

An Associated Small RNA in Rice Yellow Mottle Sobemovirus Homologous to the Satellite RNA of Lucerne Transient Streak Sobemovirus

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ABSTRACT

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A low molecular weight RNA (about 210 nucleotides) was detected in virions of some isolates of rice yellow mottle sobemovirus (RYMV). Electrophoretic analysis of virion RNAs in denaturing polyacrylamide gels resolved the presence of both linear and circular forms of this small RNA. Northern transfer and dot blot hybridization tests showed a strong

homology between the small RYMV RNA and the satellite RNA of a Canadian isolate of lucerne transient streak sobemovirus. RYMV and barley yellow dwarf luteovirus are the only two known viruses that have host ranges restricted to the monocotyledons and that support replication and encapsidation of low molecular weight RNAs.

Additional keywords: nucleic acid hybridization, RYMV genomic RNA.

Rice yellow mottle virus (RYMV) is prevalent on cultivated rice (*Oryza sativa* L.) in several countries in West Africa, Kenya and Tanzania in East Africa, and on the island of Madagascar in the Indian Ocean (2,13,19). Under natural conditions, RYMV is transmitted by chrysomelid beetles and possibly through irrigation water (2,14). Most commercial rice cultivars are susceptible to RYMV infection (2,5), although some traditional upland cultivars appear somewhat tolerant (19). Severe yield losses (84–97%) caused by RYMV infection have been recorded (18) in several rice cultivars.

RYMV is a member of the sobemovirus group (9,14). The RYMV genome is a single-stranded RNA with a molecular weight of 1.4×10^6 (approximately 4.5 kilobases), and its coat protein subunit is about 30 kDa (9,20). RYMV virions are highly immunogenic but are not related serologically to any other member of the sobemovirus group (5,9,14).

In this study, we report that some RYMV isolates have an associated low molecular weight RNA in addition to the viral genomic RNA. Moreover, the small RYMV RNA shows homology with the viroidlike satellite RNA (satRNA) of a Canadian isolate of lucerne transient streak sobemovirus (LTSV). Other than a recent report (12) of the association of a satRNA with barley yellow dwarf luteovirus, information is lacking concerning low molecular weight RNAs encapsidated by viruses that are restricted to the monocotyledons (6,7).

MATERIALS AND METHODS

Two RYMV isolates, RYMV-I and RYMV-K, which are maintained at the International Institute of Tropical Agriculture, Ibadan, Nigeria, were used for this study. These isolates were propagated in the rice cultivar IR-5. Symptomatic leaves were harvested 4 wk after inoculation and stored at -17°C . Young IR-5 seedlings (10–12 days old) were also used for infectivity assays.

RYMV virions were purified according to Hull (9) and stored at 5°C in 10 mM sodium phosphate buffer, pH 7.0, containing 0.025% sodium azide. The virion concentration was estimated spectrophotometrically (2). In some studies, virions (5 mg/ml) were treated with pancreatic ribonuclease (RNase A, 20 $\mu\text{g}/\text{ml}$)

at 25°C for 2 h and then freed of the enzyme by two successive cycles of sedimentation through a 15% sucrose cushion. Isopycnic centrifugation of RYMV (RNase-treated and untreated) in gradients of cesium chloride or cesium sulfate (in 50 mM acetate buffer, pH 5.0) was performed as described previously (15,21).

