Characterization of Rice Tungro Bacilliform and Rice Tungro Spherical Viruses

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ABSTRACT

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Nucleic acids and proteins of rice tungro bacilliform virus (RTBV) and rice tungro spherical virus (RTSV) were analyzed by gel electrophoresis. RTSV contains single-stranded RNA of 3.7×10^6 Da and two major proteins of 24 and 23 kDa. RTBV contains circular double-stranded DNA of 8.3 kbp and a single major protein of 32 kDa. The RTBV-DNA is 2.1–2.3 μm in length and has two discontinuities, one in each of the two strands, having different polarities. Endonuclease cleavage sites in the RTBV-DNA molecule are mapped.

Additional keywords: helper and dependent virus combination, leafhopper-borne viruses, rice tungro disease.

Tungro is the most serious virus disease of rice and the major constraint to rice production in South and Southeast Asia. It is a composite disease caused by rice tungro bacilliform virus (RTBV) and rice tungro spherical virus (RTSV) (10,16,17). Neither virus is mechanically transmissible, but both are transmitted in a semipersistent manner by *Nephotettix virescens* (Distant) and some other leafhopper species (7,10). Transmission of RTBV by vectors is dependent upon the presence of RTSV in source plants (8,10). Nucleic acid released from RTBV particles was found to be resistant to RNase but digested with DNase, whereas that from RTSV particles was resistant to DNase but digested with RNase (9).

This paper confirms earlier findings, reports properties of nucleic acids and proteins of RTBV and RTSV, and describes restriction endonuclease mapping of the circular double-stranded (ds) genome of RTBV.

MATERIALS AND METHODS

Viruses. RTBV and RTSV from a tungro disease source originally collected in the Philippines were transferred to the rice cultivar Taichung Native 1 and maintained in that cultivar by successive transfers using *N. virescens*. Inoculated seedlings were grown in a greenhouse for 1–2 mo and then indexed by enzyme-linked immunosorbent assay (ELISA) (2). The viruses were purified separately from plants infected with either RTBV or RTSV by the method of Cabauatan and Hibino (2) or from doubly infected plants by the method of Omura et al (16).

Electrophoresis of nucleic acids. Nucleic acid was prepared by heating a suspension of purified RTBV or RTSV in 36 mM Tris, 30 mM NaH_2PO_4, 10 mM EDTA, and 1% SDS (pH 7.8) at 55 C for 10 min. The suspension was electrophoresed in a composite gel consisting of 1.7% acrylamide and 0.5% agarose using the buffer system of Loening (15) containing 0.1% SDS. After the electrophoresis, the gel was stained with ethidium bromide (1 μg/ml) or with acridine orange (30 μg/ml) in 0.01 M phosphate buffer (pH 7.0). The gel stained with ethidium bromide was incubated with RNase A (10 μg/ml) under high (2× SSC, i.e., 0.3 M NaCl and 0.03 M sodium citrate, pH 7.0) or low (0.1× SSC) ionic strength or with DNase I (5 μg/ml) in 5 mM MgCl at 37 C for 1 h. The molecular weight standards used were *Escherichia coli* ribosomal RNAs (single-stranded [ss]: 1.0 and 0.53×10^6), rice stripe virus RNAs (ss: 1.0, 1.2, 1.5, and 4.2×10^6, and ds: 1.7, 2.1, 2.8, and 7.1×10^6) (12), and lambda phage dsDNA/*Hin*dIII digests.

Purified RTBV suspension in 1× SSC containing protease K (1 mg/ml) was incubated for 20 min at 37 C. After 0.2 volume of 10% SDS was added, the suspension was mixed with an equal volume of water-saturated phenol (pH 7.0), shaken for 15 min at room temperature, and then centrifuged for 10 min at 15,000 *g*. The aqueous phase was reextracted with phenol, and nucleic acids in aqueous solution were precipitated by incubating with 0.1 volume of 3 M sodium phosphate and 2.5 volumes of ethanol for 30 min at −70 C. The precipitate was recovered by low-speed centrifugation, dissolved in distilled water, and then used for restriction endonuclease cleavages. RTBV nucleic acid preparations were electrophoresed in a 1% agarose gel in 90 mM Tris, 90 mM boric acid, and 2 mM EDTA (pH 8.0).

