

Structural Comparison of Poa Semilatifolius Virus and Barley Stripe Mosaic Virus

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ABSTRACT

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Barley stripe mosaic virus (BSMV) and poa semilatifolius virus (PSLV) are members of the hordeivirus group. The physicochemical data presented here confirm the morphological similarity of PSLV and BSMV. The two viruses can also be distinguished serologically, and their capsid proteins differ in mobility in polyacrylamide gels. PSLV coat protein has a relative molecular mass (M_r) of 23,000, but BSMV coat protein has an M_r of 25,000.

Both viruses contain multiple RNA species, but after denaturation the three RNAs of PSLV migrate more slowly in agarose gels than the three RNAs of the ND18 strain of BSMV. The genomes of the two viruses have no significant nucleotide sequence homology as measured by nucleic acid hybridization. Thus, these results support previous suggestions that BSMV and PSLV are distinct members of the same group.

Barley stripe mosaic virus (BSMV), poa semilatifolius virus (PSLV), and Lychnis ringspot virus (LRSV) are included in the hordeivirus group because they have similar particle morphology and are related serologically (10). The three viruses have multiple RNA components that resolve into two to four species by gel electrophoresis (12). No other information is available to distinguish members of the hordeivirus group, and additional studies are needed to assess their relationships more fully. We initially intended to compare all three members of the group but were unable to recover LRSV from stored samples. However, the relationship between BSMV and PSLV is of particular interest because both viruses can infect cereals and have the potential to cause serious diseases (21).

Slykhuis (23) first suggested that BSMV and PSLV were distinct viruses. Electron microscopy showed that the two viruses differed in particle length. BSMV virions averaged 133×25 nm while those of PSLV averaged 161×26 nm (21,23). The two viruses were also distantly related as estimated by serological tests (21). In cross protection studies, BSMV protected wheat from subsequent infection by PSLV, but PSLV conferred only partial protection from infection by BSMV (21). The viruses also differed in host range because PSLV infected *Agropyron anachycaulum* and *Poa palustris* while BSMV did not.

To further define the relationship between these two viruses, we compared several properties of their virions. In addition to reexamining virus morphology and serology, we determined the sizes of coat proteins by gel electrophoresis, the electrophoretic mobility of RNAs in agarose gels, and the extent of nucleic acid homology between RNAs of the two viruses.

MATERIALS AND METHODS

Virus and plant materials. The Type, ND18, and Argentina Mild strains of BSMV were propagated in barley (*Hordeum vulgare* L.) cultivar Black Hulless (CI 666) (8). PSLV (obtained from J. T. Slykhuis) was propagated in oat (*Avena sativa* L.) cultivar Clintland 60. Viruses were purified by the procedure of Jackson and Brakke (8), except that the three short high-speed centrifugation steps were eliminated.

Electron microscopy. Virus particles were negatively stained by drawing freshly cut ends of leaves through a drop of 2% uranyl acetate on 300-mesh copper grids coated with formvar and evaporated carbon. Excess liquid was removed with a filter paper and the preparations were examined in Philips EM 200 and EM 400 electron microscopes. A diffraction grating replica with 417-nm line spacing (Ladd Research Industries, Burlington, VT) was used as the magnification standard.

Serology. New Zealand White rabbits were injected intramuscularly three times at weekly intervals with 1 mg of virus in 0.5 ml of neutral PBS (15 mM potassium phosphate, pH 7.0, 150 mM NaCl) that had been emulsified with 0.5 ml of incomplete Freund's adjuvant to elicit antisera to the viruses. The rabbits were given a fourth intravenous injection 1 wk later with 1 mg of virus in 1 ml of PBS. The antisera were then collected three times at weekly

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intervals, and the titers were determined in Ouchterlony double diffusion plates.

