Partial Characterization of a Soybean Strain of Tobacco Mosaic Virus

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ABSTRACT

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Properties of a strain of tobacco mosaic virus (TMV-S) originally isolated from soybean (*Glycine max*) were compared with those of two isolates of the common strain of TMV (ATCC PV-135 and PV-220), one TMV strain infecting bean (*Phaseolus vulgaris*) (TMV-B, ATCC PV-742), and sunnhemp mosaic virus (SHMV, ATCC PV-744). The virion capsid protein subunits of the common and soybean strains were 21 kDa. Reduced and alkylated capsid proteins of TMV-B and SHMV migrated as two bands and more rapidly than capsid proteins of the common and soybean strains in acrylamide gels containing sodium dodecyl sulfate. Host symptomatology, serological assays, particle morphology, and gel electrophoresis of the monopartite genome (6.4 kb) and whole virions demonstrated that TMV-S is closely related to the common strain of TMV but only distantly related to SHMV and TMV-B, which should be regarded as an SHMV isolate.

In 1983, soybean (*Glycine max* (L.) Merr.) plants exhibiting vein clearing and mild chlorotic mosaic symptoms were collected from a commercial field plot in Yugoslavia. Information on the incidence of the virus or disease severity is not known. The virus isolated from the plants appeared to be a new legume strain of tobacco mosaic virus (TMV) and was tentatively designated as the soybean strain (TMV-S) on the basis of host range, symptomatology, light and electron microscopy, properties in vitro, and serological tests (22). The host range of TMV-S differed from that of the common strain of TMV by inclusion of pea (*Pisum sativum* L.), red clover (*Trifolium pratense* L.), and soybean, and by exclusion of tomato (*Lycopersicon esculentum* Mill.) (Table 1). In addition to particles approximately 300 nm in length, many TMV-S particles shorter than 100 nm were observed in both leaf-dip and purified preparations, a characteristic that suggested a relationship to bean (*Phaseolus vulgaris* L.) strains of TMV. However, gel diffusion immunoassays indicated that TMV-S was closely related to, but distinct from, the common strain of TMV (TMV-C) and only distantly related to a bean strain of TMV (22).

This paper reports physicochemical properties of TMV-S and serological comparisons with American Type Culture Collection (ATCC; Rockville, MD) holdings of the bean, common, and cowpea (synonymous with sunnhemp mosaic virus [SHMV]) strains of TMV using the enzyme-linked immunosorbent assay (ELISA) and immunoblotting techniques.

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MATERIALS AND METHODS

Virus isolates and plant maintenance. All viral cultures were obtained from the ATCC and maintained in host tissues under quarantine conditions. Two TMV-C isolates (ATCC PV-135 [H. Waterworth isolate] and PV-220 [H. H. McKinney isolate]), as well as TMV-S (ATCC PV-739), were maintained in tobacco (*Nicotiana tabacum* L.) cv. Samsun. The bean strain of TMV (TMV-B, ATCC PV-742 [G. Gooding, Jr., isolate]) was increased in bean cv. Top Crop. The SHMV isolate (ATCC PV-744 [R. Toler isolate]) was maintained in cowpea (*Vigna unguiculata* (L.) Walp. cv. California Blackeye). Plants were inoculated with virus from propagation hosts and grown under conditions previously described (15), except for those infected with TMV-S. Plants containing TMV-S were maintained within a growth chamber located in the containment glasshouse with 12–14 hr of fluorescent and incandescent lighting (200 $\mu E \cdot m^{-2} \cdot s^{-1}$) at 25 ± 3 C. Plants used in this study to distinguish TMV from SHMV were soybean cv. York, tobacco (*N. tabacum* cvs. Burley 21 and Xanthi; *N. sylvestris* Speg & Comes; *N. glutinosa* L.), bean cvs. Pinto and Black Turtle Soup, and guar (*Cyamopsis tetragonoloba* (L.) Taub.) cv. Lewis. Bioassay plants used for back assays of symptomless plants were Samsun and Xanthi tobacco. Symptomless plants were assayed for the presence of infectious TMV-S virions 2–3 wk after inoculation.

Purification. Virions of all cultures were purified from 100-g lots of propagation host leaf tissue using polyethylene glycol precipitation (10). The preparation was further purified through linear 10–40% sucrose density gradients prepared in Beckman SW28 (Beckman Instruments, Inc., Palo Alto, CA) centrifuge tubes using 0.01 M sodium phosphate buffer, pH 7.2, as previously described (15). Isopycnic gradients were prepared by dissolving granular CsCl in a suspension of partially purified virus in the same buffer to 1.30 g CsCl/ml and centrifuged in a Beckman SW60Ti rotor (Beckman Instruments) at 273,000 g_{max} for 36 hr at 13 C; virion buoyant density was determined as previously described (14). Virions were concentrated from CsCl by centrifugation in a Beckman SW60Ti rotor at 273,000 *g* for 1 hr, resuspended in 0.01 M sodium phosphate buffer containing 0.05% sodium azide, and stored at −85 C. Purity of samples was assessed using transmission electron microscopy (TEM), ultraviolet spectroscopy, and sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Virion concentration was estimated by spectroscopy (A_{260} = 3 = 1 mg/ml) (28) or by using the BCA Protein Assay Reagent kit (Pierce Chemical Company, Rockford, IL) (1).