Electron microscopy of nucleic acid. RTBV nucleic acid was recovered by electroelution from agarose gel after electrophoresis and equilibrated in a hyperphase mixture (50% formamide, 0.1 M Tris, and 10 mM EDTA, pH 8.5) (4). Cytochrome c (type V, Sigma, St. Louis, MO) was added to 60 μg/ml, and the mixture was immediately spread over a drop of a hypophase mixture (0.01 M Tris, 1 mM EDTA, and 18% formamide, pH 8.5) (4). DNAs in the surface of the drop were mounted on collodion-covered grids, which were previously discharged by an ion coater (Eiko, Japan). The grids were stained with 50 μM uranyl acetate in HCl-acidified 90% ethanol, rotary-shadowed with platinum-palladium, and observed under a JEM-1200 EX electron microscope (JEOL, Japan). The length of RTBV-DNA was measured on preparations using circular Bluescript II plasmids (Stratagene, La Jolla, CA) as the length standard.

Electrophoresis of proteins. Purified virus suspension in 0.01 M Tris-HCl buffer (pH 6.8) containing 2% SDS and 2% mercaptoethanol was heated at 100 C for 3 min. The sample was electrophoresed on a 12.5% polyacrylamide gel using the buffer system of Laemmli (13) and was stained with Coomassie Brilliant Blue R.

RESULTS

Nucleic acids. Electrophoresed nucleic acids extracted from purified RTBV and RTSV were each resolved as a single band (Fig. 1). When the composite gel was stained with acridine orange, the band from RTBV fluoresced yellow-green, and that from RTSV fluoresced red. When the gel was treated with RNase A under high ionic strength, the nucleic acids making up the band from RTSV and those of the ssRNA markers were digested (Fig. 1). When the same gel was treated further with the same enzyme but under low ionic strength, the nucleic acids in the bands of dsRNA markers were digested. When the gel was treated further

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with DNase I, nucleic acids making up the bands of RTBV and those of dsDNA markers were digested, and the bands disappeared or became faint. These results indicate that RTBV contains dsDNA and RTSV contains ssRNA. The molecular size of RTSV-RNA was estimated at 3.7×10^6. Under the electron microscope, most RTBV-DNA molecules appeared to be circular filaments with uniform width (Fig. 2). The length of 43 circular molecules ranged from 1.8 to 2.5 μm, with a contour length of 2.1–2.3 μm. Based on the length of RTBV-DNA and Bluescript II plasmids (2,961 bp, measured as 0.9 μm), the size of RTBV-DNA was estimated to be 6,910–7,570 bp.

Restriction enzyme mapping of RTBV-DNA. In the agarose gel electrophoresis, RTBV-DNA was resolved as a single diffusive band that migrated slowly (Fig. 3). When treated with the restriction endonucleases *Bam*HI (Fig. 3), *Bst*XI, *Eco*47III, *Hin*cII, *Sal*I, or *Sca*I before electrophoresis, RTBV-DNA formed a single sharp band, which migrated faster than that of native DNA. This suggests that each circular RTBV-DNA molecule possesses a single cleavage site for each of these enzymes and became linear by the cleavages. The size of the linear DNAs was estimated to be 8.3 kbp. The DNA was cleaved into two bands of 5.3 and 3.0 kbp with *Pst*I treatment (Fig. 3). The total size of fragmented DNAs after treatment with other endonucleases was also 8.3 kbp. Cleavage sites of seven endonucleases in the RTBV-DNA molecule are mapped in Fig. 4. Digestion of RTBV-DNA with *Bam*HI before denaturation in 5 mM methyl mercuric hydroxide fragmented the molecule into ssDNA bands of 7.4, 5.5, 2.8, and 0.7 kb. Similar treatment but using *Hin*cII fragmented it into ssDNA bands of 8.2, 4.5, 3.8, and 0.1 kb. These results suggest that RTBV-DNA has two discontinuities (gaps), one in each strand, as indicated in the map (Fig. 4).

Proteins. In the polyacrylamide gel electrophoresis, proteins dissociated from purified RTBV were resolved as one major band of 32K and those from RTSV as two major bands of 24K and 23K (Fig. 5).