The serological relationship between PSLV and BSMV was examined with two assays. The first assay was done in Ouchterlony double diffusion plates composed of 0.6% agarose in PBS containing 0.1% sodium azide. The second assay was the Bio-Rad (Richmond, CA) Immun-Blot Goat Anti-Rabbit IgG Horseradish Peroxidase Conjugate Assay (7). Virus suspensions were diluted in tris-saline (15 mM tris-HCl, pH 7.4, 0.9% NaCl), and 1- μ l samples were spotted onto nitrocellulose filter paper. The serological reaction was conducted according to instructions supplied by the manufacturer, with the following modifications: 7.5% bovine serum albumin, instead of gelatin, was used in the blocking solution; after reaction with the first antibody, filters were washed for 5–10 min in tris-saline, twice for 5–10 min in tris-saline containing 0.05% Tween-20, and twice for 5 min in tris-saline; and after reaction with the second antibody, filters were washed for 5–10 min in tris-saline, twice for 5–10 min in tris-saline containing 0.05% Tween-20, twice for 5 min in tris-saline, and for 5–10 min in 20 mM Tris-HCl, pH 7.5, 0.5 M NaCl.

Gel electrophoresis of proteins. Purified virions were disrupted with 7.5% sodium dodecyl sulfate (SDS) at 50 C for 10 min. The products were analyzed on a 12.5% polyacrylamide gel (11,13), and after electrophoresis, the proteins were stained with Coomassie Blue. Molecular weight standards were purchased from Bio Rad.

Nucleic acid isolation. RNA was extracted from the viruses by a modification of the method described by Jackson and Brakke (8). This modification involved adjusting the virus concentration to 5 mg/ml before addition of an equal volume of 200 mM ammonium

carbonate buffer, pH 9.0, containing 2 mM disodium ethylenediaminetetraacetate (Na₂EDTA), 2% SDS, and 200 μ g of bentonite per milliliter. The samples were then extracted twice with equal amounts of a phenol mixture (40% phenol, 0.05% 8-hydroxyquinoline, 5% *m*-cresol, and 50% chloroform). Two volumes of absolute ethanol and 1/20 volume of 3 M sodium acetate were added to the aqueous phase to precipitate the RNA. After the precipitate was recovered by centrifugation, the pellet was dissolved in 1 ml of H₂O for each 10 mg of virus extracted. Three volumes of 4 M sodium acetate, pH 6.0, were added to the RNA, and the mixture was stored at 4 C for 2 hr to precipitate the RNA. The pellet resulting from subsequent centrifugation was resuspended in 2 ml of H₂O for each 10 mg of extracted virus and precipitated with two volumes of absolute ethanol. The final pellet was dissolved in H₂O to a concentration of 1 mg/ml and stored at –80 C. This extraction procedure consistently yielded high-purity RNA preparations free of detectable protein or detergent, and the RNA was suitable for a variety of hybridization, cloning, and sequencing procedures.

Hybridization of RNA. RNA samples were denatured with glyoxal and separated by electrophoresis through 1.5% agarose gels containing 25 mM tris acetate, pH 7.9, 5 mM sodium acetate and 1 mM Na₂EDTA, at 7 V/cm for 2.5 hr (2). The gels were either stained with acridine orange and photographed (16) or transferred onto nitrocellulose filter paper (25) and hybridized to ³²P-labeled cDNAs (9). Either DNase-digested calf thymus DNA (22,24) or oligo(dT)_{12–18} (6) was used to prime the cDNA reaction. Temperatures of hybridization ranged from room temperature to 65 C. Approximately 10⁵ cpm of ³²P-labeled cDNA per milliliter