Electron microscopy. Virions obtained from sucrose density and isopycnic gradients and leaf-dip samples (7–10 days after inoculation of Samsun tobacco) were stained with 0.5% aqueous uranyl acetate and examined on 300 mesh carbon-coated copper grids using TEM. Particle length distribution was determined using measurements obtained from 2.8× photographic prints of negatives magnified 37,108×.

Preparation and sources of antisera. Polyclonal antisera to SHMV (ATCC PVAS-744), TMV-B (ATCC PVAS-742), TMV-C/PV-135 (ATCC PVAS-135d), and TMV-S (ATCC PVAS-822) were prepared by a commercial firm. Briefly, a New Zealand white rabbit was injected intramuscularly in both hips with a total of 100 μg of intact virion emulsified with Hunter's TiterMax adjuvant (CytRx Corporation, Norcross, GA) according to the manufacturer's instructions. A total of five injections was administered with 2-wk intervals between injections. Due to low antibody titer in early batches of TMV-B and SHMV antisera, the injection dosage was increased to 500 μg of virions for each of the fourth and fifth injections. Beginning 7–10 days after the third and fourth injections, sera were collected weekly during a 3-wk period ending 1 wk after the fifth injection. Antisera titers were determined using indirect, antigen coated plate ELISA (6).

Serological analysis. Indirect ELISA

was performed using polystyrene microtitration plates (Dynatech Laboratories, Inc., Alexandria, VA), using highly purified virions of SHMV, TMV-B, TMV-C/PV-135, TMV-C/PV-220, and TMV-S at 4 μg/ml as antigens (6). Immunoglobulins (IgG) were purified using Protein A columns (NYGene Corp., Yonkers, NY) according to the manufacturer's instructions. Concentrations of IgG, estimated using the protein assay kit, used as primary antibody probe were 0.125, 0.25, 0.5, 1.0, 2.0, and 4.0 μg/ml in TBS-T (0.2 mM Tris-HCl, pH 7.5, 0.15 M NaCl, and 0.05% polyoxyethylene sorbitan monolaureate [Tween 20, Sigma Chemical Company, St. Louis, MO]) containing 0.4% nonfat dry milk. The secondary antibody was goat anti-rabbit IgG conjugated to alkaline phosphatase (Boehringer Mannheim Biochemicals, Indianapolis, IN) and used at 1:1,000 in TBS-T containing 0.4% nonfat dry milk.

Double antibody sandwich ELISA (DAS-ELISA) with alkaline phosphatase conjugates (Sigma) was performed as described (4) using TMV-C/PV-135, TMV-B, and TMV-S. Primary antibodies were used at 10 μg IgG/ml. Antigen concentrations were 0.125, 0.25, 0.5, 1.0, 2.0, and 3.0 μg/ml in TBS-T containing 4% nonfat dry milk. Secondary antibodies (conjugates) were diluted in TBS-T containing 4% nonfat dry milk to 1:500 for PVAS-822 (TMV-S) and 1:1,000 for PVAS-135d and PVAS-742 (TMV-B). Enzyme substrate reactions were terminated after 30 min (indirect ELISA) or 60 min (DAS-ELISA), and absorbance at 405 nm was recorded.

Electroblotting (PolyBlot Transfer System; American Bionetics, Hayward, CA) and immunological analysis of virion capsid proteins in 12% SDS-PAGE resolving gels (Bio-Rad Protean II unit; Bio-Rad Laboratories, Richmond, CA) were performed according to the manufacturers' instructions, using 0.20-μm supported nitrocellulose membranes (Schleicher & Schuell, Keene, NH) for protein immobilization. Following protein transfer, the membrane was washed once in PBS (0.1 M sodium phosphate, pH 7.2, containing 0.15 M NaCl) for 5 min, and uncoated membrane sites were blocked with PBS containing 0.05% Tween 20 (PBS-T) and 4% nonfat dry milk for 1 hr at 37 C. The membrane was washed twice for 15 min each with PBS-T. The membrane was probed with 1 μg of primary antibody per milliliter of PBS-T buffer containing 4% nonfat dry milk for 1.5 hr at 37 C with agitation. The membrane was washed, then incubated for 1 hr with goat anti-rabbit IgG conjugated to alkaline phosphatase (Boehringer Mannheim Biochemicals) at 1:1,000 in PBS-T with 4% milk at 37 C with agitation. The membrane was washed, sprayed with a liquid substrate (Visiblot AP, United States Biochemical Corp., Cleveland, OH) containing nitroblue tetrazolium and 5-bromo-4-chloro-3-indolyl phosphate, and incubated 15–30 min for color development.

