Identification of Double-Stranded RNAs Associated with Barley Yellow Dwarf Virus Infection of Oats

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ABSTRACT

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Double-stranded RNAs were identified in preparations from Coast Black oats infected with each of five characterized isolates of barley yellow dwarf virus (BYDV). Differences both in the number and electrophoretic mobility of isolate-associated double-stranded RNAs (dsRNAs) support the division of BYDV isolates into two distinct groups consisting of group 1 isolates, MAV, PAV, and SGV, and group 2 isolates, RPV and RMV. Five dsRNAs with molecular weights of approximately 3.6, 2.0, 1.2, 0.55, and 0.50×10^6 daltons were observed from oats infected with group 1 isolates. Only four dsRNAs with molecular weights of 3.8, 1.6, 1.2, and 0.55×10^6 were detected in oats infected with group 2 isolates.

Additional key word: luteovirus.

Luteoviruses are icosahedral, single-stranded RNA viruses transmitted in a circulative manner by aphids (12). Barley yellow dwarf virus (BYDV), type member of the luteovirus group, consists of several distinct isolate types distinguished on the basis of vector transmission and serology. Five characterized BYDV isolates studied in New York are RPV, RMV, MAV, SGV, and PAV (6,10,11). Although all BYDV isolates are similar in structure, mode of transmission, and induce similar symptoms in plants, recent studies of the cytopathology of BYDV-infected hosts (4) and serological analysis (11) suggest that the isolates can be classified into two distinct groups: group 1, consisting of MAV, PAV, and SGV; and group 2, consisting of RPV and RMV.

Because of the difficulty in purifying phloem-limited luteoviruses, only two of the BYDV isolates, RPV and MAV, have been partially characterized for nucleic acid content (2) and coat protein (13). Additional information on the physical composition of the BYDV isolates is not currently available for use in determining relationships among all five isolate types. The purpose of this study was to identify and compare the double-stranded RNAs (dsRNAs) involved in virus replication in plants infected with each of the five New York BYDV isolates. Comparisons of the dsRNA profiles associated with each isolate provided additional evidence for grouping BYDV isolates and suggested possible replicative strategies for luteoviruses.

MATERIALS AND METHODS

Double-stranded RNA was extracted from leaf tissue of Coast Black oats (*Avena byzantina* C. Koch) inoculated with five characterized New York BYDV isolates. The isolates used were RPV, transmitted specifically by *Rhopalosiphum padi*; RMV, transmitted specifically by *R. maidis*; MAV, transmitted by *Sitobion avenae*; SGV, transmitted specifically by *Schizaphis graminum*; and PAV, transmitted by *R. padi*, *S. avenae*, and *S. graminum* (10). Oats exposed to nonviruliferous aphids were used as healthy controls. Following a 5-day inoculation feeding by aphids, the oat seedlings were fumigated and maintained in a greenhouse at Ithaca, NY, as previously described (10). Four weeks after inoculation, the plants were harvested and shipped to Berkeley, CA, where the shoot tissue was stored frozen at −20 C until used. Samples of each group were tested by enzyme immunosorbent assay (11) against antiserum to RPV, RMV, MAV, and PAV, to confirm isolate identity.

For extraction of dsRNAs from plants infected by the five BYDV isolates, procedures described by Morris and Dodds (8) were used with modifications to allow dsRNA extraction from large amounts of tissue. Frozen leaf tissue (100 g) was ground to a powder in liquid nitrogen in a large mortar and stirred for 30 min in an extraction medium consisting of 100 ml 2× STE buffer (STE: 50 mM tris, 0.1 mM Na_2EDTA, 100 mM NaCl, pH 8.0), 100 ml of water-saturated phenol containing 0.1% 8-hydroxyquinoline, 5 ml 10% SDS, 5 ml bentonite (40 g/l), and 50 ml chloroform:pentanol (25:1). After the mixture was centrifuged at 6,000 *g* for 20 min, the aqueous phase was decanted and an additional 50–100 ml of STE buffer was stirred into the phenol phase. Following a second centrifugation, the two aqueous phases were combined and absolute ethanol was added to a final concentration of 15%. Cellulose powder (Bio-Rad Cellex N-1) was added to the 15% ethanol solution (6 g/100–200 g tissue) and stirred 2 hr at 4 C. The Cellex N-1, with bound dsRNAs, was then collected by centrifugation, washed four times in 15% ethanol-STE buffer, packed in four 1.5 × 10-cm columns, and washed with 200 ml of 15% ethanol-STE. The dsRNAs were eluted from each column with 8 ml of STE buffer without ethanol, made to 20 mM $MgCl_2$ and 2 mM $CaCl_2$, incubated 30 min at 37 C with 10 μg of DNase per milliliter, and concentrated by ethanol precipitation. When the dsRNA pellet consisted of a granular precipitate, it was dried under vacuum and then resuspended in a minimal volume of 20 mM tris, 10 mM sodium acetate, 0.5 mM NaEDTA at pH 7.5 and stored at −20 C. On some occasions the final pellet contained a clear gelatinous substance. To remove this material the pellet was resuspended and dsRNAs were precipitated with cetyltrimethylammoniumbromide (9).

