Characterization of Two QTLs Controlling Resistance to Rice Stripe Virus Detected in a Japanese Upland Rice Line, Kanto 72

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The effects of two quantitative trait loci (QTLs) controlling the resistance to rice stripe virus (RSV) identified in a Japanese upland rice line, Kanto 72, (URK 72) were evaluated using near-isogenic lines (NILs). Two NILs carrying a single QTL (QTL-NILs) on chromosomes 2 and 11, respectively, were developed by marker-assisted selection (MAS). The target QTL regions were introduced from the donor parent, Chugoku 40, which was bred from URK 72, into the genetic background of Koshihikari. Another line, which was a combined QTL-NIL, was developed from the cross between the two QTL-NILs to analyze the interaction of the two QTLs. Investigation of RSV resistance using the three NILs revealed that the effects of the two QTLs clearly differed in the reaction to RSV. The QTL on chromosome 11 exerted a major effect on reducing the infection rate of RSV. Although the QTL on chromosome 2 did not affect the infection rate, the symptoms of the diseased plants were milder. The combined QTL-NIL displayed a high level of resistance to RSV, while the infection rate and the symptom types of the diseased plants were similar to those of URK 72 or Chugoku 40. Since the major agronomic characters of the three QTL-NILs were the same as those of Koshihikari, these QTL-NILs were considered to be useful gene sources for RSV resistance rice breeding.

Key Words: rice (Oryza sativa L.), rice stripe virus (RSV), resistance genes, quantitative trait loci, near-isogenic lines.

Introduction

Rice stripe disease is one of the most important viral diseases affecting rice (Oryza sativa L.) production in the temperate regions of East Asia, especially in China, Korea and Japan. The small brown planthopper, Laodelphax striatellus Fallen, transmits the rice stripe virus (RSV), the causal agent of the disease. This disease spread in Japan in the 1960s, and it was estimated that over 500,000 hectares of paddy fields had been damaged. Recently, Sogawa (2005) has reported the occurrence of an RSV epidemic in a large area of Jiansu province in China.

Screening of RSV-resistant rice varieties was initiated in the early 1960s, and some Indica type rice varieties and Japanese upland rice varieties displayed a high level of resistance (Washio et al. 1967). They reported that an incompletely dominant gene, Stvb-i, controlled RSV resistance in Indica type varieties. Resistance in Japanese upland rice varieties was also reported to be controlled by two complementary genes, Stva and Stvb (Washio et al. 1968a, 1968b). It had been concluded that the Stvb gene was allelic to the Stvb-i gene and Stva was reported to be located on chromosome 6, based on a linkage analysis using the glutinous endosperm gene (wx) and photoperiod sensitivity-1 gene (Se1) (Washio et al. 1968c).

Breeding program of RSV-resistant rice was started using the Stvb-i gene in the Pakistan variety, Modan. The Stvb-i gene was introduced into the Japonica type paddy rice variety, Norin 8, through several backcrossings. At last, two resistant lines were selected from the BC3 population and designated as St No.1 and Chugoku 31, respectively (Toriyama et al. 1966). A large number of resistant varieties, which were subsequently bred from St No.1 and Chugoku 31, have been widely cultivated in Japan and have shown a stable resistance to RSV for the last 40 years. Consequently, almost all the resistant paddy rice varieties cultivated in Japan were expected to harbor the Stvb-i gene. However, once a virus strain changes and can overcome Stvb-i, all the rice varieties in Japan would become susceptible to the viruses. For example, the varieties harboring the resistance gene to grassy stunt virus introduced from wild rice O. nivara became susceptible to a new viral strain (Ghosh et al. 1979, Hibino et al. 1985). As a result, other resistance genes should be introduced to the breeding programs of RSV-resistant rice. The resistance genes Stva and Stvb, which have been identified in Japanese upland rice varieties, were also introduced into Japonica type paddy rice varieties and three resistant lines were bred, Chugoku 40, Chugoku 41 and Chugoku 42 (Washio et al. 1968c). These lines exhibited a strong and stable level of resistance, similar to that of the...
varieties harboring Stvb-i. However, undesirable characteristics of grains and eating quality were also introduced from the donor parent, upland rice Kanto 72 (URK 72), which is a Japonica type upland rice line.

