Identification, Characterization, and Relatedness of Luteovirus Isolates from Forage Legumes

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Accepted for publication 28 January 1999.

ABSTRACT

Damsteegt, V. D., Stone, A. L., Russo, A. J., Luster, D. G., Gildow, F. E., and Smith, O. P. 1999. Identification, characterization, and relatedness of luteovirus isolates from forage legumes. *Phytopathology* 89:374-379.

Virus isolates from forage legumes collected from eight different states were identified as luteoviruses closely related to soybean dwarf luteovirus dwarfing (SbDV-D) and yellowing (SbDV-Y) described in Japan. All isolates produced reddened leaf margins in subterranean clover and were transmitted in a persistent manner by *Acyrtosiphon pisum*, but not by *Aulacorthum solani*. Specific monoclonal antibodies raised against

SbDV-Y were differentially reactive with endemic isolates. Immunoblots probed with a SbDV-D polyclonal antiserum showed single 26-kDa coat protein bands, confirming close serological relatedness to SbDV. Analyses of genomic and subgenomic double-stranded RNAs and northern blot analyses confirmed genomic relatedness to SbDV. Based on our results, we conclude that the U.S. luteovirus isolates studied comprise a strain or strains of the soybean dwarf virus that have clovers as common hosts and the pea aphid as a common vector.

Additional keywords: vector-specific strains.

Soybean dwarf, first described as a virus disease of soybean (*Glycine max* (L.) Merr.) in Japan (36), is capable of causing serious yield losses in soybean (35,37). Two strains of the soybean dwarf luteovirus (SbDV), dwarfing (-D) and yellowing (-Y), were identified by Tamada (34) based on host range and symptoms in soybean. The SbDV-D and SbDV-Y strains, persistently aphid-transmitted by the foxglove aphid (*Aulacorthum solani* (Kaltenbach)) (33), are closely related serologically and have been reported to occur naturally in Japan only (5). Electron micrographs of infected soybean tissue have revealed high concentrations of virions only in phloem tissue (30).

A closely related virus reported from Australasia as subterranean clover red leaf virus (SCRLV) (17,40) was renamed SbDV on the basis of biological and serological relatedness (15). The latter consists of isolates transmitted by *A. solani* or *Acyrtosiphon pisum* (Harris) (13). In 1983, a SbDV-like (SCRLV-like) pathogen was isolated from legumes in California (14) that reacted with SbDV antisera and was persistently transmitted by *Acyrtosiphon pisum*. Subsequently, SbDV-like isolates have been reported from naturally infected, asymptomatic white clover (*Trifolium repens* L.), symptomatic red clover (*T. pratense* L.), alsike clover (*T. hybridum* L.), subterranean clover (*T. subterraneum* L.), and soybean (*G. max*) from 11 eastern and southeastern states in the United States (7,24). An isolate of SbDV infecting lentils was recently reported from Syria (21). We report here the biological and physico-chemical characterization of several endemic U.S. isolates and compare them with known SbDV strains. Preliminary reports on this research have been published (6-8,29).

MATERIALS AND METHODS

Virus isolates. SbDV-D and SbDV-Y were obtained from T. Tamada (Naganuma, Japan) and maintained in soybean 'Williams' through serial aphid transmissions with *A. solani*. Additional strains of SbDV were obtained from New Zealand (SbDV-NZ; J. W. Ashby, Christchurch, New Zealand) and Tasmania (SbDV-TAS; G. R. Johnstone, Hobart, Tasmania) and maintained in subterranean clover. Bean leaf roll virus (BLRV) was obtained from R. Hampton (Agricultural Research Service [ARS], Corvallis, OR) and maintained in *Pisum sativum* L. cv. Puget (1,2). Endemic isolates were obtained from asymptomatic white clover from Kentucky (KY; S. A. Ghabrial), Mississippi (MS; M. R. McLaughlin), North Carolina (NC; C. L. Campbell), South Carolina (SC; O. W. Barnett), and Maryland (MD-1 and MD-2); alsike clover from Pennsylvania (PA); red clover from New York (NY); and subterranean clover from Virginia (VA 20; S. A. Tolin). All endemic isolates were maintained in 'Mount Barker' subterranean clover through serial aphid transmissions with *Acyrtosiphon pisum*.

