Etiology

Characterization of a California Isolate of Sorghum Yellow Banding Virus

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ABSTRACT

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A small isometric virus was isolated from sudangrass (*Sorghum sudanense*) in the Imperial Valley of California. The sap-transmissible virus was found to infect a narrow range of the gramineous hosts tested. The virus was not serologically related to several other small, well-characterized isometric viruses that infect the gramineae, including panicum mosaic virus, St. Augustine decline virus, and several members of the sobemovirus group. However, it did react with antiserum to sorghum yellow banding virus, a newly discovered and uncharacterized virus affecting commerical sorghum and sorghum × sudangrass hybrids in Texas. The virions are isometric, approximately 25 nm in diameter, and have an estimated S_{20w} of approximately 109. They contain a single species of single-stranded RNA of approximately 1.5×10^6 and a single capsid protein of approximately 29,000 MW. Two prominent double-stranded RNAs were isolated from infected *Zea mays*. These were approximately 3.0×10^6 and 0.6×10^6 MW and hybridized with cDNA prepared against the virion RNA.

More than 15 viruses with small isometric particles and a single genomic RNA species have been found to infect members of the gramineae. Hull (10) proposed that six of these, phleum mottle virus (PhMV), cocksfoot mild mosaic virus (CMMV), molinia streak virus (MoSV), festuca mottle virus (FeMV), brome stem mottle virus (BrSMV), and holcus transitory mottle virus (HTMV), be placed in a group called the phleum mottle virus group. Shared properties of viruses in this group include S_{20w} in the range of 105–120, genomic single-stranded RNA (ss-RNA) approximately 1.4×10^6 to 1.5×10^6 MW, capsid protein of 25,000 to 27,000 MW, and a host range restricted to the gramineae. Hull (10) also suggested three other viruses that might belong to this group: lolium mottle virus (LoMV), panicum mosaic virus (PMV), and St. Augustine decline virus (StADV).

Several small isometric viruses also infect the gramineae that are similar to southern bean mosaic virus and would fall in the sobemovirus group. These are cocksfoot mottle virus (CfMV) and rice yellow mottle virus (RYMV) with an S_{20w} of 104–120, ss-RNA approximately 1.4×10^6 to 1.5×10^6 MW, and a capsid protein of 28,000 to 31,000 MW.

The serological interrelationships of all the above viruses (except LoMV and RYMV) and maize chlorotic mottle virus (MCMV) and cynosurus mottle virus (CyMV) were described by Paul et al (18). These viruses were found to belong to three distinct serological groups consisting of MCMV, CyMV, CfMV, and a fourth large group divided into two subgroups. The first subgroup consisted of CMMV, PhMV, BrSMV, and HTMV. PMV, StADV, and MoSV were in the second subgroup. Viruses belonging to different groups were very distantly serologically related if at all.

Francki et al (6) reviewed Hull's suggestion that PhMV and a number of other viruses with similar properties be placed in a new taxonomic group. They suggested that PhMV, BrSMV, CMMV, FeMV, and HTMV might best be considered as strains of the same virus and placed in one subgroup. Because PMV was only remotely serologically related to PhMV, it was placed in a second subgroup with StADV and MoSV. LoMV and MCMV each were viewed to have properties sufficiently similar to both PhMV and PMV to be included in the same general taxonomic group and therefore were placed in a third and fourth distinct subgroup, respectively. CfMV and CyMV were placed with members of the sobemovirus group.

In this paper we report the isolation and physicochemical characterization of a small isometric virus infecting a sudangrass hybrid (*Sorghum sudanense* (Piper) Stapf. 'Double Dwarf') in California. This virus was found to be serologically unrelated to several of the above-mentioned viruses of gramineae. While our investigation was in progress, we became aware of the work of Giorda et al (8) on sorghum yellow banding virus (SYBV) in Texas. After exchanging and testing antisera, we found that the two viruses were closely related. The virus will be referred to as the California isolate of SYBV (SYBV-CA).

MATERIALS AND METHODS

Virus maintenance and host-range studies. SYBV-CA-infected plants were collected in the Imperial Valley of California, and extracts from these plants were mechanically inoculated to a range of plant species. Inoculum, prepared by grinding infected leaves 1:3 (w/v) in cold 0.03 M potassium phosphate and 0.1% Na_2SO_3, pH 7.0, was rubbed onto Celite-dusted leaves. Subsequent transfers were made by inoculating *Zea mays* L. seedlings at the two- to three-leaf stage. Infected tissue was harvested 15–20 days later and stored at −20 C for further analysis. Inoculated plants that did not develop symptoms were tested for SYBV-CA infection by immunodiffusion.