Recent advances in DNA marker technologies have contributed to the development of new breeding techniques such as marker-assisted selection (MAS). This breeding method has become a powerful tool to remove unfavorable characters that are linked to target genes in backcross breeding programs. Near-isogenic lines (NILs) developed using MAS are useful for evaluating the effects of target genes or QTLs. For example, the effects of QTLs that control heading dates identified in the Indica type rice variety, Kasalath, were investigated using NILs (Lin et al. 2000, 2002 and 2003). In these reports, interactions in pairs of QTLs for heading date were also analyzed using two or three combined QTL-NILs.

Maeda et al. (2004) who performed a QTL analysis for RSV resistance using F2 lines derived from a cross between Nipponbare and URK 72, detected two QTLs on chromosomes 2 and 11. The LOD peak of the QTL on chromosome 11 was detected near a RFLP marker G257, which was closely linked to the Stvb-i gene (Hayano-Saito et al. 1998). This QTL was assumed to correspond to the Stvb gene. However, the relationship between the QTL detected on chromosome 2 and the Stva gene was uncertain, because the Stva gene was reported to be located on chromosome 6 (Washio et al. 1968c). No QTL for RSV resistance had been detected on chromosome 6 in the previous report (Maeda et al. 2004).

The Stvb-i gene from the same donor, Modan, had been introduced into almost all the RSV-resistant paddy rice varieties cultivated in Japan. Varieties with a single resistance gene may lose their resistance once the virulence of a virus strain changes. It is thus necessary to use new gene sources for resistance to RSV in rice breeding programs. Though the relationship between the QTL detected on chromosome 2 (Maeda et al. 2004) and the Stva gene (Washio et al. 1968c) remains uncertain, two QTLs identified in URK 72 were thought to be useful for the breeding of RSV-resistant rice. In the present study, attempts were made to confirm the effects and interaction of the two QTLs using NILs, in which segments of the regions with each QTL had been introgressed into the genetic background of a susceptible variety, Koshihikari.

Materials and Methods

Plant materials

A susceptible Japonica type paddy rice variety, Koshihikari, a resistant Japonica type upland rice line, URK 72, and a resistant paddy line, Chugoku 40, were used in the present study. Chugoku 40 was bred from URK 72 (Fig. 1) (Washio et al. 1968c). Two QTL-NILs, which had been introduced segments of a single target QTL regions detected on chromosomes 2 and 11 respectively, were selected from the backcrossed progenies of the BC1F2 population derived from the cross between Koshihikari and Chugoku 40. These two QTL-NILs were designated as NIL-STV2 and NIL-STV11, respectively. Another combined NIL (NIL-STV2/STV11), which harbored the two QTLs, was also selected from the F2 generation derived from the cross between NIL-STV2 and NIL-STV11. In the three QTL-NILs, RSV resistance was investigated.

NIL-STV2 carried an undesirable chromosomal segment from Chugoku 40. In order to remove this chromosomal segment, new QTL-NILs were selected from the backcrossed progenies of the BC1F2 population derived from the cross between Koshihikari and Chugoku 40. These two QTL-NILs were designated as NIL-STV2 (BC5) and NIL-STV11 (BC5), and their resistance to RSV was investigated.

Investigation of agronomic characters of the three QTL-NILs

Major agronomic characters of the three QTL-NILs were investigated. The three QTL-NILs and Koshihikari were transplanted to the 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 using ten plants in each plot.

Evaluation of RSV resistance based on the seedling test method

The seedling test method (Washio et al. 1967) was employed in the present study. Thirty seeds of each QTL-NIL or check varieties/lines were sown in a Petri dish filled with soil. At the 1.5-leaf stage, the Petri dish was covered with a glass cylinder, and two hundred nymphs of the viruliferous small brown planthopper 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 six groups (A, B, Bt, Cr, C and D) at 30 days after inoculation based on the symptom types. The disease rating index was calculated according to the severity of symptoms as follows (Washio et al. 1967):

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

The test was repeated six times. This disease rating index was influenced by the condition of the viruliferous small brown planthoppers. Therefore, the susceptible check variety To-to was used for testing. Ratio of the disease rating index

![Pedigree of a resistant paddy line, Chugoku 40. □ denotes RSV-resistant lines.](image-url)
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The RSV resistance was estimated based on the RDRI values obtained by the seedling test method: Plants with RDRI values of 0 to 30.0 were classified into “resistant”, those with RDRI values over 60 were classified into “susceptible”, and those with RDRI values of 30.1 to 59.9 were classified into “moderately resistant”.