Aphid vectors. Colonies of *A. solani* were maintained on curly dock (*Rumex crispus* L.). *Acyrtosiphon pisum* was obtained from R. Hampton and maintained on broad bean (*Vicia faba* L.). All aphid species were monitored periodically to insure colonies were free of any contaminant viruses. The identity of each aphid population was verified by M. Stoetzel (ARS Systematic Entomology Laboratory, Beltsville, MD).

Aphid populations were tested for their ability to transmit known strains of SbDV and the endemic forage legume isolates by placing 50 to 500 aphids on infected clover or soybean leaflets for a 48-h acquisition access period (AAP), followed by a 48-h inoculation access period (IAP), on healthy 3-week-old subterranean clover seedlings utilizing 20 or more aphids per seedling. Seedlings were sprayed with a solution of Malathion (0,0-dimethyl phosphorodithioate of diethylmercapto succinate; 0.2 g a.i./liter) to remove the aphids and placed on glasshouse benches for 30 days at 20 to 25°C for symptom development.

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Publication no. P-1999-0310-01R

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Purification. Endemic virus isolates were purified from 'Puget' pea by an enzyme extraction method similar to that previously described for SbDV (12,19). Modifications of the SbDV purification protocol were the use of 100 mM sodium citrate extraction buffer with 10 mM EDTA, pH 8; precipitation of chloroform/butanol-clarified supernatant with 8% polyethylene glycol 6000 (PEG) with 1% NaCl; and centrifugation of the resuspended high-speed pellets on a 30% sucrose pad (9,26). The resultant pellets were resuspended overnight at 4°C in 1.0 ml of 10-mM sodium phosphate, pH 7.6. Virus preparations were layered onto 10 to 40% sucrose gradients and centrifuged in an SW 41 rotor (Beckman Instruments, Inc., Palo Alto, CA) at $248,000 \times g$ for 2 h. Gradients were scanned with an ISCO UA-5 absorbance monitor at 254 nm (ISCO, Lincoln, NE). Absorption peak fractions were collected, concentrated by centrifugation in a type 75 Ti rotor (Beckman Instruments, Inc.) at $223,000 \times g$ for 1 h, resuspended in 250 μ l of resuspension buffer for at least 3 h, and then stored frozen at -20 or -80°C.

Electron microscopy. Purified virus preparations of endemic isolates were used to determine virion morphology and size by transmission electron microscopy. Formvar carbon-coated 300-mesh grids were incubated on 20- μ l drops of virus preparations for 5 min, rinsed twice with distilled water, incubated 3 min on 2% aqueous uranyl acetate, and air-dried. Grids were examined at a magnification of 30 to 50 K on a JEOL 1200 TEM (JEOL USA, Inc., Peabody, MA) at the Biotechnology EM Facility for the Life Sciences, Pennsylvania State University (State College, PA). Particle size was determined by measuring the diameter of 50 virions on photographic plates and calculating the mean diameter.

To examine plant tissues for virus localization, young 'Mount Barker' leaf tissue infected with endemic isolates and exhibiting mild reddening symptoms and 'Williams' soybean leaves infected with SbDV-Y were cut into 1- to 2-mm slices while submerged in fixative consisting of 1% formaldehyde and 2% glutaraldehyde in 2-mm sodium cacodylate buffer (pH 7.5). The fixative was infiltrated under vacuum for 5 min, and the tissue was allowed to fix at atmospheric pressure for 2 h at 4°C. Following three buffer rinses, the tissues were postfixed in 1% osmium tetroxide, dehydrated in acetone, and embedded in an eponaraldite plastic. Ultrathin sections of leaf mesophyll and vascular bundles were stained for 30 min in 2% uranyl acetate/isobutanol (9:1) and 15 min in 0.4% lead citrate and examined for the presence of virions.

Infectivity. Virions of each endemic isolate were resuspended in purification buffer amended with sucrose to make an 18% sucrose/virus preparation. Each preparation (3 to 5 ml) was placed between two stretched Parafilm membranes. Nonviruliferous *Acyrtosiphon pisum* were allowed a 48-h AAP on the membrane, after which, groups of 10 or more aphids were transferred to 'Mount Barker' seedlings for a 48-h IAP. The aphids were killed and the plants placed in the glasshouse for 30 days at 20 to 25°C for symptom development.