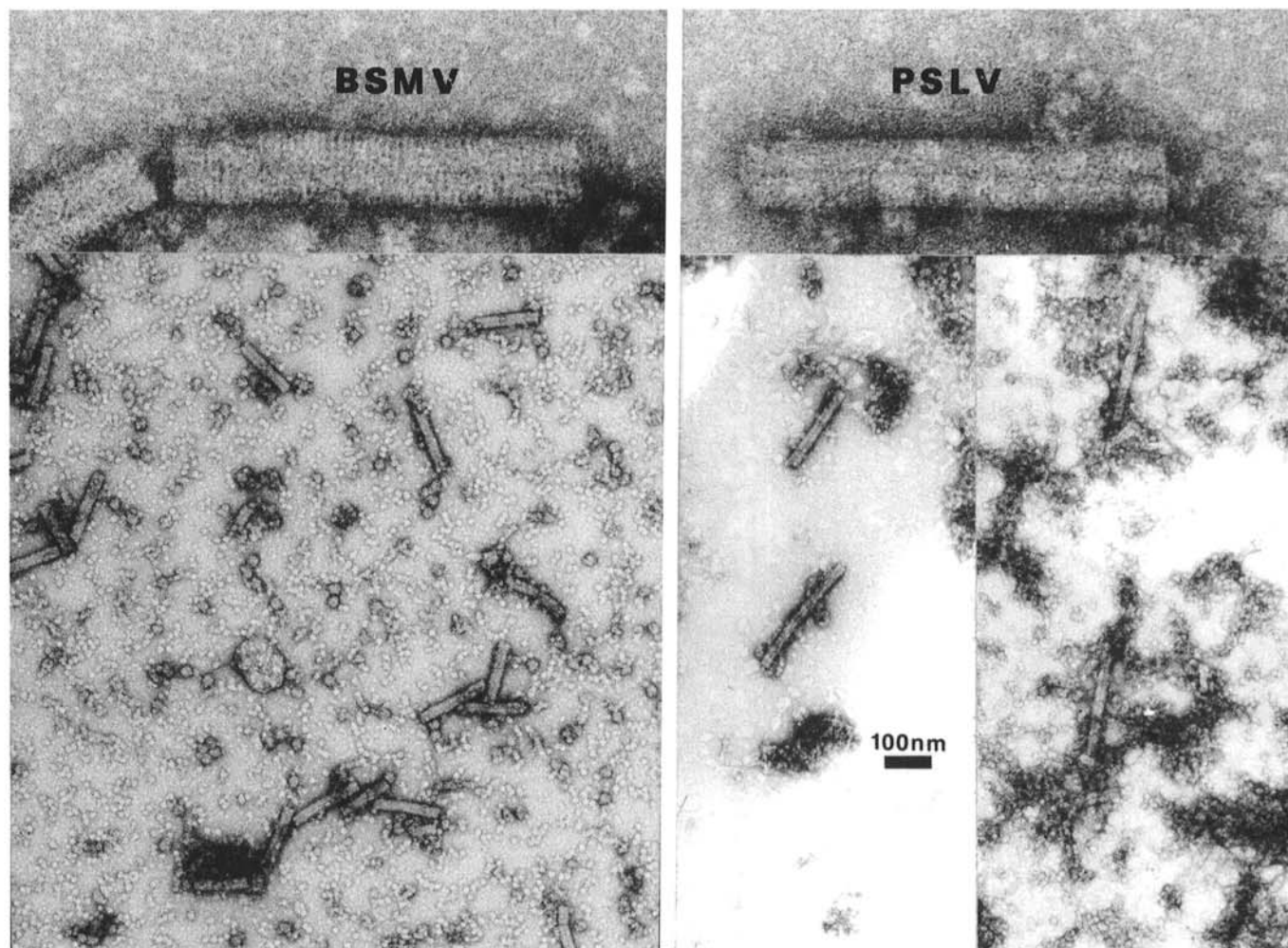


Fig. 1. Electron micrographs of leaf dip preparations from plants infected with barley stripe mosaic virus (BSMV) or poa semilatifolius virus (PSLV) negatively stained with 2% uranyl acetate. The bar in the lower panel represents 100 nm. The top frames are enlarged an additional $\times 5.5$ to illustrate detail of the particles.

were added to the hybridization reactions consisting of 0.08% polyvinylpyrrolidone (Sigma, mw 40,000); 0.08% bovine serum albumin (Sigma); 0.08% ficoll (Sigma, mw 400,000); $3 \times$ SSC (SSC 150 mM NaCl, 15 mM Na citrate); 0.1% SDS; and 30 μ g/ml of denatured calf thymus DNA that had been sheared by ultrasonic treatment. Reactions were conducted for 14–18 hr, then the filters were washed twice at room temperature and twice at the hybridization temperature in $2 \times$ SSC containing 0.1% SDS. The filters were dried and then autoradiographed at -80 C.

RESULTS

Electron microscopy. To verify purity of virus cultures, sap from infected plants was examined by electron microscopy (Fig. 1). Both PSLV and BSMV cultures contained short rigid rods similar in size to those measured by previous investigators (21,23). Because of end-to-end aggregation of purified virus particles, particle lengths could not be accurately measured. Such aggregation has caused problems in previous studies of hordeiviruses (1,10). However, our measurements of diameters of 26.3 nm for BSMV and 24.4 nm for PSLV, as well as the 2.6 nm pitch of BSMV subunits and 2.7 nm pitch of PSLV subunits, are within ranges previously reported and summarized by Jackson and Lane (10).