Viral capsid protein subunits. The relative molecular mass (M_r) of TMV-S virion capsid protein subunits was determined using 12% resolving gels (14 × 16 cm, 1.0 mm thick) in SDS-PAGE with low M_r protein markers (14.2, 20.1, 24, 29, 36, 45, and 66 kDa); gel proteins were stained with silver nitrate (15). Protein samples were also reduced and alkylated using *N*-ethylmaleimide (11).

Whole virion electrophoresis. Comparative mobility of SHMV and TMV virions was examined by electrophoresis of 50–60 μg of virions per lane in a 1.2% agarose gel (40 mM sodium borate buffer, pH 8.0, containing 0.25 mM EDTA, and 0.25 M urea) for 16–23 hr at 25 V at room temperature. Virion proteins in the gel were stained with 0.1% Coomassie Brilliant Blue G-250 (25) for 30–45 min, then destained in 20% methanol and 6% glacial acetic acid (18) or stained with silver nitrate (27).

Nucleic acid. Virion nucleic acid was extracted, purified, and denatured with glyoxal for M_r determination, as previously described (15). Double-stranded RNA was extracted and purified from 5.0 g of Samsun tobacco tissue harvested 4–6 days after inoculation with TMV-C/PV-135 and TMV-S, and samples were examined using electrophoresis as previously described (16).

RESULTS

Symptomatology. Vein clearing and mild chlorotic mosaic symptoms developed on noninoculated leaves of soybean within 3 wk after TMV-S inoculation (Table 1). Back assay inoculations to Xanthi tobacco resulted in formation of few lesions, suggesting a low titer of infectious TMV-S in soybean. In addi-

Table 1. Composite summary of plant responses to inoculation by bean (TMV-B, ATCC PV-742), common (TMV-C [23], TMV-C/PV-135, and TMV-C/P-220), and soybean (TMV-S, ATCC PV-739) strains of tobacco mosaic virus (TMV) and sunnhemp mosaic virus (SHMV, ATCC PV-744)

Plant	SHMV	TMV-C	TMV-C PV-135	TMV-C PV-220	TMV-B	TMV-S
Chenopodium spp.[a]	...	LCL[b]	...	...	LCL	LCL
Cucumis sativus[a]	...	LCL	...	...	O	LCL
Datura stramonium[a]	...	LNL	...	...	LNL	LNL
Glycine max cv. York[a]	O	O	O	O	O	SVC, SMM
Gomphrena globosa[c]	...	...	...	...	...	LNL
Lycopersicon esculentum						
cvs. Marglobe & Rutgers[a]	...	SM	...	...	O	O
cv. Big Boy[c]	...	...	...	...	...	O
Nicotiana glutinosa[c]	NLL	...	NLL	NLL	NLL	NLL
N. rustica[a]	...	SYM	...	...	O	SCM, NM
N. tabacum						
cvs. Burley 21 & Xanthi nc[c]	NLL	...	NLL	NLL	NLL	NLL
cv. Samsun[c]	SM	...	SM	SM	SM	SM
Phaseolus vulgaris						
cv. Bountiful[a]	...	O	...	...	SM	O
cvs. Black Turtle Soup & Pinto[c]	SM, RS	...	NLL	NLL	SM, RS	NLL
cv. Top Crop[c]	...	...	...	...	...	O
Pisum sativum						
cv. Little Marvel[a]	...	O	...	...	SM	SM
Trifolium pratense[a]	...	O	...	...	...	SMM
Vigna unguiculata						
cv. California Blackeye[a]	...	O	...	...	SM	O

[a] As reported for TMV-C, TMV-B, and TMV-S by Taraku and Tolin (23).

[b] LCL = Local chlorotic lesion, LNL = local necrotic lesion, NM = necrotic mosaic, O = no infection, RS = ringspots, SCM = systemic chlorotic mosaic, SM = systemic mosaic, SMM = systemic mild mosaic, SVC = systemic vein clearing, SYM = systemic yellow mosaic, and ... = no data.

[c] As performed in this study for the strains as noted. All plant responses to inoculation with TMV-S as reported previously (23) were reconfirmed, except for those that were not tested: *L. esculentum* cvs. Marglobe and Rutgers, *Pisum sativum*, and *T. pratense*.

tion to York soybean, guar and *N. sylvestris* served as diagnostic hosts for the test viruses (Table 2). While TMV-C/PV-135, TMV-C/PV-220, and TMV-S did not infect guar, dark brown lesions (1–2 mm diameter) formed on cotyledonary leaves 2–4 days after inoculation with SHMV or TMV-B. Mosaic symptoms were observed on *N. sylvestris* inoculated with TMV-C/PV-135, TMV-C/PV-220, and TMV-S, but not in those inoculated with SHMV and TMV-B. Infectious SHMV and TMV-B particles were recovered from local chlorotic lesions but not from noninoculated leaves of *N. sylvestris* using back assay plants.