Virion purification. Virions were purified using modifications of methods for PMV (3) and brome mosaic virus (BMV) (2). Virions were further purified, regardless of the initial purification steps, by sedimentation in a 10–40% sucrose density gradient in 0.1 M potassium phosphate, pH 7.0, for 2.5 hr at 113,000 *g* (maximum) in an SW27 rotor (Beckman Instruments, Inc., Fullerton, CA). Gradients were fractionated using an ISCO Model 183 density gradient fractionation (ISCO, Inc., Lincoln, NE). Fractions containing SYBV-CA were pooled and centrifuged for 2 hr at 142,000 *g* (maximum) in a Beckman type 40 rotor (Beckman Instruments, Inc., Fullerton, CA). Pellets were resuspended in 0.02 M potassium phosphate, pH 7.0, and stored at 4 C.

Virions of turnip yellow mosaic virus (TYMV), PMV, and the cowpea strain of southern bean mosaic virus (CP-SBMV) were purified and used for standards. TYMV and CP-SBMV were purified as described by Bancroft et al (2), and PMV was purified as described by Buzen et al (3).

Electron microscopy. Purified SYBV-CA virions were placed on Formvar-coated grids, stained with 0.1% ammonium molybdate, and examined with a Zeiss EM 109 electron microscope (Carl Zeiss, Inc., Thornwood, NY).

Serology. Antisera were provided as follows: PMV, wheat streak mosaic virus (WSMV), and FeMV, C. L. Niblett; StADV and SYBV, R. W. Toler; PhMV and CMMV, H. L. Paul; maize white line mosaic virus (MWLMV), G. De Zoeten; and MCMV, S. A. Lommel. Antisera to maize stripe virus (MStV), maize mosaic virus (MMV), maize rayado fino virus (MRFV), BMV, and cucumber mosaic virus (CMV) were prepared previously in our laboratory. Plants infected with PMV and satellite panicum mosaic virus (SPMV) were provided by A. O. Jackson.

Antiserum was prepared to SYBV-CA virions in a New Zealand white rabbit. Equal volumes of purified SYBV-CA virions and Freund's adjuvant were mixed and the rabbit was injected intramuscularly once a week for 4 wk with 300 μg of virus in 600 μl of emulsion. Freund's complete adjuvant was used for the first injection and incomplete adjuvant was used in all subsequent injections. Two booster injections identical to previous injections were given 4 wk after the fourth injection. Initial bleedings began 2 wk after the last injection and at intervals for 3 wk.

Immunodiffusion tests were performed in 1% agarose and 0.1% sodium azide in 0.02 M potassium phosphate, pH 7.0. Antigen samples were prepared in 0.1 M potassium phosphate, pH 7.0. Antisera were used undiluted.

Analysis of virion protein and nucleic acid. SYBV-CA capsid protein was prepared for electrophoresis by disrupting virions in 10% ficoll, 2 M urea, and 1% sodium dodecyl sulfate (SDS). Proteins were reduced and alkylated with dithiothreital and iodoacetamide, respectively. Bromophenol blue was added as a tracking dye, and samples were analyzed by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) (11) in a mini slab unit (Idea Scientific, Corvallis, OR) at 200 V constant voltage for 1 hr at room temperature. The 0.8-mm-thick gel was composed of a 10-mm-high 5% stacking gel above a 55-mm-high 10, 12, or 15% resolving gel. Proteins were visualized by silver staining (17), and the SYBV-CA capsid protein molecular weight was estimated by comparing its mobility to low molecular weight standards (Bio-Rad Laboratories, Richmond, CA).

Nucleic acids were extracted from purified SYBV-CA virion preparations by mixing 1 v of the nucleoprotein with 1 v of 0.2 M Tris-HCl, 0.02 M ethylenediaminetetraacetic acid (EDTA), 1.5% SDS, 1.5% *n*-lauroyl sarcosine, pH 8.0, and 0.1 v of purified bentonite (5). After 20 min, 2 v of phenol (pH 8.0) containing 0.1% 8-hydroxyquinoline was added; the sample was shaken for 10 min and centrifuged at 10,810 *g* (maximum) for 5 min. The aqueous phase was reextracted with 2 v of phenol and 1 v of chloroform:pentanol (25:1), and the nucleic acids were precipitated with ethanol. Tobacco mosaic virus (TMV), BMV, TYMV, PMV, and SPMV virion RNAs were extracted by the same method and used as molecular weight markers.