Host range. Initial transmissions of virus isolates were accomplished with *Acyrtosiphon pisum* to 'Mount Barker'. Development of symptoms in 'Mount Barker' and positive enzyme-linked immunosorbent assay (ELISA) were criteria for selection of isolates for further study. All host-range studies were conducted with *Acyrtosiphon pisum*, utilizing 10 or more aphids per test plant with 48-h AAPs and IAPs. Seed for test plant species was obtained from U.S. Department of Agriculture Regional Plant Introduction Stations and colleagues.

Serological assays. Plant tissue samples of the original isolates, greenhouse-propagated plant tissue, and purified virions were tested in double-antibody sandwich (DAS)-ELISA (3,4,11) using polyclonal antisera to SbDV-D, SbDV-Y, BLRV (produced at the U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, MD), and beet western yellows virus (BWYV; Agdia, Inc., Elkart, IN). Monoclonal antibodies (MAbs) to highly purified whole virions of SbDV-Y were produced by standard procedures (10,18,29). The MAbs were screened against SbDV-D,

SbDV-Y, BLRV, BWYV, and brome mosaic bromovirus (BMV) to select MAbs specific for SbDV. Triple-antibody sandwich (TAS)-ELISA (22) was used to determine the presence of common epitopes among the endemic isolates, SbDV-D, and SbDV-Y. All TAS-ELISA volumes were 150 μ l per well in Maxisorp microplates (A/S Nunc, Kamstrup, Denmark), and plates were read at 405 nm using a Molecular Devices V-Max Reader (Molecular Devices Corp., Menlo Park, CA).

Immunoblot analysis. Extracts from sucrose gradient-purified luteovirus preparations (12), representing 2 μ g of SbDV-D, SbDV-Y, MD-2, and VA 20 and 4 μ g of BLRV and BWYV, were resuspended in Laemmli sample buffer (20) and electrophoresed on 4 to 20% acrylamide gradient sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) minigels using Laemmli buffers. Proteins were transferred to nitrocellulose membranes (pore size, 0.2 μ m) in transfer buffer (38) at 10 V for 15 h. After transfer, blots were blocked in 3% (wt/vol) gelatin in Tris-buffered saline (TBS; 25 mM Tris-HCl, pH 7.2, and 150 mM NaCl), probed with Affi-Gel blue-purified (12) anti-SbDV-D polyclonal immunoglobulin G (IgG) for 3.5 h at 25°C on a shaker at 50 rpm. Following incubation with the antisera, blots were washed in TBS and probed with goat anti-rabbit IgG conjugated to alkaline phosphatase at 1:3,000 dilution. Avidin conjugated to alkaline phosphatase was included at 1:3,000 dilution to detect biotinylated molecular weight standards and detected with kit reagents according to the manufacturer's protocols (Bio-Rad Laboratories, Richmond, CA).

Double-stranded (ds) RNA analysis. Virus-infected 'Mount Barker' clover or 'Puget' pea tissue was harvested 20 to 22 days after inoculation and processed immediately for dsRNA extraction. PAGE of dsRNA and northern blot hybridization analysis were conducted as described (25,32). A SbDV-Y plasmid (pSbDV-Y13), containing a 2.2-kilobase pair (kbp) cDNA downstream from the coat protein gene but including the readthrough protein gene and the 3' untranslated region of the SbDV-Y genome, was used as a hybridization probe (31). Single-stranded RNA size markers (Gibco BRL, Gaithersburg, MD) were detected by hybridization to lambda DNA.

Fig. 1. Virions from purified preparations of soybean dwarf luteovirus (SbDV) stained with 2% uranyl acetate, magnified $\times 150,000$. **A**, SbDV-Y (yellowing) and **B**, SbDV-US (United States; VA 20 isolate). Bar = 100 nm.