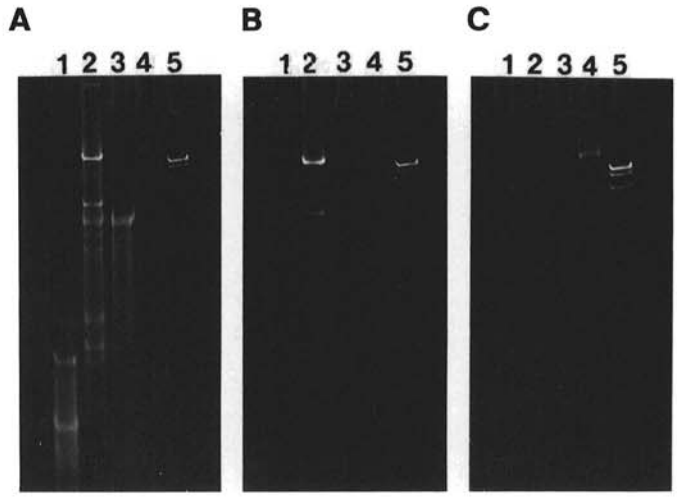

Fig. 1. Electrophoresis of nucleic acids released from rice tungro bacilliform virus (RTBV) or rice tungro spherical virus (RTSV): **A**, in a composite gel consisting of 1.7% acrylamide and 0.5% agarose; **B**, in the same gel treated after electrophoresis with 0.1% RNase A in 0.3 M NaCl and 0.03 M sodium citrate (2× SSC) for 1 h at 37 C; and **C**, in the same gel treated after electrophoresis with 0.1% RNase A in 2× SSC and then in 0.1× SSC for 1 h each at 37 C. Lane 1, *Escherichia coli* ribosomal RNAs; lane 2, rice stripe virus RNAs; lane 3, nucleic acid released from RTSV; lane 4, nucleic acid released from RTBV; and lane 5, lambda-DNA/*Hin*dIII digest.

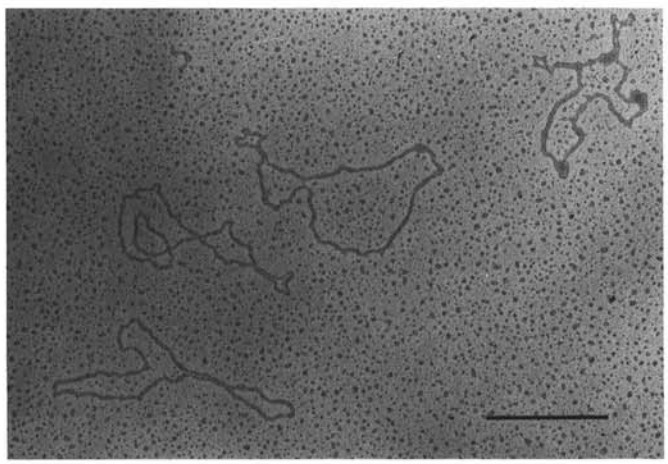

Fig. 2. Electron micrograph of rice tungro bacilliform virus DNA showing circular filaments with uniform width. Rotary-shadowed with platinum-palladium and stained with uranyl acetate. Bar represents 0.5 μm.

DISCUSSION

Because of characteristic interactions between RTBV and RTSV and their vectors (8,10), the two viruses have frequently been studied together. RTBV and RTSV are different in morphology

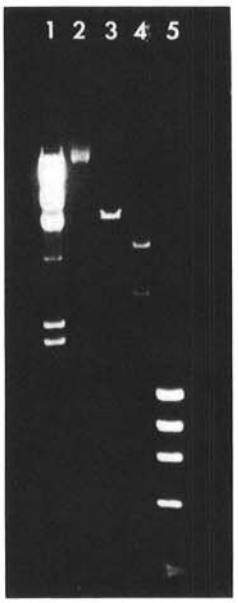

Fig. 3. Agarose gel electrophoresis of rice tungro bacilliform virus (RTBV) DNA before and after treatment with restriction endonuclease *Bam*HI or *Pst*I. Lane 1, lambda-DNA/*Hin*dIII; lane 2, RTBV-DNA; lane 3, RTBV-DNA/*Bam*HI; lane 4, RTBV-DNA/*Pst*I; lane 5, Phi X 174-RF DNA/*Hae*III.

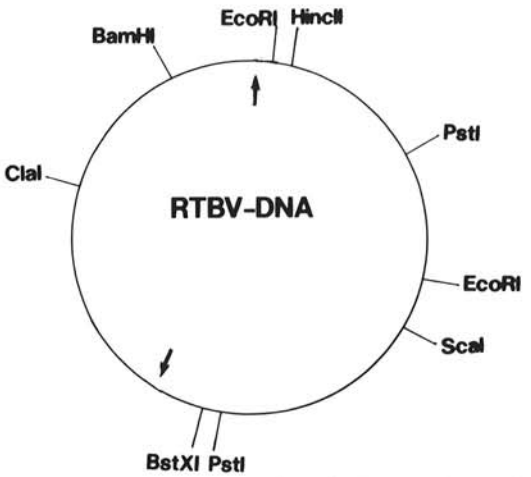

Fig. 4. Restriction endonuclease map of the circular double-stranded DNA of rice tungro bacilliform virus indicating the cleavage sites of seven endonucleases and two discontinuities, in one or the other of the two polarity strands (arrows).

