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Coding strategy of rice stripe virus and genetic analysis of rice stripe resistance

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Summary

Rice stripe virus (RSV) is a member of the tenuivirus group of plant viruses. The filamentous shape and biological properties of RSV are similar to those of the other viruses of this group, such as maize stripe virus (MSpV) and rice grassy stunt virus (RGSV). These three viruses have four (RSV and RGV) or five (MSpV) RNAs of various size as their genomes. When either RSV or MSpV is extracted with SDS-phenol and analyzed by gel electrophoresis, a mixture of various single-stranded (ss-) and double-stranded (ds-) RNAs is detected. Therefore, this suggested that both (+) and (-) RNAs are separately encapsidated in virus particles, although one polarity of each size RNA occurs in excess. It is unclear whether this virus group belongs to the positive- or negative-strand RNA viruses.

A rice stripe resistance gene from the *indica* rice (*Oryza sativa*) cv. 'Modan' was introgressed into several Japanese rice cultivars. The gene, *Stvb-i*, has provided stable resistance to rice stripe virus since it was first introgressed into *japonica* paddy rice cultivars about 30 years ago. However, the precise chromosomal location of this resistance gene has not been not determined.

In this study, the coding strategy of two major protein genes of rice stripe virus (RSV) was revealed. For positional cloning of the stripe resistance gene *Stvb-i* derived from 'Modan', genetical and physical analyses of the rice stripe disease resistance gene *Stvb-i* using molecular markers was performed. Furthermore, DNA marker-assisted selection (MAS) of rice stripe disease resistance using a *Stvb-i*-linked marker was examined.

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1. Coding strategy of rice stripe virus

In RSV-infected plants as in MSpV-infected maize, two major proteins, coat protein and major nonstructural protein (NS), are detected. However, the genes encoding these two proteins have not yet been identified. To characterize the RSV genome segments, these two proteins were purified and their partial amino acid sequences were determined. The purified protein was digested with proteinase trypsin or lysyl endopeptidase. The peptides fractionated by high-performance liquid chromatography (HPLC) were subjected to automated Edman degradation using a protein sequencer. Both sense oligonucleotides were synthesized on the basis of the amino acid sequence information of the two proteins and used as probes for Northern blot analyses of four ss-RNA species (segments 1-4) and four ds-RNA species of the RSV genome. The results of hybridization with ss-RNAs indicated that the coat protein and the major NS of RSV

were encoded on two different segments in opposite polarities. Briefly, segment 3 encoded the coat protein in the negative sense, while segment 4 encoded the major NS protein in the positive sense. Furthermore, the results of hybridization with both ss- and ds-RNA species suggested that the amounts of ss-RNA species are different. The amount of the species encoding two proteins was more abundant in virus particles than was the opposite sense species. Therefore, it is thought that the RSV genome consists of ssRNAs and one polarity of ssRNAs exists in excess. The coding strategy of RSV, i.e., that different viral ss-RNA species code for the coat protein and the major NS in opposite senses, as described above, is similar to the coding strategies of ambisense viruses (arenaviruses and phleboviruses). The expression of these two proteins may require different mechanisms as has been suggested for the ambisense genes of Punta Toro phlebovirus.

2. Genetic mapping of the *Stvb-i* gene introgressed from an *indica* cultivar 'Modan' to Japanese paddy rice

To identify the chromosomal segment introgressed from the *indica* rice 'Modan' into a resistant progeny, I performed RFLP analysis using 9 resistant and 6 susceptible cultivars ('Modan', 'St. No. 1', 'Mineyutaka', 'Shimahashirazu', 'Aichi 21', 'Musashikogane', 'Aichi 6', 'Aoisora', 'Hoshinohikari', 'Norin 8', 'Sachihikari', 'Wasekirakogane', 'Tamakei 56', 'Kanto 98' and 'Koshihikari'). The number of RFLP markers that hybridized with 'Modan'-specific bands decreased with each succeeding generation, and the introgressed segment derived from 'Modan' was only on chromosome 11 commonly in all tested resistant cultivars. Therefore, the segment on chromosome 11 was ascribable to 'Modan' and is associated with stripe resistance. The introgressed segment was located at 28 to 36 cM of genetic distance on chromosome 11.