RYMV RNAs were extracted from purified virions by EDTA-SDS-heat treatment followed by phenol extraction and precipitation with ethanol (15) or by the EDTA-LiCl-freeze-thaw method (4). Total RNAs were electrophoresed in 1% agarose ($1\times$ MOPS-EDTA buffer, pH 7.0) or 4% polyacrylamide, 7 M urea gels ($1\times$ TBE buffer: 20 mM Tris-HCl, 20 mM boric acid, 5 mM EDTA, pH 8.0), along with total virion RNA from LTSV. RNA size markers were generated by runoff transcription with either T7 or T3 RNA polymerases. A 301-nucleotide (nt) T7 RNA transcript was derived from a cDNA clone of papaya mosaic virus (kindly provided by T. Sit) linearized with the restriction endonuclease *Sph*I; a 250-nt T7 RNA transcript was generated from an *Nco*I-linearized cDNA clone of potato aucuba mosaic virus (kindly provided by D. Leclerc); and a 177-nt T3 RNA transcript was derived from the same potato aucuba mosaic virus clone linearized with *Mlu*I. Total virion RNAs from RYMV were fractionated with 2 M LiCl into high and low molecular weight RNAs (11), precipitated with ethanol, and then dissolved in sterile distilled water. These RNAs were purified further by agarose gel electrophoresis (15), eluted from the gels, precipitated with ethanol, and dissolved in water. In some studies, the redissolved RNAs were submitted to an additional cycle of agarose gel electrophoresis and then stored at -60°C . Glyoxal-denatured RNAs were used in Northern transfer and dot blot tests (22), and the nitrocellulose membranes were probed with a ^{32}P -labeled cDNA clone of the LTSV satRNA of specific activity 10^8 cpm/ μg of DNA (1). Hybridization and washing conditions were as described (16).

Immunodiffusion tests were performed in 1.0% agarose gels in 20 mM sodium phosphate buffer, pH 7.0, 0.85% sodium chloride, and 0.025% sodium azide with a rabbit polyclonal RYMV antiserum. The titer of this antiserum, estimated by the gel diffusion test, was 1:4096.

RESULTS AND DISCUSSION

Electrophoretic analysis of virion RNA in agarose gels (Fig. 1) showed the presence of a faster migrating, low molecular weight

RNA in addition to the full-length genomic RNA (indicated by arrows) in the RYMV-K and RYMV-I isolates. Several minor intermediate-sized RNAs were also detected. RYMV virions treated with RNase prior to RNA extraction yielded the low molecular weight RNA and genomic viral RNA, indicating that both were contained within the viral capsid.

Upon isopycnic centrifugation in cesium sulfate solution, the RNase-treated or untreated RYMV virions were resolved into

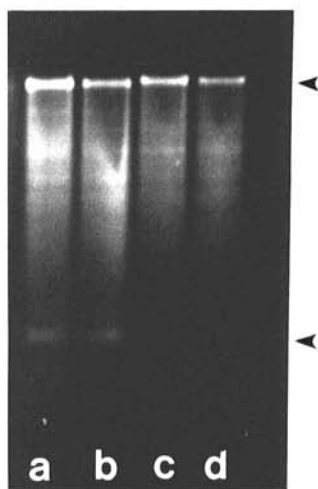


Fig. 1. Agarose gel electrophoresis of virion RNAs of rice yellow mottle virus (RYMV) isolates I (lanes a and b) and K (lanes c and d). RNAs isolated by EDTA-SDS-heat treatment followed by phenol extraction (lanes a and c) or by EDTA-LiCl-freeze-thaw treatment (lanes b and d). Approximately 1 μ g of RNA per lane was electrophoresed in 1% agarose at 50 volts (1 \times MOPS buffer) for 4 h at 5 C and then stained with ethidium bromide. The top arrow identifies genomic RYMV RNA while the bottom arrow indicates the position of the low molecular weight RNA.

