Conserved terminal nucleotide sequences in the genome of rice black streaked dwarf virus

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The terminal regions of the dsRNA genome segments of rice black streaked dwarf virus (RBSDV) were sequenced. The individual dsRNAs, which were 32P-labelled at their 3′ termini by incubation with [32P]pCp and T4 RNA ligase, were separated by 5% PAGE, and the 10 dsRNA segments were sequenced by two-dimensional electrophoresis. The common 3′-terminal sequences ---GUC Y and ---AAAAACUU 3′ were found in the plus and minus strands, respectively. The strictly conserved terminal sequences (5′ AA-GUUUU....GUC 3′) of the genome segments of RBSDV differ from those of the phytoreoviruses and rice ragged stunt virus.

Plant reoviruses have been placed in three different genera, *Phytoreovirus*, *Fijivirus* and the rice ragged stunt virus (RRSV) group (a possible genus), on the basis of morphological differences, number of dsRNA genome segments and vector species (Boccardo & Milne, 1984). Members of the genus *Phytoreovirus*, which have 12 dsRNA segments, share the conserved terminal oligonucleotide sequences 5′ GG(U/C)A-....-(U/C)GAU 3′ (Asamizu et al., 1985; Kudo et al., 1991). Therefore it is tempting to hypothesize that the conserved terminal oligonucleotide sequences of a virus are genus-specific. We now report the conserved terminal nucleotide sequences of all 10 dsRNA genome segments of rice black streaked dwarf virus (RBSDV), a member of the genus *Fijivirus*.

RBSDV was propagated in maize plants cv. golden cross bantam and purified by a method modified from Redolfi & Boccardo (1974). Genomic dsRNA was extracted from the purified subviral particles by the method of Uyeda & Shikata (1984). Purified dsRNA was labelled at the 3′ terminus using cytidine 3′,5′-bisphosphate (pCp; 3000 Ci/mmol; Amersham) and T4 RNA ligase (Takara Shuzo) as described by England & Uhlenbeck (1978). The labelled dsRNA genome segments were separated by 5% PAGE in 40 mM-Tris, 195 mM-sodium acetate and 2 mM-EDTA. This gel system separated all the genome segments except S3 and S4. Individual genome segments were localized by autoradiography, eluted from the crushed gel and precipitated with 2-5 volumes of ethanol.

The 3′-terminal nucleotide of both complementary strands of the genome was identified using the method described by Kudo et al. (1991). The 32P-labelled dsRNAs were hydrolysed by using RNase T2 (Yamasa Shoyu). The complete digests were run on a PEI-cellulose sheet in 0.4 M-LiCl in a moist chamber, and analysed by autoradiography of the chromatograph. All 10 segments were found to have uridine or cytidine at their termini.

Fig. 1. Autoradiogram of PEI-cellulose chromatography of RNase T2-digested 3′-end-labelled complementary strands of all 10 genome segments of RBSDV. The arrow shows the origin.
These data showed that the 5' and 3' termini of the plus strands of all segments are adenine and cytidine, respectively, because the 3' termini of the plus strand of genome segments S10 (Azuhata et al., 1990; Uyeda et al., 1990), S8 and S7 (data not shown) were identified as cytidine by cDNA analysis.

The nucleotide sequences of terminal regions of the genome were analysed by the wandering spot method (de Wachter & Fiers, 1972; Rensing & Schoenmakers, 1973) after partial alkaline hydrolysis of 32P-labelled dsRNA in 100 mM-NaHCO3 for 1 h at 100 °C (Nomoto & Imura, 1979; Kudo et al., 1991). Using this gel system, the sequence of 10 residues at the 3' terminus of each strand was determined (Fig. 2). The terminal sequences of the plus strand deduced using the wandering spot analysis were consistent with those determined from cDNA sequencing in the case of genome segments S10 (Azuhata et al., 1990), S8 and S7 (data not shown), showing that the interpretation of the spots was reliable. The nucleotide sequences of the terminal regions of all 10 genome segments were determined and found to be identical. Although the pattern of spots derived from the 3'-terminal region is similar between segments for up to 10 nucleotides, only the last three nucleotides, ---GUC 3' are strictly conserved. These segments contain the conserved terminal oligonucleotide sequences 5' AA-GUUUUU- and -GUC 3'.

These terminal sequences are also shared by genome segments S6, S7 and S8 of maize rough dwarf virus (S' AAGUUUUUU- and -UGUC 3'), another member of the genus Fijivirus (Marzachi et al., 1991). It has been reported that the terminal oligonucleotide sequences [S' GG(U/C)A- and -(U/C) GAU 3'] of all three viruses in the genus Phytoreovirus are highly conserved (Kudo et al., 1991). RRSV, a member of a possible third genus, possesses the distinct conserved terminal oligonucleotide sequences 5' GUAAA- and -GUGC 3' (Yan et al., 1992). Analysis of the terminal sequences of other members of the genus Fijivirus will be of interest for the confirmation of their significance as a criterion for the taxonomy of plant reoviruses.

References


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