Serology. PSLV and BSMV samples reacted positively only to their homologous antisera in gel diffusion tests (Fig. 2). However, the two viruses cross reacted when tested by a sensitive immun-blot assay with enzyme linked antibodies (Fig. 3). The immun-blot assay also revealed serological differences between the viruses because heterologous reactions were weaker than homologous reactions. When a dilution series of either antisera or antigens was tested, peroxidase activity was lost more quickly in the heterologous reaction than in the homologous reaction. Homologous reactions usually had at least a 10-fold greater titer than the heterologous reactions. The reactions were virus-specific because preabsorption of antisera with sap from healthy barley or oat plants did not affect the results (*unpublished*). Neither antiserum reacted with tobacco mosaic virus (TMV). Thus, these results indicate that PSLV and BSMV differ serologically, but also have some common antigenic determinants.

Polyacrylamide gel electrophoresis of coat proteins. Polyacrylamide gel electrophoresis of SDS-disrupted virus preparations revealed only one major polypeptide (Fig. 4). The BSMV polypeptide had an M_r of 25,000 and the M_r of the PSLV protein was 23,000. Thus, both mobility and serological differences show that the capsid proteins of the two viruses differ in their physical and antigenic properties.

Analysis of viral RNAs. The denatured RNAs of PSLV separated into three species during electrophoresis in agarose gels (Fig. 5), but these RNAs migrated more slowly than the corresponding RNAs of the ND18 strain of BSMV. This result suggested that the genome of PSLV is tripartite as are all strains of BSMV, despite the differences in the numbers of RNAs associated with the three strains of BSMV shown in Fig. 5. See Gustafson et al (6) and McFarland et al (15) for a discussion of the complexity of the RNAs of these three BSMV strains.

The relationships of the RNAs of BSMV and PSLV were further compared by nucleic acid hybridizations with 32 P-labeled cDNAs copied from the RNAs of PSLV and the ND18 strain of BSMV. Fig. 6 shows that at 50 C, hybridization occurred only between homologous nucleic acids. Similar results were obtained at all hybridization temperatures between 65 C and room temperature, showing that the genomes of BSMV and PSLV differ considerably in sequence relations. However, longer exposure of the nitrocellulose filters revealed faint bands in the lanes containing RNAs heterologous to the cDNAs (*unpublished*). Subsequent tests showed that the faint bands resulted from nonspecific hybridization of the cDNA probe to RNA bound on the filter because similar results were obtained in control tests with ribosomal RNA from tobacco. This artifact was observed at all stringencies of hybridization tested, and could easily have been interpreted as indicating limited homology between BSMV and PSLV RNAs if appropriate controls had not been included.

Another anomaly, resulting from the preparation of the cDNA probe, was the weak hybridization of the third PSLV genomic RNA to the homologous cDNA probe (Fig. 6). The hybridization shown in Fig. 6 was conducted with a cDNA probe derived by priming the viral RNAs with oligo (dT) (6). In contrast, when PSLV cDNA probes derived by priming RNA with calf thymus DNA were used, the third PSLV RNA hybridized efficiently, but BSMV RNA still failed to hybridize (results not shown). Similar differences in reverse transcription efficiency of individual RNAs

Anti-PSLV **Anti-BSMV**

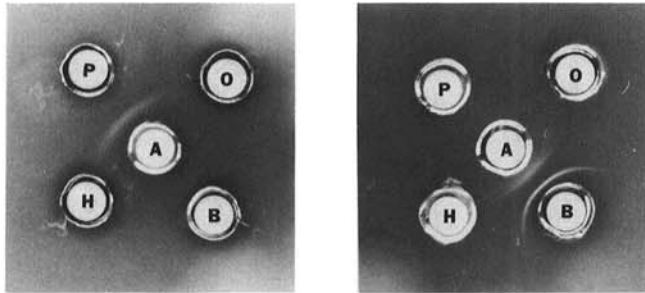


Fig. 2. Reaction of antisera raised against either poa semilantent virus (PSLV) or barley stripe mosaic virus (BSMV) to sap from various plants in Ouchterlony double diffusion plates. The antisera in the center wells (A) were preabsorbed with either sap from healthy oats (anti PSLV) or healthy barley (anti BSMV). The outer wells contain sap from PSLV-infected oats (P), healthy oats (O), BSMV-infected barley (B), or healthy barley (H).