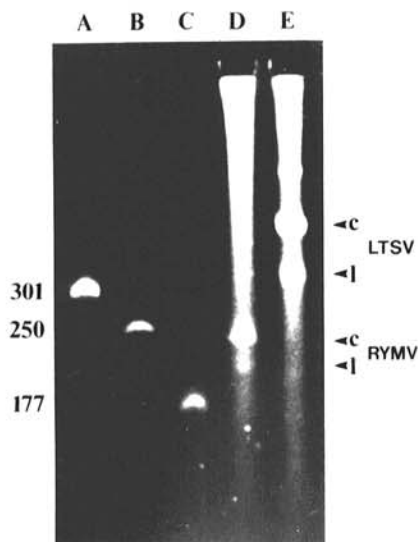


Fig. 2. Denaturing polyacrylamide gel electrophoresis of total virion RNAs from rice yellow mottle virus (RYMV) (lane D) and lucerne transient streak sobemovirus (LTSV) (lane E). Approximately 0.5 μ g of RNA per lane was electrophoresed in 4% polyacrylamide, 7 M urea gels at 100 V (1 \times TBE buffer: 20 mM Tris-HCl, 20 mM boric acid, 5 mM EDTA, pH 8) for 1.5 h and stained with ethidium bromide. Lane A, a 301-nucleotide (nt) T7 RNA transcript derived from an *Sphi*-linearized cDNA clone of papaya mosaic virus. Lane B, a 250-nt T7 RNA transcript derived from an *Nco*I-linearized cDNA clone of potato aucuba mosaic virus (PAMV). Lane C, a 177-nt T3 RNA transcript derived from the above PAMV cDNA clone linearized with *Mlu*I. Arrows in the right margin identify bands corresponding to circular (c) and linear (l) forms of the low molecular weight RNAs of RYMV and LTSV. Note that in the LTSV RNA preparations the presence of a dimeric form of the satellite RNA has been previously reported (10).

two discrete fractions with densities of 1.22 and 1.27 g/ml. Virions from both fractions, upon capsid disruption, yielded the low molecular weight RNA as well as genomic RNA. In cesium chloride gradients, RYMV virions (RNase-treated and untreated) banded homogeneously at a density of 1.36 g/ml, and the virions also contained both RNA types. These observations suggest that the low molecular weight RNA and the genomic RYMV RNA are cocapsidated. The sedimentation patterns of RYMV (a paucidisperse pattern in cesium sulfate but a monodisperse pattern in cesium chloride gradients) are similar to the reported behaviors of most sobemoviruses (8,9,21).

When total RYMV RNA was electrophoresed under denaturing conditions in 4% polyacrylamide, 7 M urea gels, two closely migrating bands were visible at the approximate position of the low molecular weight RNA (Fig. 2). Because the presence of a second small RNA band was not resolved in non-denaturing agarose gels, this result suggests that the low molecular weight RYMV RNA occurs in both circular and linear forms, as is the case with the LTSV satRNA, and that the circular (and more prevalent) RNA migrates more slowly under denaturing conditions. The faster, linear low molecular weight RYMV RNA migrated to a position that corresponded to an apparent size of about 210 nt between the 177- and 250-nt RNA transcripts (Fig. 2).

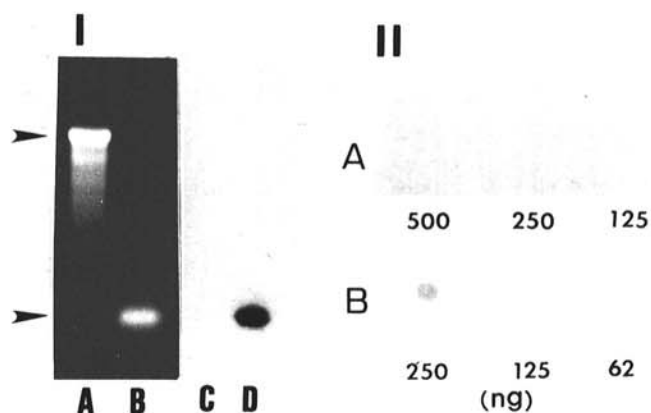


Fig. 3. Sequence homology between the low molecular weight RNA of rice yellow mottle virus (RYMV) and the viroidlike satellite RNA of lucerne transient streak sobemovirus (LTSV). Panel I, Agarose gel electrophoresis of purified RYMV genomic (lanes A and C) and low molecular weight (lanes B and D) RNAs. About 2 μ g of RNA per lane was electrophoresed as described in Figure 1. Agarose gel was stained with ethidium bromide (lanes A and B), or RNAs in the gel were transferred onto a nitrocellulose filter and hybridized with a 32 P-labeled cDNA probe specific to the LTSV satellite RNA (lanes C and D). The top arrow identifies genomic RNA, and the bottom arrow shows the position of the small associated RNA. Panel II, Dot blot hybridization test; A, purified genomic RYMV RNA and B, low molecular weight RYMV RNA spotted onto a nitrocellulose membrane and probed with a 32 P-labeled cDNA of the LTSV satellite RNA. Note a detectable hybridization signal with 62–125 ng of the small RYMV RNA but no such signal with even 500 ng of genomic RYMV RNA.