RESULTS

Purification. Initial attempts to purify the endemic SbDV isolates from subterranean clover tissue were unsuccessful. Bioassays of intermediate purification steps indicated the presence of infectious virions; however, no virus pellets were observed in high-speed preparations. Increasing the pH of the extraction buffer to pH 8, changing the extraction buffer from sodium phosphate to sodium citrate, and incorporating a PEG precipitation step produced yields

TABLE 1. Determination of specificity of monoclonal antibodies (MAbs) raised to the yellowing strain of soybean dwarf luteovirus (SbDV-Y) using indirect enzyme-linked immunosorbent assay

Virus isolate ^a	SbDV-Y MAbs		
	1C9	1C6	1F8
SbDV-Y	3.317 ± 0.01 ^b	3.659 ± 0.02	3.804 ± 0.19
SbDV-D	3.494 ± 0.17	3.548 ± 0.08	3.530 ± 0.21
BLRV	0.013 ± 0.00	0.037 ± 0.00	0.041 ± 0.01
BWYV	0.00	0.001 ± 0.00	0.002 ± 0.00
PLRV	0.010 ± 0.01	0.013 ± 0.00	0.003 ± 0.01
BMV	0.007 ± 0.00	0.017 ± 0.01	0.00
Buffer blank	0.00	0.00	0.00

^a SbDV-D = soybean dwarf luteovirus, dwarfing; BLRV = bean leaf roll luteovirus; BWYV = beet western yellows luteovirus; PLRV = potato leaf roll luteovirus; and BMV = brome mosaic bromovirus.

^b Average absorbance values (A_{405}) for duplicate wells (200 ng of purified virus per well). Monoclonal antibodies screened against healthy soybean with no cross-reactivity.

TABLE 2. Reactivity of soybean dwarf virus (SbDV) monoclonal antibodies (MAbs) to exotic and endemic luteovirus isolates from forage legumes by triple-antibody sandwich enzyme-linked immunosorbent assay

Isolate ^a	Polyclonal capture antiserum	SbDV MAbs		
		1C9	1C6	1F8
SbDV-Y	SbDV-D	++++ ^b	+++	+++
	SbDV-Y	+++	+	+
SbDV-D	SbDV-D	++++	+++	+++
	SbDV-Y	+++	++	++
SbDV-NZ	SbDV-D	++++	++++	+++
	SbDV-Y	+++	++	+
SbDV-TAS	SbDV-D	+++	++	+
	SbDV-Y	++	+	±
MD-1 ^c	SbDV-D	+++	±	—
	SbDV-Y	++	—	—
MD-2	SbDV-D	+++	+	±
	SbDV-Y	+++	+	—
VA 20	SbDV-D	+++	+	±
	SbDV-Y	++	±	—
MS	SbDV-D	+++	±	—
	SbDV-Y	++	—	—
NY	SbDV-D	—	+	+
	SbDV-Y	—	+	+
PA	SbDV-D	+++	+	—
	SbDV-Y	+++	+	—
SC	SbDV-D	++	±	—
	SbDV-Y	++	±	—
NC	SbDV-D	++	±	—
	SbDV-Y	++	±	—
BLRV	SbDV-D	—	—	—
	SbDV-Y	—	—	—
Healthy soybean	SbDV-D	—	—	—
	SbDV-Y	—	—	—

^a SbDV-Y = yellowing; SbDV-D = dwarfing; -NZ = New Zealand; -TAS = Tasmania; MD-1 and MD-2 = isolates from white clover from Maryland; VA 20 isolates from Virginia; MS = isolates from Mississippi; NY = isolates from New York; PA = isolates from Pennsylvania; SC = isolates from South Carolina; NC = isolates from North Carolina; and BLRV = bean leaf roll luteovirus.

^b Scale (absorbance units at A_{405}): — = 0 to 0.2, ± = 0.2 to 0.5, + = 0.5 to 1.0, ++ = 1.1 to 2.0, +++ = 2.1 to 3.0, and ++++ = 3.1 to 4.0.

^c Endemic isolates show reactivity to a lesser extent than characterized SbDV strains.

ranging from 100 to 200 µg of infectious virions per kg of plant tissue for all isolates.

Each isolate sedimented as a single component band in sucrose gradients. Gradient fractions containing virus were collected at a depth of 5.5 to 6 cm, which corresponded to the narrow peaks obtained by ultraviolet scanning of the gradients.