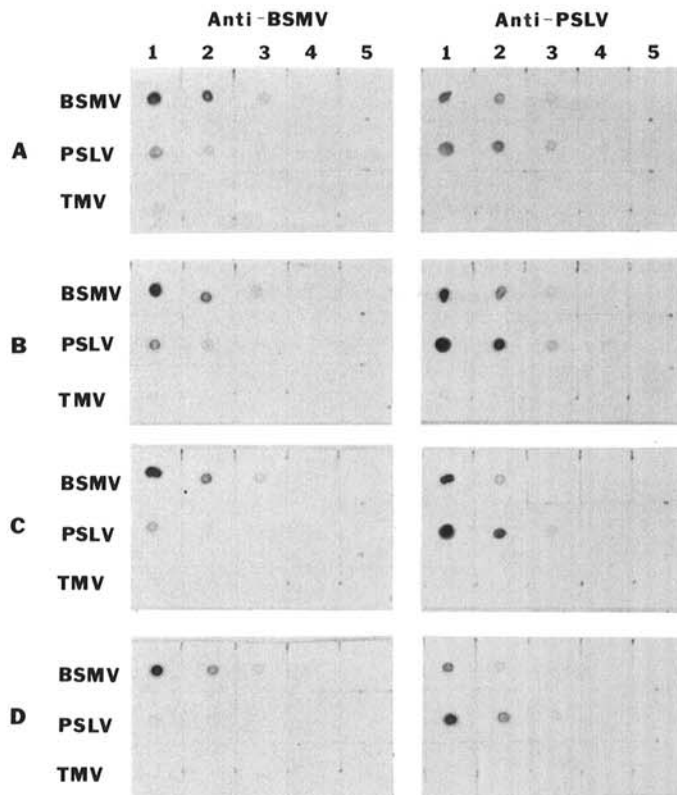


Fig. 3. Immun-blot assay of reactions between antisera and purified virus. Columns 1–5 contain dilutions of purified virus spotted onto nitrocellulose filter paper: column 1, 70 ng; column 2, 7 ng; column 3, 700 pg; column 4, 70 pg; and column 5, 7 pg. The spots in the upper row on each filter contain purified barley stripe mosaic virus (BSMV) (ND18 strain), the middle row contains poa semilantent virus (PSLV), and the lower row contains TMV. The filters on the left were reacted with serum raised against BSMV, and the filters on the right were reacted with serum raised against PSLV. The antisera for the filters in row A were diluted 1/20; in row B, 1/40; in row C, 1/400; and in row D, 1/4,000.

have previously been observed (*unpublished*) when BSMV RNAs were primed with oligo (dT).

DISCUSSION

In the hordeiviruses, only limited evidence exists for determining relatedness of viruses within the group. Although there is much information about the hordeivirus prototype, BSMV, only four comparative studies are available for assessing its relationship to PSLV and LRSV (3,12,21,23). Our results with electron microscopy confirm previous results showing morphological similarities of BSMV and PSLV (23,21). In addition, our immunoblot experiments verify the weak heterologous reaction observed in microprecipitin or ring interface precipitin tests (21,23). Our other results show that PSLV and BSMV may also be distinguished by differences in the relative electrophoretic mobility of their capsid

proteins and RNAs. Of greater significance from a taxonomic standpoint is the inability of the PSLV and BSMV RNAs to cross hybridize with their respective cDNAs. Several earlier studies have illustrated the usefulness of nucleic acid hybridization as a supplemental tool for evaluating relatedness of plant viruses (4,5,17-20,26,27). These studies and our own work show that judicious application of hybridization assays in combination with direct comparisons by classical techniques should enable more precise determination of relationships among viruses. The present study thus increases our confidence that the previous proposals (3,21,23) for including PSLV and BSMV as distinct members of the hordeivirus group are valid. A more quantitative assessment of their relationships may eventually be obtained by direct comparison of their nucleotide sequence, but this was beyond the scope of our investigation.

