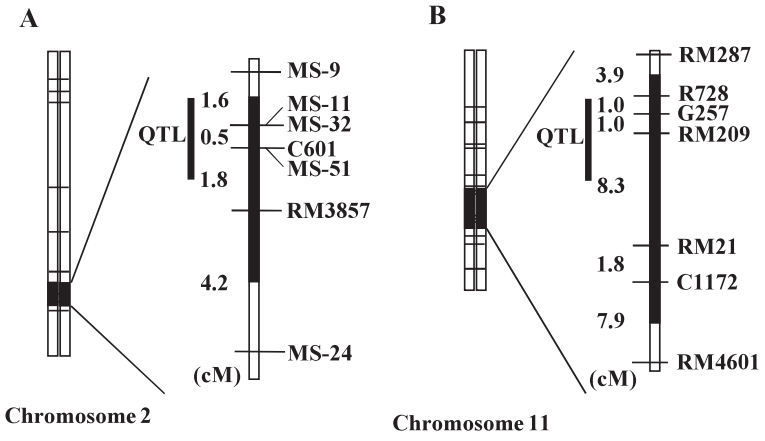

Fig. 3. Introgressed chromosomal segments of the two QTL regions
(A) QTL region on chromosome 2, and (B) QTL region on chromosome 11. Hatched bars and solid bars denote the chromosome regions derived from Koshihikari and Chugoku 40, respectively.

Table 1. RSV resistance of three QTL-NILs and comparative cultivars, Koshihikari and Chugoku 40 line

Lines and cultivars	Number of seedlings classified by symptom type[3)]							Infection rate (%)[1)]	RDRI[1, 2)]
	A	B	Bt	Cr	C	D	No symptoms		
Koshihikari	10.7	2.7	1.7	0.0	0.0	0.3	14.7	51.1[ab]	71.2[a]
NIL-STV2	2.3	2.0	3.7	3.3	1.3	0.0	17.3	42.2[b]	39.8[b]
NIL-STV11	2.7	0.7	1.0	0.0	0.0	0.0	25.7	14.4[c]	19.6[bc]
NIL-STV2/STV11	0.0	0.0	0.0	0.0	0.8	1.8	27.3	8.9[c]	1.3[c]
Chugoku 40	0.0	0.0	0.0	0.0	0.7	2.0	27.3	8.9[c]	1.2[c]
URK 72	0.0	0.0	0.0	0.0	1.0	2.2	26.8	10.6[c]	1.6[c]
To-to	14.3	5.3	1.3	0.0	0.0	0.0	9.0	70.0[a]	(100)

[1)]: Means with the same letter are not significantly different at the 5% level (Tukey test).

[2)]: RDRI: Ratio of the disease rating index was calculated compared to that for To-to.

The desease rating index was calculated as follows:

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

[3)]: Symptom types were classified into six groups (A: dead - D: slight symptoms).

3. Agronomic characters of three QTL-NILs

Major agricultural traits of three QTL-NILs and the recurrent parent, Koshihikari, are shown in Table 2. The five agronomic characters investigated, heading and maturing dates, culm length, panicle length, and 1,000-grain weight, of three QTL-NILs were not significantly different from Koshihikari at the 5% level.

Discussion

1. QTL analysis for rice stripe resistance

QTL analysis was performed using RFLP and SSR markers to map QTLs related with RSV resistance in URK 72, and two QTLs were detected on chromosomes 2 and 11. Washio et al.[17] reported that the RSV resistance of upland rice cultivars depends on a pair of complementary dominant genes, *Stva* and *Stvb*. The *Stvb* gene was thought to be allelic with the other RSV resistance gene *Stvb-i*, which was mapped on chromosome 11 by Hayano-Saito et al.[4]. Ando et al.[1] reported that some RFLP markers on chromosome 11 associated significantly with the RSV resistance of Japanese upland rice. Based on their reports, it is likely that the QTL detected on chromosome 11 is thought to correspond to the *Stvb* gene. However, it seemed to provide a larger RSV resistance effect than that described by Washio et al.[16]. QTL analysis based on the IR obtained in the net-house test, the QTL detected on chromosome 11 explained 61.4 % of total phenotypic variation. This result suggested that the URK 72 allele at the QTL on chromosome 11 had a major effect on the infection of RSV.