A total of 120 F_2 individuals from a cross of 'Koshihikari' (susceptible) / 'Asanohikari' (resistant) were used for the linkage analysis between RFLP markers and stripe resistance. I performed a bioassay for rice stripe resistance in F_3 lines of the F_2 individuals using infective small brown planthoppers. F_3 lines segregated into 26:56:38 for susceptible homozygous (S), heterozygous (H) and resistant homozygous (R). This segregation fitted the expected 1(s):2(H):1(R) ratio (chi-square = 2.93, $0.10<p<0.30$). Thirteen polymorphic RFLP markers between the crossing parents were used to investigate the F_2 population. Based on the segregation for these markers and for stripe resistance in the F_2 population, the genetic distance and best-fit gene orders were determined using the computer program MAPL. The *Stvb-i* resistance gene was mapped into the introgressed segment on chromosome 11 and was located in a 1.8-cM interval between XNpb220 and XNpb257/XNpb254. *Stvb-i* was also linked to an RFLP marker, ST10, with 0.0 cM of genetic distance.

3. Fine physical mapping of the *Stvb-i* gene

For positional cloning, I constructed a physical map spanning 1.8-cM distance flanking markers, consisting of 18 bacterial artificial chromosome (BAC) clones, around the *Stvb-i* locus on rice chromosome 11. The 18 clones were isolated by screening of a BAC library derived from a *japonica* cultivar, 'Shimokita', with three *Stvb-i*-linked RFLP markers and *Dra*I-digested DNAs of a yeast artificial chromosome (YAC) clone. The results of Southern hybridization and restriction enzyme analysis indicated that these BAC clones were contiguous and covered about a 700-kb region containing *Stvb-i* allele. Utilizing end and internal fragments of the BAC insert

DNAs, 34 molecular markers were generated within a small chromosomal region including the *Stvb-i* locus. Genotyping analysis with these markers of a resistant cultivar and four nearby-recombinants selected from 120 F_2 individuals indicated that *Stvb-i* is contained within an approximately 286-kb region covered with two overlapping BAC clones. Using the molecular markers located in the 286-kb region, we analyzed 2090 recombinant inbred lines (RILs, F_7 generation) derived from a cross of 'Koganebare' (susceptible) / 'Tsukinohikari' (resistant). From the genotyping analysis of the RILs, 8 lines in which the recombination occurred within the 286-kb region were selected. The close relation between the graphical genotypes and stripe-resistance phenotypes of the 8 RILs indicated that the locus conferring stripe resistance existed within an approximately 120-kb region covered by one BAC clone, 220B5, on the physical map. The results described here provide the basis for isolation of the *Stvb-i* gene.

4. A SCAR marker, ST10, for marker-assisted selection of stripe resistant rice individuals

A stripe resistance gene, *Stvb-i*, was introduced from the *indica* cultivar Modan into *japonica* cultivars in Japan. A set of primers for PCR assay was designed on the basis of sequence information of the RFLP marker ST10, which is linked by 0.0 cM to the *Stvb-i* gene. This set of primers, which generate a SCAR (sequence-characterized amplified region) marker, was called ST10 primers. In PCR with the ST10 primers, a 727-bp DNA fragment was amplified specifically from all tested rice plants having the *Stvb-i* gene derived from Modan: cultivars, lines and F_1 individuals from a cross between 'Koganebare' (susceptible) and 'Tsukinohikari' (resistant). On the other hand, the fragment was not amplified in susceptible cultivars. In 120 F_2 individuals obtained from a cross between 'Koshihikari' (susceptible) and 'Asanohikari' (resistant), the resistant homozygous and heterozygous individuals showed a positive reaction in the PCR assay with the ST10 primers. The 727-bp fragment was identified as a part of the ST10 fragment (745 bp) by sequence analysis. In foreign cultivars, the ST10 primers amplified the 727-bp fragment in 3 out of 9 tested resistant cultivars that have been reported to harbor the same resistance gene, *Stvb-i*. The 3 cultivars were Modan, IR8 and Mudgo. The 727-bp fragment was not amplified in a foreign cultivar, 'Zenith', and Japanese upland cultivars harboring other resistance genes, *Stva* and *Stvb*. This SCAR marker will be useful for marker-assisted selection (MAS) of stripe resistant individuals having the *Stvb-i* gene derived from Modan. Therefore, stripe resistance can now be screened by using a vector insect-free bioassay.