Samples of dsRNA were electrophoresed in 5, 6, or 7% polyacrylamide slab gels (1.5 mm thick, 16 cm long, and 14 cm wide) in 40 mM tris, 20 mM sodium acetate, 1 mM EDTA, pH 7.5, at 20 mA for 18 hr at 15 C. Following staining in ethidium bromide

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(20 ng/ml), dsRNAs were visualized by exposing the gel to ultraviolet light (302 nm) and photographed. Some gels were then fixed and stained with silver nitrate (7).

Double-stranded RNAs, extracted from brome mosaic virus (BMV)-infected cultivar Briggs barley (*Hordeum vulgare* L.) or cultivar Coast Black oats infected with the SGV isolate of BYDV, were purified and tested for RNase A sensitivity by the methods of Zelcer et al (14). Following incubation in 50 ng RNase A per milliliter diluted in high- or low-salt conditions, the RNase was digested with proteinase K and the dsRNAs were precipitated with two volumes of ethanol at −20 C prior to analysis.

RESULTS AND DISCUSSION

Profiles of virus-specific dsRNAs isolated from BYDV-infected oats showed two major and two or three minor classes of dsRNA molecules associated with each BYDV isolate (Fig. 1). Nucleic acid bands were never observed in control preparations made from healthy oats in any of 10 gels. Mobility differences consistently were observed for dsRNA-1 and dsRNA-2 between isolates of group 1 (MAV-PAV-SGV) and group 2 (RPV-RMV). An estimated molecular weight of $3.6–3.8 \times 10^6$ daltons (d) for dsRNA-1 (Table 1) was approximately double the value reported (2) for the MAV and RPV genomic single-stranded RNA (1.85×10^6 d) and probably represents the replicative form of the viral genome. The additional smaller dsRNAs observed most likely represent subgenomic species like those observed for some other small RNA plant viruses (5). The dsRNA-2 molecules showed the greatest difference between the two BYDV groups, with group 1 dsRNA-2 comigrating with dsRNA-2 of BMV, which is 2×10^6 d, and group 2 dsRNA-2 (1.6×10^6 d) migrating slightly slower than BMV dsRNA-3, which is 1.5×10^6 d. The dsRNAs 1 and 2 were detected consistently in all preparations and in the greatest amounts, as suggested by intensity of ethidium bromide and silver staining. The apparent difference in mobility for MAV compared to PAV and SGV (Fig. 1) did not occur in any of four other preparations. This particular gel was selected because it showed to best advantage dsRNA profiles of all five isolates. Minor dsRNA bands, staining much less intensely than the two major bands, occurred in all preparations from plants infected by each of the five BYDV isolates. These minor bands varied in staining intensity among different preparations, but gave consistent patterns in various gels. A minor band of 1.2×10^6 d (dsRNA-3) and a doublet of about 0.55×10^6 d (dsRNA-4) occurred in samples extracted from tissue infected with isolates of both groups, but a fifth class of dsRNA molecule, of about 0.5×10^6 d, occurred only in tissue infected by group 1 isolates.

The double-stranded nature of these RNAs was verified by RNase treatment under high- and low-salt conditions (Fig. 2). The dsRNA purified from BMV-infected barley and BYDV-infected oats was degraded by RNase A when incubated in 15 mM NaCl, 1.5 mM trisodium citrate, but was unaffected by RNase incubation in 300 mM NaCl, 30 mM trisodium citrate.

The observed molecular weights reported for each class of BYDV-associated dsRNA were determined by relative mobility compared to a variety of coelectrophoresed markers (Table 1). It is

TABLE 1. Molecular weight estimates of dsRNAs associated with barley yellow dwarf virus (BYDV) infection in oats[a]

dsRNA designation	Molecular weight ($\times 10^6$ daltons (d)) ± S.D.[b]	
	Group 1	Group 2
	MAV-PAV-SGV	RPV-RMV
ds-1	3.60 ± 0.08	3.80 ± 0.05
ds-2	2.00 ± 0.10	1.60 ± 0.05
ds-3	1.20 ± 0.03	1.20 ± 0.05
ds-4	0.55 ± 0.05	0.55 ± 0.06
ds-5	0.50 ± 0.03	···

[a] Molecular weight estimates determined from the mobilities of BYDV dsRNAs on 5, 6, and 7% polyacrylamide gels relative to dsRNA markers of tobacco mosaic virus (4.1×10^6 d), tomato bushy stunt virus dsRNA-1 (3×10^6 d), brome mosaic virus (2.2, 2.0, 1.5, and 0.6×10^6 d), RNA-4 of alfalfa mosaic virus (0.56×10^6 d), and RNA-2 of carnation ringspot virus (10^6 d).