On the other hand, the QTL on chromosome 2 was detected using the RDRI obtained in the seedling test. In the seedling test, diseased plants were classified into 6 symptom types, A (dead) to D (slight mosaic symptoms). Susceptible plants usually show symptom type A, B or Bt, while resistant plants show Cr, C or D. The disease

Table 2. Agronomic characters of the three QTL-NILs and Koshihikari

Lines and cultivar	Heading date	Maturing date	Culm length (cm)	Panicle length (cm)	1,000- grain weight (g)
Koshihikari	8.13	9.23	91	19.0	22.2
NIL-STV2	8.14	9.24	88	19.4	22.0
NIL-STV11	8.13	9.24	89	19.7	21.7
NIL-STV2/STV11	8.14	9.23	93	19.8	22.2

These characters were investigated in 2005. The sowing date was May 27.

The five characters of the three QTL-NILs were not significantly different from those of Koshihikari at the 5% level (Tukey test).

rating index was calculated by giving each symptom type a weighted value according to the severity of symptoms. This result indicated that QTL on chromosome 2 may affect the type of symptoms after infection by RSV. In order to clarify the effects of the two QTLs on chromosomes 2 and 11, we attempted to evaluate their effects using near-isogenic lines of the two QTLs.

2. Characterization of two QTLs controlling rice stripe resistance

Three QTL-NILs showed different reactions against RSV in the investigation using the seedling test method. Among them, NIL-STV11 showed a low infection rate equivalent to that of URK 72, but its diseased plants were classified into seriously damaged classes of A or B (Table 2). The infection rate of NIL-STV2 was not significantly lower than that of Koshihikari, but the diseased plant types of NIL-STV2 differed markedly from those of Koshihikari. As compared with the symptom types of Koshihikari, the number of diseased plants classified in A and B was remarkably reduced, and that in Bt and Cr increased. These findings indicated that the QTL detected on chromosome 11 has a major effect on controlling the infection rate and that the QTL on chromosome 2 has an effect on suppressing symptoms after RSV infection. NIL-STV2/STV11 showed stable resistance to RSV in the infection rate and the diseased plant types, therefore, the two QTLs have a complementary effect against RSV.

Washio et al.[17,18] reported that the resistance genes, *Stva* and *Stvb*, were complementary dominant genes and each gene has no effect against RSV independently. The complementary effect of the two QTLs corresponds to the *Stva* and *Stvb* genes, but each QTL provided effects on the suppression of RSV infection and on the suppression of symptoms after infection of RSV, respectively. The *Stvb* gene was allelic to the *Stvb-i* gene, which was mapped on chromosome 11[2]. The QTL located on chromosome 11 might correspond to the *Stvb* gene, however, this QTL showed a large effect against RSV. The *Stva* and *Stvb* genes were identified using the populations derived from the cross between a susceptible cultivar, Kibiyoshi, and upland rice cultivars[17]. In this study, the effects of the three QTL-NILs were confirmed in the genetic background of Koshihikari. In order to clarify the relationship between the QTLs and two resistance genes, *Stva* and *Stvb*, we are now conducting QTL analyses for RSV resistance using populations derived from the cross between Kibiyoshi and URK 72.

The molecular mechanisms of the two QTLs remain unknown. To clarify the molecular mechanisms of RSV resistance, it is necessary to isolate resistance genes. The obtained information related to the effects for RSV resistance will be useful to identify resistance genes from candidate genes in the map-based cloning strategy. Moreover, MAS of the two QTL regions were possible using the DNA markers shown in Figure 3 and these DNA markers were useful to the pyramiding of the two QTLs.

3. Agronomic characters of three QTL-NILs

Major agricultural traits of the three QTL-NILs were not significantly different from those of Koshihikari (Table 2). We also tried to evaluate the grain and eating qualities of the three QTL-NILs, and their qualities are equivalent to those of Koshihikari (data not shown). Out of them, NIL- STV11 and NIL-STV2/STV11 showed high resistance against RSV. From these results, these lines are thought to be useful in rice breeding for RSV resistance. NIL- STV11 and NIL-STV2/STV11 were designated as "Chugoku IL1" and "Chugoku IL2", respectively. We are now investigating their local adaptability in Japan.

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