Nucleic acids were electrophoresed using the glyoxal-DMSO (dimethyl sulfoxide) denaturing system (15), except that buffers and the gel contained 0.02 M *N*-(2-hydroxyethyl)piperazine-*N*'-2-ethanesulfonic acid (HEPES) buffer, pH 7.0, and 1 mM EDTA. Electrophoresis was at room temperature in 10-cm-long × 6.5-cm-wide × 3-mm-thick horizontal slab gels of 1.0% agarose for 1.5 hr at 100 V. Nucleic acids were stained with ethidium bromide, visualized over ultraviolet illumination, and photographed using Polaroid Type 665 positive-negative film.

Extraction and analysis of total and double-stranded RNAs from plant tissue. Double-stranded (ds) RNAs were extracted and purified from SYBV-CA-infected *Z. mays* using the method of Morris and Dodds (16). Ds-RNAs then were precipitated with ethanol and analyzed by electrophoresis on mini slab gels composed of 6% polyacrylamide using the Tris-sodium acetate buffer system of Loening (12). Electrophoresis was for 3 hr at 100 V constant voltage at room temperature. After electrophoresis, gels were stained as for the virion RNAs. Ds-RNAs from CP-SBMV, PMV, SPMV, TMV, and BMV were used to estimate molecular weights.

Total RNAs were extracted from healthy *Z. mays* by the method of Dawson (4). Ss-RNAs and ds-RNAs were separated by 2 M LiCl fractionation (1). Total ss-RNAs were resuspended in sterile water and stored until further analysis.

cDNA synthesis and northern hybridization. An oligodeoxynucleotide mixture, pd(N) (Pharmacia, Piscataway, NJ), was used to randomly prime SYBV-CA virion RNA, and first-strand cDNA was synthesized with cloned Moloney murine leukemia virus (M-MLV) reverse transcriptase (BRL, Gaithersburg, MD). Ten μg of SYBV-CA RNA from virions purified by the method of Buzen et al (3) was incubated in 50 mM Tris-HCl, pH 8.3, 75 mM KCl, 3 mM MgCl2, 10 mM dithiothreitol, 500 μM each of 2'-deoxyadenosine 5'-triphosphate (dATP), 2'-deoxycytidine 5'-triphosphate (dCTP), 2'-deoxyguanosine 5'-triphosphate (d GTP) and 2'-deoxy-thymidine-5'-triphosphate, (dTTP), 2 μg of pd(N), 50 μCi of ^{32}P-dCTP, and 500 units of M-MLV in a 50-μl volume at 37 C for 1 hr. The resulting RNA-DNA hybrid then was subjected to an alkaline hydrolysis (14) and stored until further analysis.

RNA samples (SYBV-CA virion ss-RNAs, ds-RNAs, and total ss-RNAs from healthy *Z. mays*) were denatured with glyoxal, electrophoresed on agarose gels as described above, and transferred to nitrocellulose (19). After the blots were baked, they were prehybridized at 43 C for 3 hr in a solution containing 50% formamide, 4 × SSPE (20 × stock = 3.6 M NaCl, 0.2 M sodium phosphate, pH 7.0, 0.2 M EDTA), 1% SDS, 0.5% BLOTTO (10% stock = 10 g of nonfat powdered milk in 100 ml of sterile water), and 0.5 mg/ml of salmon sperm DNA. ^{32}P-labeled cDNA was added and hybridized overnight at 43 C. Blots then were washed at room temperature for three 15-min periods in solutions of 2 × standard saline citrate (SSC)/0.1% SDS (20 × SSC stock, 3 M NaCl, 0.3 M trisodium citrate), 0.5 × SSC/0.1% SDS, and 0.1 × SSC/0.1% SDS. The final wash was at 50 C for 30 min in 0.1 × SSC/1% SDS. The hybridized blots were exposed on Kodak X-ray film at −70 C.