[b] Values are averages determined from four to six gels for each isolate.

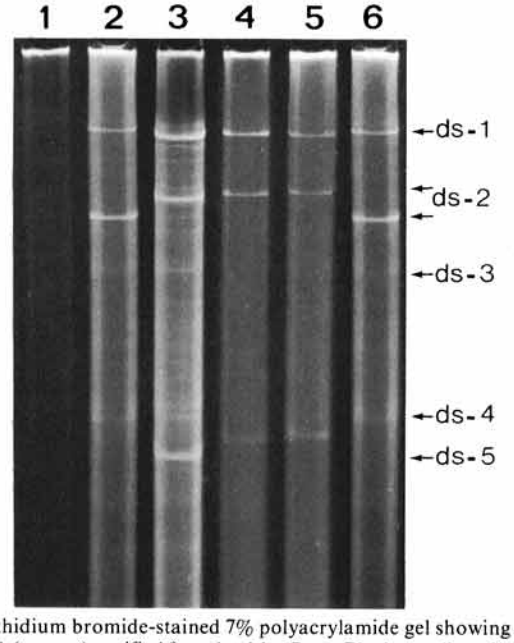

Fig. 1. Ethidium bromide-stained 7% polyacrylamide gel showing profiles of dsRNA (arrows) purified from healthy Coast Black oats (lane 1), or from oats infected with the RMV (lane 2), MAV (lane 3), PAV (lane 4), SGV (lane 5), or RPV (lane 6) isolates of barley yellow dwarf virus.

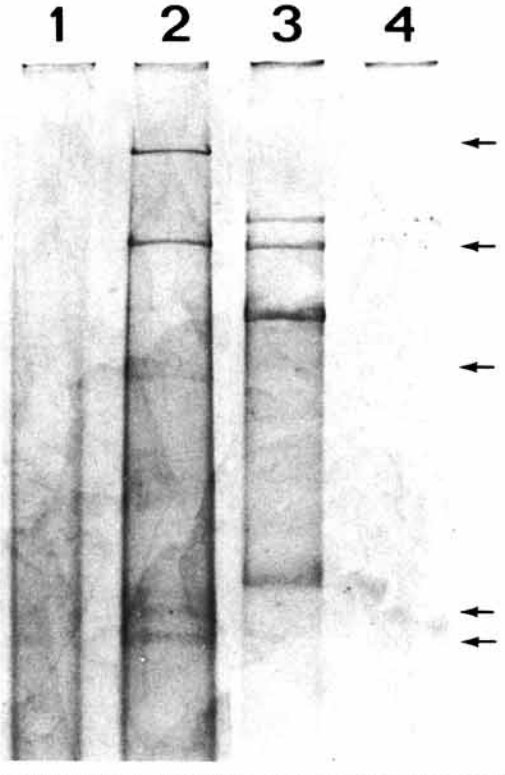

Fig. 2. Electrophoretic profile of RNase-treated dsRNA from oats infected with the SGV isolate of barley yellow dwarf virus (lanes 1 and 2) or from barley infected with brome mosaic virus (BMV) (lanes 3 and 4). Samples were incubated 30 min at 37 C in 0.05 μg/ml ribonuclease A diluted in 15 mM NaCl, 0.15 mM trisodium citrate (lanes 1 and 4) or in 300 mM NaCl, 30 mM trisodium citrate (lanes 2 and 3). The gel was stained with silver nitrate, following initial staining with ethidium bromide, and photographed with transmitted light. Arrows indicate relative positions of SGV dsRNAs.

uncertain that the difference in mobility between dsRNA-1 of groups 1 and 2 reflects a real size distinction. Small differences in mobility and sedimentation between virion single-stranded RNA of MAV and RPV have been attributed to conformational differences (2) and a nonlinear relationship between molecular weight and mobility of dsRNAs has been reported (1). We also have observed anomalous migration of dsRNAs from alfalfa mosaic virus and carnation ringspot virus-infected tissues, based on expected mobilities predicted from ssRNA values. Others (3) have suggested that various factors may influence dsRNA migration in polyacrylamide gels.

These results support the division of BYDV isolates into two distinct groups based both on the number of dsRNA molecules produced during BYDV replication and consistent differences in their electrophoretic mobilities. The significance of the various dsRNA molecules is presently unknown. Their occurrence in infected tissue, however, does suggest that subgenomic messenger RNAs may be involved in the replication of luteoviruses. Further understanding of the replicative strategy, however, must await hybridization studies to identify sequence homology between the dsRNAs obtained from infected oats and genomic RNA from each isolate type. The results reported here indicate that differences in dsRNA content occur in oats infected with various BYDV isolates; and that these differences agree with previous evidence, based on serology (11) and cytopathology (4), suggesting each BYDV isolate belongs to one of two distinct groups.

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