TABLE 1. Infectivity assays of the purified virion RNAs of rice yellow mottle virus

Inoculum	Concentration (μ g/ml)	Experiment ^a			Total	Percentage
		1	2	3		
Genomic RNA	10	6/20	9/40	15/35	30/95	31.5
Small RNA	50	0/45	0/50	0/70	0/165	0.0
Genomic + small RNA	10 + 50	7/40	6/35	14/25	27/100	27.0
Total virion RNAs	10	6/30	10/35	9/30	25/95	26.3

^aNumber of plants infected per number of plants inoculated. RNAs were diluted in 20 mM Tris-HCl buffer, pH 7.5, containing 50 mg/ml of purified bentonite and 50 mg/ml Celite. Inocula were kept ice-cold during inoculations.

Purified genomic and low molecular weight RYMV RNA fractions were not cross-contaminated, as shown by dot blot hybridization (Fig. 3, panel II). Full-length genomic RNA, free from the small RNA, was infectious, and its infectivity was comparable to that of total virion RNA (Table 1). Plants inoculated with either purified genomic RNA or total virion RNAs showed symptoms that were typical of RYMV infection (2), except that plants inoculated with total virion RNAs were somewhat more stunted than those inoculated with purified genomic RNA alone. Agarose gel electrophoresis of RNA extracted from purified virions isolated from plants inoculated with genomic RNA alone did not show any detectable low molecular weight RNA (data not shown). The low molecular weight RYMV RNA, when inoculated to plants alone, was noninfectious (i.e., no symptoms were visible on inoculated plants; Table 1), even when assayed at five times the concentration of genomic RYMV. However, when the purified low molecular weight RNA was coinoculated with purified genomic RYMV, agarose gel electrophoresis showed that both genomic and small RNAs were present in purified virions (data not shown). The infectivity of the genomic RNA remained unaltered when coinoculated with the low molecular weight RNA (Table 1). Immunodiffusion tests indicated the absence of any detectable RYMV virions extracted from plants inoculated with the purified small RNA alone. These results suggest that the replication and infectivity of genomic RYMV RNA is independent of the low molecular weight RNA. Moreover, the small RNA appears to depend on genomic RYMV for its replication and packaging.

The results of Northern hybridization tests (Fig. 3, panel I) show that a strong homology exists between the low molecular weight RYMV RNA and the satRNA of LTSV. Likewise, only the small RYMV RNA (and not genomic RNA) was found to hybridize with the LTSV satRNA probe in the dot blot hybridization tests (Fig. 3, panel II). The lack of sequence homology between genomic RYMV RNA and the small RYMV RNA, in addition to the apparent dependency of this small RNA on genomic RYMV for its replication and packaging, suggests that the low molecular weight RYMV RNA is a satRNA. Further hybridization studies are being conducted to unequivocally prove this hypothesis.

It is perhaps significant that the low molecular weight RNA associated with RYMV, a virus with a host range restricted to monocotyledons, shows a strong structural homology with the satRNA of LTSV, a virus that affects only dicotyledonous plants. Our recent observations (16) indicate that the LTSV satRNA can replicate effectively in divergent dicotyledonous and monocotyledonous species in the presence of a suitable helper sobemovirus. It is of interest to determine whether LTSV will support the replication and encapsidation of the small RYMV RNA because LTSV is more versatile than any other sobemovirus in supporting the replication and encapsidation of satRNAs (3,6,7, 11,16). Furthermore, an elucidation of the primary structure of the small RYMV RNA should permit its direct comparison with the other four circular satRNAs associated with the sobemoviruses (6). Our results suggest that the low molecular weight RYMV RNA appears to be considerably smaller than any of the circular satRNAs or viroids reported to date (3).

With the addition of RYMV, five sobemoviruses of approximately 15 are found to naturally contain the associated low molecular weight RNAs. Finally, not all RYMV isolates appear to have associated small RNAs. Two other Nigerian RYMV isolates examined by us, including the one deposited with the American Type Culture Collection (PV 515, G. Thottappilly), lack such RNAs. In contrast, isolates devoid of satRNAs have

not been found (6) in the other sobemoviruses that normally have associated and encapsidated satRNAs.

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