Electron microscopy. Purified preparations of endemic SbDV isolates and SbDV-Y contained isometric virions of similar morphology with mean diameters of 25 to 26 nm (Fig. 1). Virions were detected in leaf tissues of subterranean clovers infected with endemic isolates and showing leaf-margin reddening. High concentrations of virions were observed in a few phloem sieve elements and companion cells of vascular bundles, but not in xylem parenchyma or leaf mesophyll cells of diseased plants.

Infectivity assays. 'Mount Barker' test plants inoculated with partially purified preparations of isolates MD-1, MD-2, VA 20, MS, NY, PA, SC, or NC, which had been acquired by *A. pisum* through stretched Parafilm membranes, developed leaf-reddening symptoms 13 to 15 days after inoculation. Symptoms were identical with those produced by the original isolates in 'Mount Barker' clover.

Host range. All endemic virus isolates induced reddening of leaf margins in 'Mount Barker' subterranean clover; chlorotic mosaic, slight stunting, and premature desiccation of leaf margins in 'Puget' pea; mild chlorotic mottling and growth reduction in 'Williams' soybean; and no symptoms in 'Ladino' white clover. The symptoms were more pronounced on the upper surface of initial leaves produced after infection; later leaves exhibited almost no reddening. Symptom expression in 'Mount Barker' was less for PA and NY isolates compared with the others. All isolates induced leaf-margin reddening and stunting in 'Crimson' clover (*T. incarnatum* L.) and alsike clover; slight reddening in red clover leaves; interveinal chlorosis in fava bean (*V. faba*) leaves; reddened and yellowed leaflets and slight stunting in lentils (*Lens culinaris* Medik. cv. Laird); and slight chlorosis in peanuts (*Arachis hypogaea* L. cv. Florunner).

Vectors. All luteovirus isolates of U.S. origin were transmitted by *Acyrtosiphon pisum* from any known susceptible host to any susceptible test host on which *Acyrtosiphon pisum* would feed and none were transmitted by *A. solani*. SbDV-D and SbDV-Y were never transmitted by *Acyrtosiphon pisum*.

Fig. 2. Immunoblot analysis using a polyclonal antiserum prepared to soybean dwarf luteovirus, dwarfing (SbDV-D). Molecular weight (M) markers are in outside lanes. SbDV-Y = soybean dwarf luteovirus, yellowing; MD-2 = isolate from Maryland and VA 20 = isolate from Virginia. Lanes 1 and 2, Type strains; lanes 3 and 4, endemic isolates; and lanes 5 and 6, beet western yellows (BWYV) and bean leaf roll luteoviruses (BLRV), respectively.

Serological assays. Polyclonal antisera produced against SbDV-D and SbDV-Y virions did not differentiate among known strains of SbDV. There was slight cross-reactivity with BLRV (Fig. 1). We produced several MABs to highly purified whole virions of SbDV-Y. Three MABs (designated 1C9, 1C6, and 1F8) reacted strongly to SbDV-D, SbDV-Y, SbDV-NZ, and SbDV-TAS, but not to BLRV, BWYV, and the nonluteo BMV (Table 1). Also, the MABs reacted differentially to the endemic SbDV isolates tested (Table 2). MAB 1C6 bound all endemic isolates tested. MAB 1C9 bound all isolates except NY, and MAB 1F8 only bound isolates MD-2, VA 20, and NY. There was no reaction to healthy controls, and there was no difference between polyclonal antibody capture using antisera to either SbDV-D or SbDV-Y (Table 2).

Immunoblot analysis. All isolates except BWYV reacted with SbDV-D antisera on Western blots (Fig. 2). Four isolates (SbDV-D, SbDV-Y, MD-2, and VA 20) exhibited a 26-kDa coat protein band, and BLRV exhibited a 27-kDa protein band. The BLRV isolate exhibited weak cross-reactivity with SbDV-D antisera. A 51- to 52-kDa protein band was visible with SbDV-D, SbDV-Y, and VA 20, but not with BLRV. The MD-2 isolate exhibited a much less intense 51- to 52-kDa protein band, but had additional 44- and 31-kDa bands.

dsRNA analysis. Gel profiles of dsRNA preparations of SbDV-D, SbDV-Y, the endemic isolates, and BLRV are shown in Figure 3; BMV and tobacco mosaic virus (TMV) are known size markers. All luteovirus isolates exhibited two distinct bands representing the genomic and subgenomic dsRNAs. The genomic and subgenomic bands for SbDV-Y are slightly larger than those of SbDV-D. Among the endemic isolates, the genomic and subgenomic dsRNAs of isolates NY, PA, SC, and VA 20 appear to be similar to SbDV-D, and those from the MD-1, MD-2, MS, KY, and NC isolates are more like SbDV-Y. The genomic and subgenomic BLRV dsRNAs (Fig. 3, barely visible) were consistently larger than those of the other isolates.