The infection rate was calculated based on the total number of diseased plants (A to D) and seedlings examined.

RFLP and SSR marker analyses

Whole genomic DNA was extracted from young seedling leaves according to the modified CTAB method (Murray and Thompson 1980). Extracted DNA was digested with ten restriction enzymes (ApaI, BamHI, BglII, DraI, EcoRI, EcoRV, HindIII, KpnI, PstI and XbaI), electrophoresed using 0.8% agarose gel, and blotted onto positively charged nylon membrane by the capillary transfer method. Four RFLP markers on chromosomes 2 and 11 were provided by the Rice Genome Research Program (RGP) of Japan. For Southern hybridization and signal detection, ECL direct nucleic acid labeling and detection systems were used according to the manufacturer’s instructions (Amersham Pharmacia Biotech, Buckinghamshire, UK).

Polymerase chain reaction (PCR) was performed with a 20 µl reaction mixture containing 10 ng of DNA, 1 pmol of each primer, and 0.4 unit of Taq polymerase. Thermal cycling was carried out using a GeneAmp PCR System 9700 (Perkin Elmer, Boston, USA) programmed for 9 min at 95°C, 35 cycles of 1 min at 95°C, 1 min at 55°C and 1 min at 72°C, and 5 min at 72°C for a final extension. The PCR products were separated on 4% polyacrylamide denaturing gels and the banding patterns were visualized using a silver staining method, as described by Panaud et al. (1996).

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 the three selected QTL-NILs were analyzed using 528 SSR markers distributed on 12 chromosomes (Temnykh et al. 2000, McCouch et al. 2002).

Results

Development of QTL-NILs by marker-assisted selection

Out of 528 SSR markers distributed on the 12 chromosomes surveyed, 99 markers showed polymorphisms between Koshihikari and Chugoku 40. These 99 SSR markers were distributed on all the genomic chromosomes, except for the short arm of chromosome 4 and on the long arm of chromosomes 9, 10 and 12. Using these markers, the three QTL-NILs were selected and their graphical genotypes were investigated (Fig. 2A–C). Genotypes of QTL-NILs were homozygous for the Chugoku 40 alleles in the QTL regions on chromosomes 2 and 11 and homozygous for the Koshihikari alleles in the other chromosomal regions. Introgressed segments of the two QTL regions are shown in Fig. 3A, B.

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.

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.
the three QTL-NILs, introgression of the undesirable chromosomal segment of Chugoku 40 was observed on chromosome 8 in NIL-STV2 (Fig. 2A).

**Agronomic characters of the three QTL-NILs**

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

**RSV resistance of QTL-NILs**

The respective RSV resistance of the three QTL-NILs and check varieties/lines based on the seedling test method is depicted in Table 2. 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 11.7% and 6.1%, respectively. These results indicated that the QTL on chromosome 11 exerted a major effect on the decrease of the infection rate. Based on the seedling test method, diseased plants were classified into six groups from A (dead) to D (slight symptom). The symptoms of most diseased plants of the susceptible variety, Koshihikari, were classified into serious damage types, whereas the symptoms of the diseased plants of the resistant line, Chugoku 40, were classified into mild types, such as C or D. Although the diseased plants of the NIL-STV11 were few, they were classified into serious damage types (A to Bt). In contrast, the symptoms of the NIL-STV2 diseased plants ranged from A to D. Especially, the numbers of seriously damaged plants classes in the A and B groups were few, while those in the medium damage groups (Bt and Cr) increased compared to those of Koshihikari. NIL-STV2/STV11 had displayed a high level of resistance to RSV in terms of infection rate and diseased plant types.