A composite summary of plant response to inoculation by test viruses is presented in Table 1. The majority of plant responses to inoculation by TMV-S reported previously (23) were reconfirmed in this study (Table 1). Necrotic local lesions (2–3 mm diameter) formed within 5 days after inoculation of TMV-C (PV-135), TMV-C (PV-220), and TMV-S to *N. glutinosa* and bean cvs. Black Turtle Soup and Pinto, with similar lesions of larger diameter (5–7 mm) expressed on Burley 21 and Xanthi nc tobacco (Table 2). Infectious TMV-S was not recovered from these leaves using back assay plants.

Purification. Approximately 10 mg of highly purified TMV-S virions was recovered from 100 g of fresh, infected Samsun tobacco tissue. Similar yields of SHMV and the other TMV strains were obtained. The ultraviolet (254 nm) absorbance profile of a centrifuged sucrose density gradient containing TMV-S showed good separation of virions from host contaminants with one distinct peak and two more rapidly sedimenting multimers, each containing particles. To increase virion yield, particles were recovered from the pellets of the centrifuged sucrose density gradient tubes and separated from contaminants using isopycnic gradient centrifugation. Virions in centrifuged isopycnic gradients were detected in a single band having a mean buoyant density of 1.29 gm/cm^3 ± 0.002 g/cm^3 (mean and standard deviation of five measurements). The $A_{260/280\ nm}$ of purified preparations, corrected for light scatter, was approximately 1.21.

Electron microscopy. Mean and median lengths of TMV-S particles partially purified using sucrose density gradients were 170 and 141 nm, respectively, (number of measurements (n) = 1,331, standard deviation (s) = 115 nm, skewness = 0.8) with a range of 23–737 nm (Fig. 1). In the frequency polygon (Fig. 1), particles of approximately 50 nm, 120 nm, and 280–290 nm were noted. Nearly 78% of the particles were less than 270 nm in length, with approximately 14% of the particles within the range of 30–50 nm, 32% within 30–80 nm, and 10% within 280–320 nm. Mean and median lengths obtained from cesium chloride gradients were 207 and 221 nm, respectively, (n = 742, s = 128 nm, skewness = 0.5), with a range of 17–798 nm. Approximately 55% of particle lengths were less than 270 nm, with nearly 9% within the range 30–50 nm, 21% within 30–80 nm, and 12% within 280–320 nm. Mean and median measurements from the few particles examined by leaf-dip preparations were 239 and 265 nm, respectively, (n = 44, s = 67 nm, skewness = −1.3) with a range of 77–318 nm.

Serological analysis. Antibody titers to homologous virions and healthy plant proteins, respectively, were: SHMV (1:64,000 and 1:800), TMV-B (1:2,048,000 and 1:1,600), TMV-C/PV-135 (1:2,000,000 and 1:1,600), and TMV-S (1:512,000 and 1:800).

Results of indirect ELISA showed that TMV-S antibody reacted strongly with TMV-C/PV-135 and TMV-C/PV-220, but reacted little with SHMV or TMV-B antigen until antibody probe concentration reached 4 μg/ml (Fig. 2A). Antibody to TMV-B reacted well with TMV-C/PV-135, TMV-C/PV-220, and TMV-S antigens at the highest antibody concentration, but reacted only weakly at antibody less than 1 μg/ml. Reaction of antibody to TMV-B with TMV-B and SHMV was strong even at the lowest (0.125 μg/ml) antibody dilution (Fig. 2B). Antibody to TMV-C/PV-135 reacted well with TMV-C/PV-135, TMV-C/PV-220, and TMV-S antigens, but did not react with either TMV-B or SHMV antigens at antibody less than 1 μg/ml (Fig. 2C). Antibody to SHMV reacted strongly with SHMV and TMV-B, but weakly with other test antigens (Fig. 2D).

Strains TMV-S and TMV-C/PV-135 were closely related serologically, and neither respective homologous antibody reacted with TMV-B in DAS-ELISA (Fig. 3A and B). Antibody to SHMV reacted strongly with TMV-B but did not react with TMV-C/PV-135 or TMV-S (Fig. 3C).