RESULTS

Host range. Naturally infected sudangrass showed chlorotic streaks on the leaves (Fig. 1). Symptoms in the greenhouse were similar, regardless of host species, and appeared 15–20 days after inoculation. In some cases the plants continued to grow while older leaves lost their symptoms and yellow streaks appeared on new

leaves. In other cases the plants became dwarfed, chlorotic, and eventually died.

SYBV-CA was mechanically transmitted to 5–60% of inoculated *S. sudanense* 'Piper' and 'Double Dwarf,' *Z. mays* 'SDP 2' and 'N28,' *Setaria italica* (L.) P. Beauv., and *Pennisetum glaucum* R. Br. The following plants were not hosts of SYBV-CA: *Cynodon dactylon* (L.) Pers., *Setaria lutescens* (Wiegel) Huff., *Sorghum bicolor* (L.) Moench, *Phaseolus vulgaris* L. 'Black Bean,' *Vigna unguiculata* (L.) Walp. 'California Blackeye #5,' *Chenopodium amaranticolor* Coste & Reyn., *Chenopodium quinoa* Willd., *Nicotiana clevelandii* A. Gray, *Hordeum vulgare* L. 'Barley 21,' *Avena sativa* L. 'California Red Oats,' and *Z. mays* 'NK 199' and 'Golden Bantam.' No symptomless hosts were detected by immunodiffusion.

Virion purification. A single sedimenting component was detected after centrifugation in sucrose gradients, whereas similar components were not present in preparations from healthy *Z. mays* (Fig. 2). This component co-sedimented with PMV which has an S_{20w} of 109 S. Yields of purified virions were approximately 15 mg/100 g of tissue using the extinction coefficient of 5.8 given for SBMV (9).

Electron microscopy. Examination of purified virus consistently revealed isometric particles approximately 25 nm in diameter (Fig. 3). Both electron-dense and electron-lucent particles were observed in the same single band removed from sucrose density gradients.

Serology. SYBV-CA antigens failed to react with antisera to BMV, WSMV, PMV, StADV, CMV, MRFV, MWLMV, MStV, MMV, FMV, CMMV, MCMV, and PhMV in immunodiffusion tests. SYBV-CA antigens did react with antiserum made to the Texas isolate of SYBV. The antiserum made to purified SYBV-CA reacted with SYBV-CA-infected plant tissue and purified SYBV-CA, but not with extracts from healthy plants. The SYBV-CA antiserum also reacted with SYBV-Texas-infected tissue (R. W. Toler, *personal communication*).

Analysis of virion proteins and nucleic acid. A single virion capsid protein of approximately 29,000 MW was resolved by SDS-PAGE on 12% polyacrylamide gels (Fig. 4). The SYBV-CA protein migrated slightly faster than CP-SBMV and PMV capsid proteins. Their relative mobilities were identical on 10 and 15% polyacrylamide gels. No proteins were found in similar preparations from healthy plants.

A single major species of ss-RNA was seen when SYBV-CA virion RNA was run in 1% agarose gels using the glyoxal-DMSO denaturing system (Fig. 5). By comparing the mobility of SYBV-CA RNA to ss-RNAs from TMV, BMV, PMV, and SPMV, we estimated SYBV-CA RNA to be approximately 1.5×10^6 MW. RNA preparations from virions purified using potassium phosphate contained two less prominent ss-RNAs (1.0×10^6 and 0.4×10^6 MW) below the 1.5×10^6 virion RNA (Lane 5, Fig. 5).

Fig. 1. Leaf symptoms of the California isolate of sorghum yellow banding virus on *Sorghum sudanense*.