The ability to distinguish BSMV and PSLV RNAs by molecular hybridization is also of some diagnostic importance because it

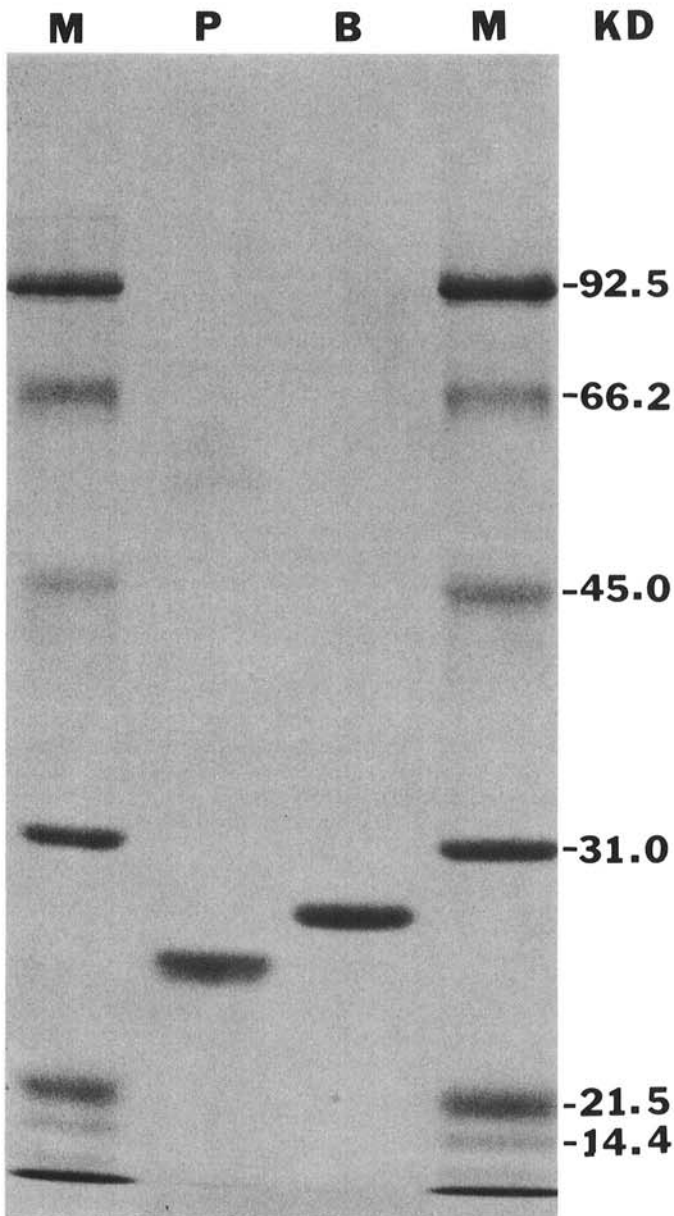


Fig. 4. Electrophoretic mobility of barley stripe mosaic virus (BSMV) and poa semilantent virus (PSLV) coat protein in polyacrylamide gels. Purified virions (2 μ g) were disrupted with 7.5% SDS, and the products were separated in a 12.5% polyacrylamide gel as described in Materials and Methods. The gel was then stained with Coomassie Blue. Lane P contains the PSLV coat protein and lane B contains the BSMV coat protein. The outer lanes (M) contain molecular weight standards whose size estimates are indicated at the right of the gel.