Differences among virion capsid proteins of the different strains detected by ELISA were also clearly distinguishable by immunoblotting (Fig. 4). Antibody to TMV-S reacted strongly with proteins of TMV-C/PV-135, TMV-C/PV-220, and TMV-S, and to a fair degree with proteins of SHMV and TMV-B (Fig. 4A). Antibody to TMV-C/PV-135

Table 2. Diagnostic plant response to inoculation with bean (TMV-B, ATCC PV-742), common (TMV-C, ATCC PV-135 and PV-220), and soybean (TMV-S, ATCC PV-739) strains of tobacco mosaic virus (TMV), as well as with sunnhemp mosaic virus (SHMV, ATCC PV-744)[a]

Plant	SHMV	TMV-B	TMV-C PV-135	TMV-C PV-220	TMV-S
Cyamopsis tetragonoloba	NLL[b]	NLL	O	O	O
Glycine max cv. York	O	O	O	O	SVC, SMM
Nicotiana sylvestris	NCL	LCL	C, SM	C, SM	C, SM

[a]Symptoms recorded during 21-day observation period after inoculation. Experiment repeated at least three times with 10 plants inoculated per replicate.
[b]C = Local chlorosis, LCL = local chlorotic lesion, NLL = necrotic local lesion, O = no infection, SM = systemic mosaic, SMM = systemic mild mosaic, and SVC = systemic vein clearing.

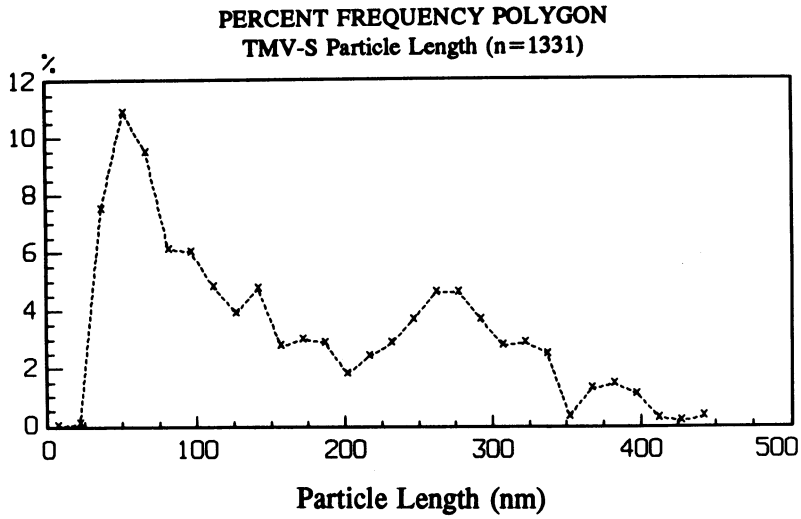

Fig. 1. Length distribution (percent frequency polygon) of TMV-S virions purified through sucrose gradients, stained with 0.5% aqueous uranyl acetate, and examined using transmission electron microscopy. Percent total frequencies is plotted on the y-axis against the midpoints of the class intervals on the x-axis. Mean and median lengths of particles were 170 and 141 nm, respectively.

reacted moderately with capsid proteins of TMV-C/PV-135, TMV-C/PV-220, and TMV-S, but very weakly with SHMV and even less with TMV-B (Fig. 4B). Antibody to TMV-B reacted strongly with proteins to TMV-B and SHMV, less with that of TMV-S, and weakly with those of TMV-C/PV-135 and TMV-C/PV-220 (Fig. 4C). Antibody to SHMV reacted more strongly to TMV-B than to SHMV but did not detect the proteins of the other strains (Fig. 4D).

Viral capsid protein subunits. The M_r of nonalkylated TMV-S capsid protein was 20.77 ± 0.35 kDa (95% confidence interval) from eight measurements. Reduced and alkylated capsid proteins of TMV-C/PV-135 and TMV-C/PV-220 each migrated distances identical to that of TMV-S in adjacent lanes. Proteins of SHMV and TMV-B migrated as two bands and more rapidly than the other samples, even when virions were treated and tested immediately after purification (Fig. 5).

Whole virion electrophoresis. Whole virion protein electrophoresis patterns were identical for TMV-C/PV-135, TMV-C/PV-220, and TMV-S, but SHMV and TMV-B virions did not enter the gel matrix under the conditions used (Fig. 6). After electrophoresis for 23 hr,