Northern blot hybridization analysis. Nucleic acid hybridization of the pSbDV-Y13 cDNA probe with both genomic and subgenomic RNAs of SbDV-D, SbDV-Y, and nine endemic luteovirus isolates is illustrated in Figure 4. The pSbDV-Y13 probe hybridized strongly to both genomic and subgenomic RNAs of SbDV-Y, MD-1, MD-2, MS, and KY and weakly to the genomic RNAs of SbDV-D, NC, NY, PA, SC, and VA 20 isolates. There was no hybridization to BLRV, indicating a lack of homology between the 3' ends of the SbDV-Y and BLRV genomes.

DISCUSSION

All the luteoviruses isolated from subterranean, white, red, and alsike clovers produced reddening of subterranean clover leaf margins, were transmitted by *Acyrtosiphon pisum* in a persistent manner, reacted to SbDV polyclonal and monoclonal antibodies, produced dsRNA profiles similar to SbDV-D or SbDV-Y, hybridized to a SbDV-Y 3' probe, and had coat proteins that reacted to antibodies raised against the SbDV-D coat protein.

Specific SbDV-Y MAB reactivity data indicate that none of the endemic isolates tested share the identical array of epitopes as the previously characterized Japanese and Australasian SbDV strains (7).

Immunoblot analyses using SbDV-D polyclonal antisera indicated that the coat proteins of isolates MD-2 and VA 20 were very similar to SbDV-D or SbDV-Y, but not to BLRV or BWYV. The SbDV-D, SbDV-Y, and VA 20 isolates produced distinctive 51- to 52-kDa bands that may represent the readthrough protein. The less intense 51- to 52-kDa band and the two additional 44- and 31-kDa bands seen with the MD-2 isolate could possibly be the result of proteolytic cleavage of the readthrough protein during isolation. Similarly, the weak 21-kDa protein band exhibited by SbDV-D (SbDV-Y, MD-2, and VA 20) is likely associated with partial proteolysis of coat protein. The BLRV coat protein was weakly cross-reactive against the SbDV-D antiserum (Fig. 2), but no readthrough

Fig. 3. Polyacrylamide gel electrophoresis of the double-stranded (ds) RNAs associated with known and undescribed luteovirus isolates in forage legumes. Size markers shown on the left margin are from tobacco mosaic virus (TMV) and brome mosaic virus (BMV). Y1 and D1 refer to genomic dsRNAs, and Y2 and D2 indicate subgenomic dsRNAs. No bands were detected in extracts of healthy clover, pea, and soybean on a control gel (data not shown). SbDV-D = soybean dwarf luteovirus, dwarfing; -Y = yellowing; and BLRV = bean leaf roll virus. Clover isolates were from New York (NY), Pennsylvania (PA), Maryland (MD-1 and MD-2), Mississippi (MS), Kentucky (KY), South Carolina (SC), Virginia (VA), and North Carolina (NC).

protein band was evident. Also, BLRV was not detectable with the 2.2-kbp 3' SbDV-Y probe, which includes the readthrough protein gene (Fig. 4). The absence of either coat protein or readthrough bands by BWYV indicates a lack of relatedness to SbDV.

Examination of dsRNA profiles and northern blot hybridization analyses indicates that sizes of genomic and subgenomic RNAs of endemic isolates are similar to those of either SbDV-D or SbDV-Y. Based on mobility of genomic and subgenomic RNAs in polyacrylamide gels, there appear to be two populations of endemic isolates that are more similar to either SbDV-D (NY, PA, SC, and VA 20) or SbDV-Y (MD-1, MD-2, MS, KY, and NC). Host-range studies (5,33) have shown that SbDV-Y infects white clover asymptotically but not red clover; SbDV-D infects red clover but not white clover; and both infect other clovers. The MD-1, MD-2, MS, KY, and NC isolates came from white clover, and the remaining isolates came from red, alsike, and subterranean clovers.