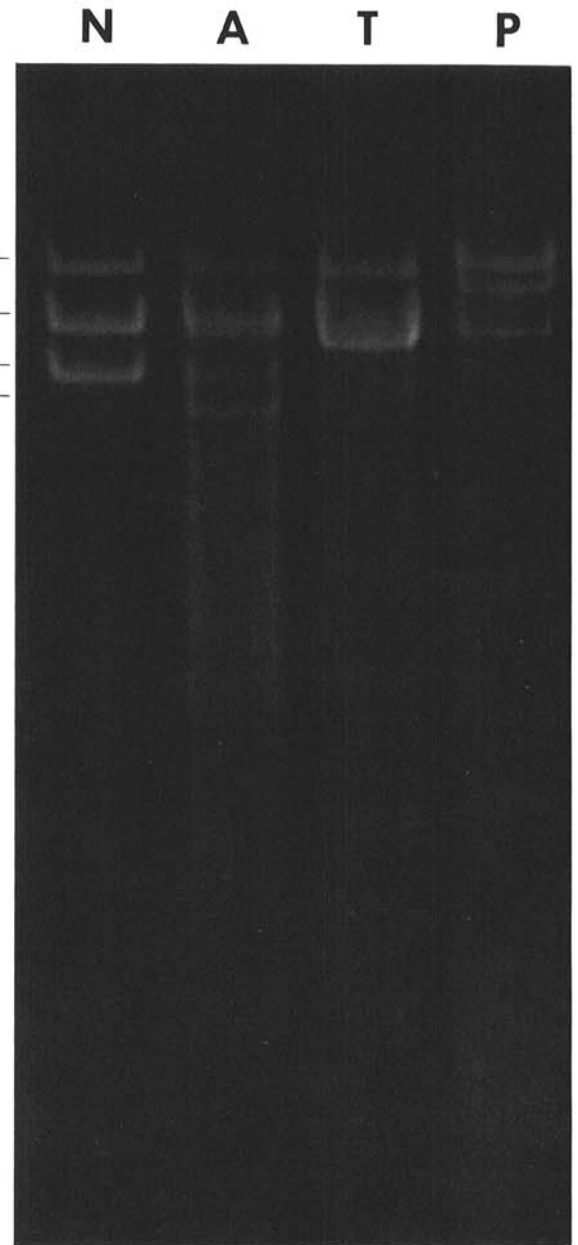


Fig. 5. Analysis of viral RNAs in agarose gels. Purified RNAs (2 μ g) from the ND18 (N), Argentina Mild (A), and Type (T) strains of barley stripe mosaic virus (BSMV) and poa semilantent virus (PSLV) (P) were denatured with glyoxal and separated in a 1.5% agarose gel. The gel was stained with acridine orange and photographed. Estimates of the lengths (in nucleotides) of the BSMV RNAs are indicated at the left of the gel.

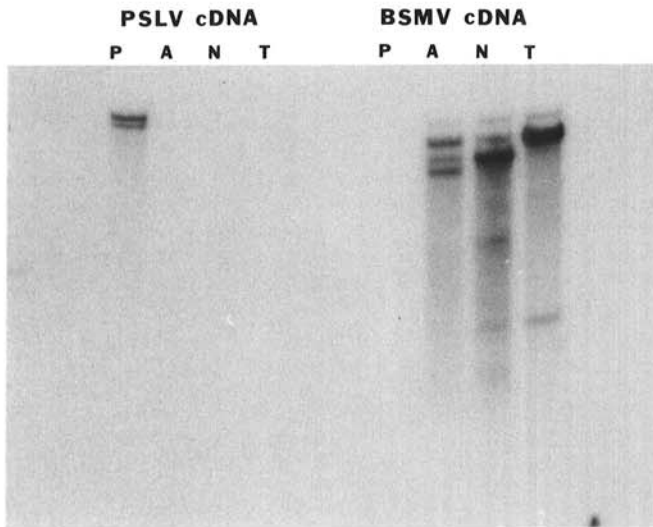


Fig. 6. Hybridization analysis of viral RNAs. Purified RNAs (250 ng) were denatured with glyoxal, separated in 1.5% agarose gels, and transferred to nitrocellulose filters. The filters were hybridized with cDNAs derived from either poa semilatifolius virus (PSLV) RNA or barley stripe mosaic virus (BSMV) RNA that had been primed with oligo(dT)₁₂₋₁₈. This hybridization was performed at 50 C. Abbreviations: P, PSLV RNA; A, Argentina Mild RNA; N, ND18 RNA; and T, Type RNA.

provides the basis for sensitive field assays for the viruses by "dot blot" techniques. PSLV has been recovered from two widely separated locations in Alberta; in both cases, the hosts were symptomless carriers of the virus (23). Thus, it is possible that PSLV is widely distributed in native grasses, and that under favorable conditions it may cause damage to cereals. Such damage might be difficult to distinguish from BSMV infections by techniques of electron microscopy or serology. However, the presence and distribution of either virus and its disease potential should be readily assessed by dot-blot hybridization assays (17). Although recombinant DNA clones would be preferable for use in such assays, recovery of viral RNA of high purity permits synthesis of a highly specific cDNA probe that should be useful for routine diagnosis.

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