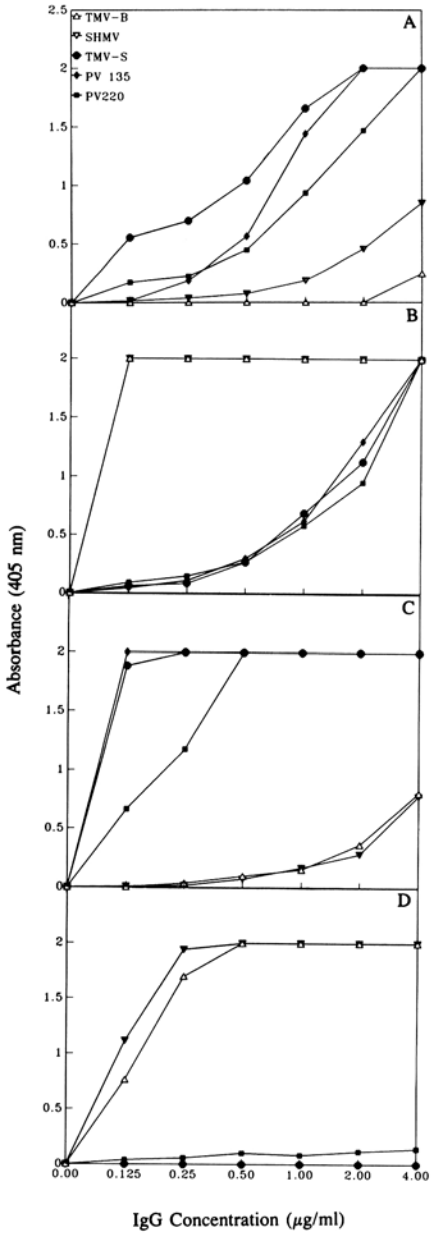

Fig. 2. Indirect (antigen coated plate) enzyme-linked immunosorbent assay (ELISA) results using purified virions of the bean and soybean strains of tobacco mosaic virus (TMV-B and TMV-S), sunnhemp mosaic virus (SHMV), and two common strain isolates of TMV (PV-135 and PV-220). The primary antibodies were used at six dilutions and were to: **(A)** TMV-S, **(B)** TMV-B, **(C)** PV-135, and **(D)** SHMV. The reporter molecule was goat anti-rabbit IgG conjugated to alkaline phosphatase. Absorbance (405 nm) of well contents was recorded after 30 min of incubation.

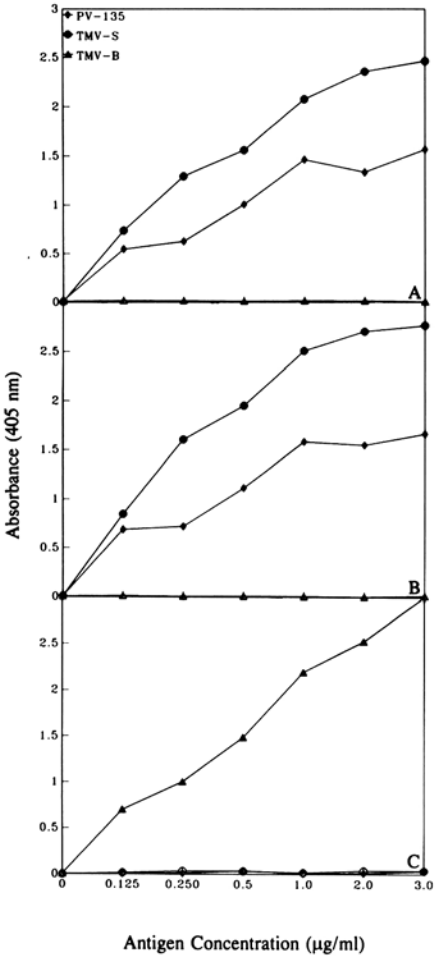

Fig. 3. Double antibody sandwich ELISA results using antibody (10 μg/ml of carbonate buffer) to the **(A)** common strain of tobacco mosaic virus (TMV-C/PV-135), **(B)** soybean strain of TMV (TMV-S), and **(C)** sunnhemp mosaic virus to coat microtitration plates. Purified virions of the bean strain (TMV-B), common strain (PV-135), and soybean strain (TMV-S) were added to wells in indicated amounts, with antibody conjugated to alkaline phosphatase used as secondary antibody. Absorbance (405 nm) of well contents was recorded after 60 min of incubation.