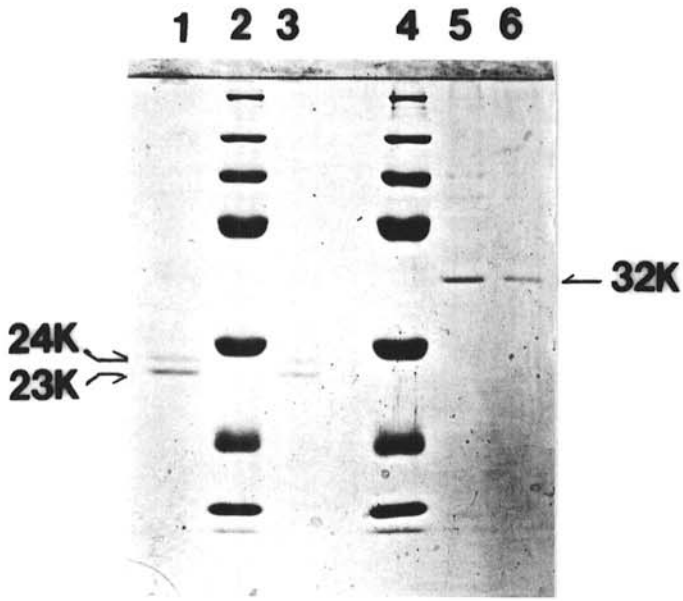

Fig. 5. Polyacrylamide gel electrophoresis of proteins released from purified rice tungro bacilliform virus (RTBV) or rice tungro spherical virus (RTSV) by heating for 3 min at 100 C in 2% SDS and 2% mercaptoethanol. Lanes 1 and 3, proteins from RTSV; lanes 2 and 4, molecular weight markers: myosin H chain (200 kDa), phosphorylase b (97.4 kDa), bovine serum albumin (68 kDa), ovalbumin (43 kDa), α-chymotrypsinogen (25.7 kDa), β-lactoglobulin (18.4 kDa), and cytochrome c (12.3 kDa); and lanes 5 and 6, proteins from RTSV. Arrows indicate the positions of the 24- and 23-kDa proteins from RTSV and the 32-kDa protein from RTBV.

and are serologically unrelated (16,17). These experiments demonstrated that they are also distinct from each other in their compositions; that is, RTBV consists of a circular dsDNA of 8.3 kbp with two discontinuities and a major protein of 32K, and RTSV consists of an ssRNA of 3.7×10^6 Da and proteins of 24K and 23K. This combination of genome types, particle types, and vector relationships is unique.

The molecular size of RTBV-DNA estimated by measuring length was smaller than that obtained by electrophoresis. Further analysis on RTBV-DNA prepared by different procedures is required to determine the real size of the molecule. Commelina yellow mottle virus (CYMV), which is similar to RTBV in morphology, has circular dsDNA 2.1–2.3 μm long (14).

In earlier works, RTSV was found to have an unusually high sedimentation coefficient (173 S) (17), buoyant density (1.551) (16), and A_{260}/A_{280} ratio (1.5–1.75) (2,16) for a small isometric virus (30 nm in diameter). These are expected values for a virus with an ssRNA genome of 3.7×10^6 Da, which is larger than those of other isometric viruses of similar size. Similar values have been reported for four small isometric viruses, namely, maize chlorotic dwarf virus (3), dandelion yellow mosaic virus (1), parsnip yellow fleck virus (5), and Anthriscus yellows virus (6). The sizes of the proteins of these viruses so far analyzed (5,6) are not comparable with those of RTSV, although possible minor proteins in RTSV were not analyzed.

Caulimoviruses (11) and nonenveloped bacilliform viruses (14) are known plant viruses that contain circular dsDNA. RTBV and caulimoviruses are similar in size and type of dsDNA but are different in the number of gaps in the DNA molecules, particle morphology, vector species, and their relations to their vectors (11). Lack of mechanical transmission of RTBV also distinguishes it from caulimoviruses, which are mechanically transmissible.

Several nonenveloped bacilliform viruses similar to RTBV have been reported in plants. Of these viruses, CYMV is reported to contain circular dsDNA of 7.3 kbp with two gaps, one in each strand, in different polarity; banana streak, Canna yellow mottle, and Kalanchoe top-spotting viruses are reported to contain dsDNA of similar size (14). Component proteins and vector species of the four viruses are not known. Of the four viruses, CYMV and banana streak virus are mechanically transmissible. RTBV and these small bacilliform viruses can be tentatively grouped, because of the similarity in their particle morphology and the properties of their genome, although further investigation of their biological and biochemical properties will be required to determine their true taxonomic relationships.

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