Luteoviruses exist as vector-specific and nonspecific strains (39). The SbDV type strains SbDV-D and SbDV-Y, transmitted specifically by *A. solani*, have been reported from Japan only. Related strains from New Zealand and Tasmania (SbDV-NZ and SbDV-TAS, respectively) are transmitted by either *A. solani* or *Acyrtosiphon pisum*, but not by both. The endemic isolates are transmitted by *Acyrtosiphon pisum* (7,8,23,24), but not by *A. solani*. Preliminary transmission data with *Myzus persicae* (Sulzer) has indicated it may transmit several of the endemic isolates in a persistent manner. Whether that indicates vector-nonspecific strains or a mixture of vector-specific strains remains to be determined.

We have found more than 25% of symptomless white clover from 11 states in the southeastern United States to be infected with SbDV, based on reaction to SbDV antisera (7). Others (14,24) have

found high levels of SbDV infection in white clover and subterranean clover; in Japan, white clover acts as the overwintering host of SbDV (35).

In Japan, where soybean dwarf is a serious disease of soybean, the migratory generation of the oriental biotype of *A. solani* acquires the virus before leaving white clover, its primary host, and flies to soybeans, its secondary host. This results in high levels of infection in soybean by virus strains that are highly virulent to soybean (16,33). There are no aphid species in the western world or Australasia that colonize soybean (5). We were able to infect 'Williams' soybean with several endemic isolates utilizing *Acyrtosiphon pisum* (also *M. persicae*; V. D. Damsteegt, and A. L. Stone, unpublished data) under artificial conditions, producing very mild symptoms resembling those of SbDV-D or SbDV-Y. We occasionally observe soybean plants growing near forage legumes with typical SbDV-like symptoms; however, we have never observed spread beyond the border of a soybean field.

SbDV appears to be common in white clover in many areas of the world. Similar to barley yellow dwarf luteovirus (BYDV), which was named for the symptoms produced on barley from which it was first identified, soybean dwarf was named for soybean in Japan. Based on tradition, BYDV strains were named for the specific vector relationship (28), and SbDV strains were named for the symptoms produced in soybean (34). Rathjen et al. (27) used a geographic acronym, SbDV Tas-1, to identify an *A. solani*-transmitted isolate in Tasmania and SbDV AP-1 for an *Acyrtosiphon pisum*-specific strain. Although we have identified two populations of SbDV isolates in the United States, we propose to use one acronym for endemic isolates, SbDV-US, until further molecular characterization is conducted.

Fig. 4. Northern blot hybridization of the RNAs associated with known and undescribed luteovirus isolates. The hybridization was conducted using a 2.2-kilobase pair (kbp) soybean dwarf luteovirus, yellowing (SbDV-Y) cDNA clone downstream from the coat protein gene and corresponding to the readthrough protein gene and the untranslated 3' end. Lanes 1, 2, and 3, Double-stranded RNA extracted from 10 g of infected tissue of known strains; and lanes 4 to 12, double-stranded RNA from 10 g of previously uncharacterized isolates. The mobility of genomic SbDV-Y and SbDV-D (dwarfing) are shown on the right as Y1 and D1 and the subgenomic RNAs as Y2 and D2. The position of bean roll leaf virus (BLRV) single-stranded RNA size markers ranging from 0.24 to 9.5 kb are shown on the left. Clover isolate were from New York (NY), Pennsylvania (PA), Maryland (MD-1 and MD-2), Mississippi (MS), Kentucky (KY), South Carolina (SC), Virginia (VA), and North Carolina (NC).

ACKNOWLEDGMENTS

We thank M. R. McLaughlin, O. W. Barnett, S. A. Tolin, C. L. Campbell, S. A. Ghabrial, T. Tamada, and R. O. Hampton for virus isolates; A. D. Hewings and S. A. Tolin for antisera; Brotherton Seed Company, Moses Lake, WA, for donation of 'Puget' pea seed for virus increase; C. Schmidt for purifying one of the isolates; and the many students whose diligent work has made this project possible.

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