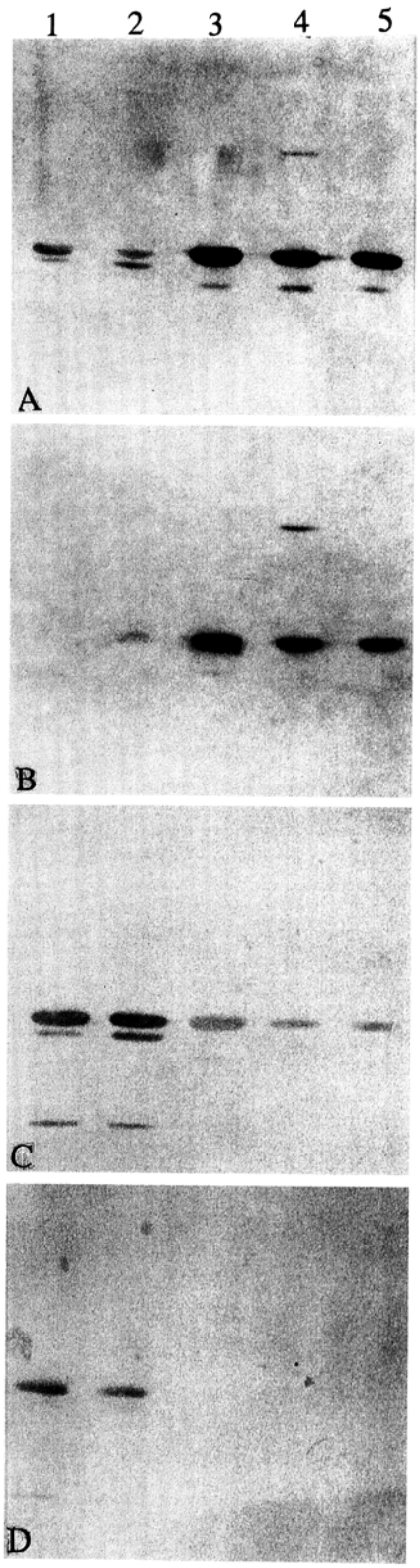

Fig. 4. Reduced and alkylated virion capsid proteins were separated in a 12% polyacrylamide gel containing sodium dodecyl sulfate and electroblotted to a nitrocellulose membrane. Membranes were probed with polyclonal antibodies to: **(A)** soybean strain of tobacco mosaic virus (TMV-S), **(B)** common strain of TMV (PV-135), **(C)** bean strain of TMV (TMV-B), and **(D)** sunnhemp mosaic virus (SHMV). Virion capsid proteins are from (lane 1) TMV-B, (lane 2) SHMV, (lane 3) TMV-S, (lane 4) PV-135, and (lane 5) PV-220 (TMV, common strain isolate).

SHMV and TMV-B virions were each visualized in single bands a few millimeters from their points of gel entry.

Nucleic acid. The TMV-S nucleic acid migrated primarily as one genomic species (band) in denaturing gels (Fig. 7) with M_r estimated as 6.43 kb from three measurements. The presence of several diffusely staining areas of gel below the prominent genomic species suggests that low RNA concentrations in a range of sizes are present, perhaps due to the diversity in the virion length distribution. The dsRNA profiles of TMV-C/PV-135 and TMV-S were identical.

DISCUSSION

Since SHMV may infect soybean (17) and was the first tobamovirus found to have two definitive particle sizes (i.e., 40 and 300 nm), its properties (24), as well as those of ATCC TMV-B and TMV-C isolates, were compared to those of TMV-S. This study presents further evidence to support classification of TMV-S as a new strain of TMV rather than of SHMV. In addition, evidence that TMV-B is an isolate of SHMV is presented.

The TMV-S host range determined from this and a prior study (22) distinguish TMV-S from common and legume strains of TMV (5,24). The TMV-S host range also differs from SHMV (17,24), especially in TMV-S inability to infect bean systemically (24).

Yield and purity of TMV-S virions isolated from tissues were comparable to those previously obtained (22). Nucleoprotein absorbance ratio and buoyant density of TMV-S virions in CsCl were similar to those reported for the type strain of TMV (28).

Although many factors affect particle length distribution in TMV preparations (7,8,21,22), the large percentage of particles ranging from 30 to 50 nm observed from diverse samples suggests it is unlikely that short TMV-S particles arose solely as a result of breakage, although a comparable examination of TMV-C particle length was not performed in our study. Since Taraku and Tolin (22) demonstrated that short TMV-S particles were noninfectious, and our results showed that both short and long particles had identical buoyant densities, the short particles may have encapsidated subgenomic RNA. In this study, few short particles were observed in two leaf-dip preparations from tobacco, but the results perhaps were influenced by age of tissue, preparation methodology, or small sample size. However, in the original study done in one author's laboratory (22), short TMV-S particles were readily observed in numerous leaf-dip preparations from tobacco and soybean tissues. Since short particles have not been reported from TMV-C preparations (12,26), we conclude that TMV-S is a novel strain of TMV with particles of two or more size-classes.

Data from ELISA and immunoblots demonstrated that TMV-S was serologically closely related to TMV-C/PV-135 and TMV-C/PV-220, but only distantly related to SHMV and TMV-B. The SHMV and TMV-B isolates were closely related to each other, but only antibodies to TMV-B reacted, albeit only at high primary antibody concentrations, with TMV-S and the TMV-C isolates with indirect ELISA. The immunoblot data support this finding. Since a distant serological relationship was reported between TMV and SHMV (13), whose capsid protein amino acid sequence differs by two-thirds from that of TMV (19), our serological data further support grouping TMV-S with the two common strain TMV isolates and grouping TMV-B with SHMV.

Our SDS-PAGE estimate of TMV-S capsid protein subunit M_r was higher (20.7 kDa) than the published value determined by amino acid analysis for the TMV type strain (17.5 kDa) (23) but within the range of SDS-PAGE estimates (18.5–21 kDa) reported for TMV-C capsid protein (3). Furthermore, the