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**Discussion**

We attempted to evaluate the effects of the two QTLs for RSV resistance identified in URK 72, using two QTL-NILs and one combined QTL-NIL of Koshihikari. Among
them, although NIL-STV11 showed a low infection rate, the diseased plants were classified into the serious damage types A and B (Table 2). On the other hand, 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. The numbers of diseased plants with the serious damage types A and B were remarkably reduced and those of the plants with the moderate damage types Bt and Cr increased compared to those in Koshihikari. These results indicated that the QTL detected on chromosome 11 exerted a major effect on the control of the infection rate and that the QTL on chromosome 2 enabled to alleviate the symptoms after RSV infection. The combined QTL-NIL, NIL-STV2/STV11, showed a stable level of resistance to RSV in terms of infection rate and diseased plant types, comparable to that of the donor parent, Chugoku 40, and URK 72. Therefore, the two QTLs exerted a complementary effect on RSV.

The effects on the RSV resistance of NIL-STV2 (BC5) and NIL-STV11 (BC5), which were selected from the BC$_1$F$_2$ population, were the same as those of NIL-STV2 and NIL-STV11, respectively (Table 3). Although introgression of the undesirable chromosomal segment on chromosome 8 occurred in NIL-STV2, this chromosomal region was not related to the RSV resistance. Evaluation of RSV resistance was affected by the condition of the viruliferous small brown planthoppers, and the symptom types of the diseased plants dependent on the growth conditions after inoculation. Therefore, the inoculation conditions might have influenced the effects of the two QTLs. In the present study, the effects of the QTLs on chromosomes 2 and 11 were evaluated based on two inoculation tests (Table 2 and Table 3). The effects of the QTLs on chromosomes 2 and 11 were stable under the different inoculation conditions.

The combined QTL-NIL, NIL-STV2/STV11, showed a high level of resistance similar to those of the donor parent, Chugoku 40, and URK 72 (Table 2). This result suggested that the RSV resistance identified in URK 72 was associated with the complementary effect of the two QTLs located on chromosomes 2 and 11. Washio et al. (1968a, 1968c) reported that the resistance genes, Stva and Stvb, were complementary dominant genes that did not affect RSV independently. Although the complementary effect of the two QTLs was corresponding to the Stva and Stvb genes, each QTL exerted separate effects on the suppression of RSV infection and on the alleviation of the symptoms after infection with RSV. The Stvb gene was allelic to the Stvb-i gene, which was mapped on chromosome 11 (Hayano-Saito et al. 1998). The QTL located on chromosome 11 might correspond to the Stvb gene, however, this QTL showed a large effect on RSV. The averaged RDRI value of the NIL-STV11 plants was 15.0, which were classified into “resistant”. The Stva and Stvb genes were identified using the populations derived from the cross between a susceptible variety, Kibiyoshi, and upland rice varieties (Washio et al. 1968a). In the present study, the effects of the three QTL-NILs were confirmed in the genetic background of Koshihikari. In order to elucidate the relationship between the QTLs and the two resistance genes, Stva and Stvb, we are currently performing QTL analyses on RSV resistance using populations derived from the cross between Kibiyoshi and URK 72.

The present study was carried out to confirm the effects of the two QTLs identified in URK 72 in the previous report (Maeda et al. 2004). The two QTL-NILs with a single QTL showed a different response to RSV and the combined QTL-NIL exhibited a high and stable level of resistance. Major agronomic characters of the three QTL-NILs were not significantly different from those of Koshihikari (Table 1). If the grain and eating quality of these three QTL-NILs were to be similar to those of Koshihikari, these lines could become useful for rice breeding for RSV resistance. MAS of the two QTL regions could be performed using the DNA markers shown in Fig. 3A, B, and these DNA markers could be useful for the pyramidation of the two QTLs.

The molecular mechanisms of the two QTLs remain unknown. It will thus be necessary to isolate the resistance genes in order to elucidate the molecular mechanisms of RSV resistance. The obtained information related to the effects on RSV resistance will be useful for identifying resistance genes from candidate genes in a map-based cloning strategy.

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Literature Cited