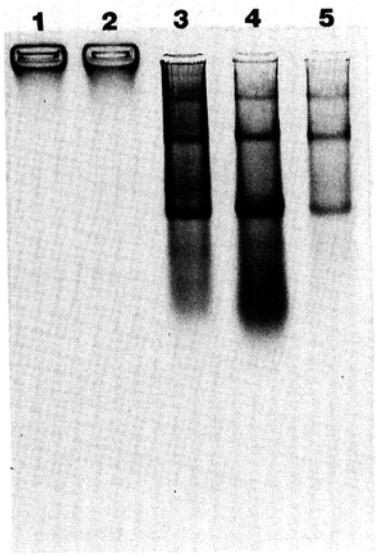

Fig. 6. Comparison of electrophoretic mobility of whole virions of tobacco mosaic virus (TMV) strains and sunnhemp mosaic virus (SHMV) in 1.2% agarose gels containing 40 mM borate buffer, pH 8.0, 0.25 mM EDTA, and 0.25 M urea. Whole virion (50–60 μg per lane) samples of TMV-B (lane 1), SHMV (lane 2), TMV-S (lane 3), and two common strain isolates of TMV, TMV-C/PV-135 (lane 4) and TMV-C/PV-220 (lane 5), were loaded into wells. Electrophoresis was conducted for 16 hr at 25 V at room temperature. The gel proteins were stained with Coomassie Brilliant Blue G-250.

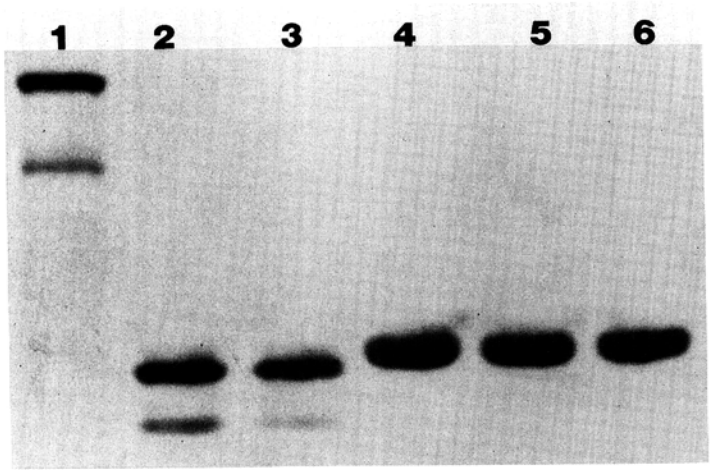

Fig. 5. Virion capsid protein subunits of purified tobacco mosaic virus (TMV) and sunnhemp mosaic virus (SHMV) were resolved in a 12% polyacrylamide gel containing sodium dodecyl sulfate. Standard marker proteins (lane 1, 24- and 29-kDa proteins are visible) and capsid proteins of TMV-B (lane 2), SHMV (lane 3), TMV-S (lane 4), TMV-C/PV-135 (lane 5), and TMV-C/PV-220 (lane 6) were stained with silver nitrate. Molecular mass of the TMV-S polypeptide was estimated to be 20.7 kDa.

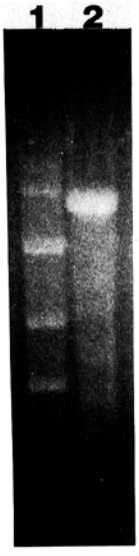

Fig. 7. Glyoxal-denatured RNA of commercial standard markers (9.49-, 7.46-, 4.4-, 2.37-, and 1.35-kb markers are visible in lane 1) and genomic RNA extracted from purified TMV-S virions (lane 2) were resolved in a 1.4% agarose gel. The gel was stained with ethidium bromide.

migration patterns of the TMV-S capsid protein subunits were identical to those of the TMV-C isolates tested. The capsid proteins of unfractionated SHMV preparations migrated more rapidly than those of TMV-C and as double bands when resolved by SDS-PAGE, as reported (26). The TMV-B capsid protein migration profile was identical to that of SHMV, presenting an additional similarity between these two viruses.

Electrophoretic mobility of whole virions in crude extracts or purified preparations has been used in TMV isolate characterization to separate virions and host constituents on the basis of particle size, surface charge, and sample heterogeneity (2,18,20). The TMV-S, TMV-C/PV-135, and TMV-C/PV-220 electrophoretic patterns were identical but easily distinguished from those of TMV-B and SHMV. Since the amino acid residues of the SHMV capsid protein differ markedly from those of the common strain of TMV (19), this may account for the difference in whole-virion mobility of the SHMV, as well as that of TMV-B, in the agarose gel.

The TMV-S nucleic acid M_r (6.4 kb) was similar to that reported for the type strain (6.395 kb) (9). The profiles of dsRNA from tobacco infected with TMV-C/PV-135 and TMV-S were identical, with no extraneous dsRNA detected in tobacco infected with TMV-S.

Although TMV-S was originally isolated from systemically infected soybean, our results demonstrated that it is a new strain of TMV and closely related serologically to TMV-C, but not to reported legume-infecting SHMV and TMV-B isolates. This study also showed that TMV-B is an isolate of SHMV. The presence of two or more particle sizes has not been reported for TMV-C, so further study of TMV-S is necessary. The nucleotide sequence of the TMV-S genome will be compared to those of other tobamovirus members in future studies, and the nature of the RNA in the short particles will be determined.

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