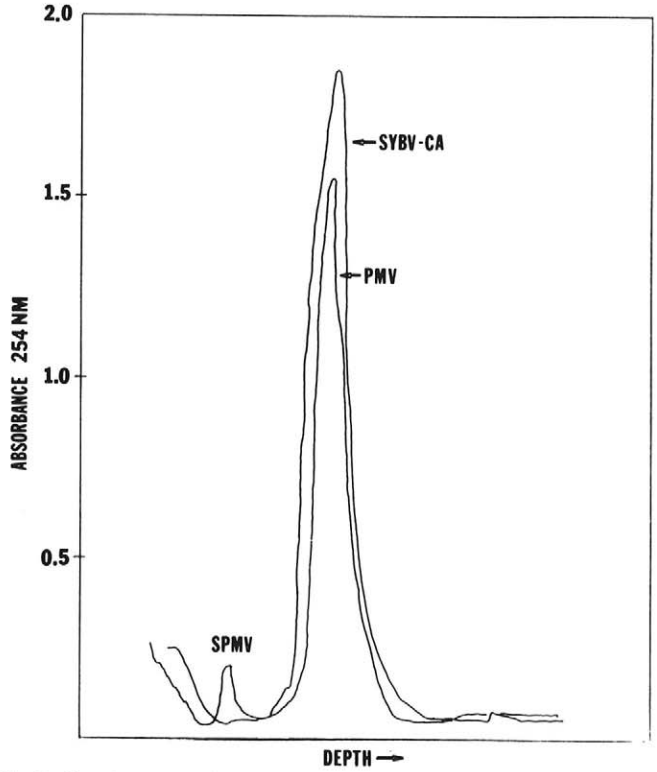

Fig. 2. Absorbance profile of fractionated sucrose density gradients after purified virions of California sorghum yellow banding virus (SYBV-CA) were centrifuged for 2.5 hr at 113,000 *g* (maximum) in an SW 27 rotor at 4 C. Panicum mosaic virus (PMV) (109 S) and satellite panicum mosaic virus (SPMV) (42 S) virions were used as markers.

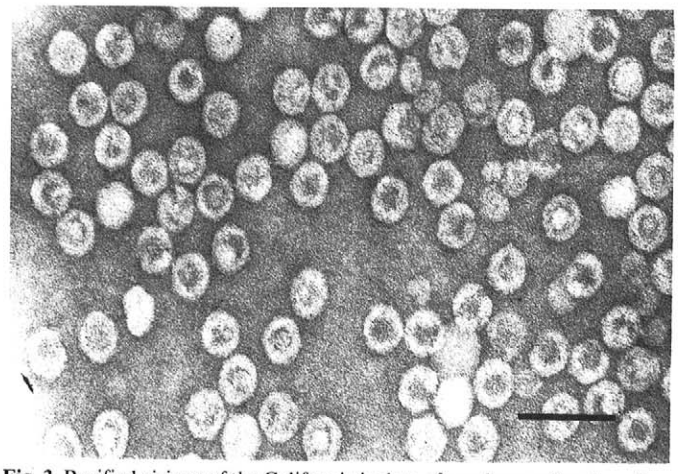

Fig. 3. Purified virions of the California isolate of sorghum yellow banding virus negatively stained with 0.1% ammonium molybdate. The bar represents 50 nm.

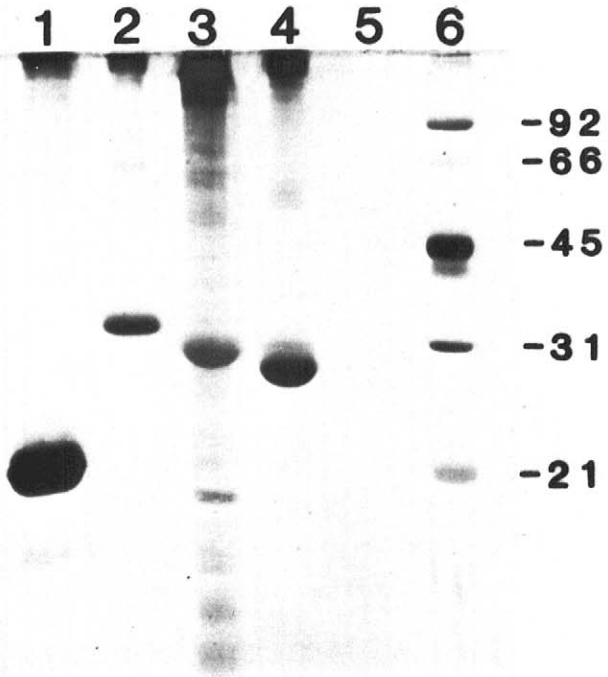

Fig. 4. Sodium dodecyl sulfate-polyacrylamide (12% acrylamide) slab gel showing virion capsid proteins. Lane 1, turnip yellow mosaic virus; 2, cowpea strain southern bean mosaic virus; 3, panicum mosaic virus and satellite panicum mosaic virus; 4, California isolate of sorghum yellow banding virus; 5, extract from healthy *Zea mays*; and 6, molecular weight standards in order of decreasing mass: phosphorylase B, bovine serum albumin, ovalbumin, carbonic anhydrase, and soybean trypsin inhibitor.

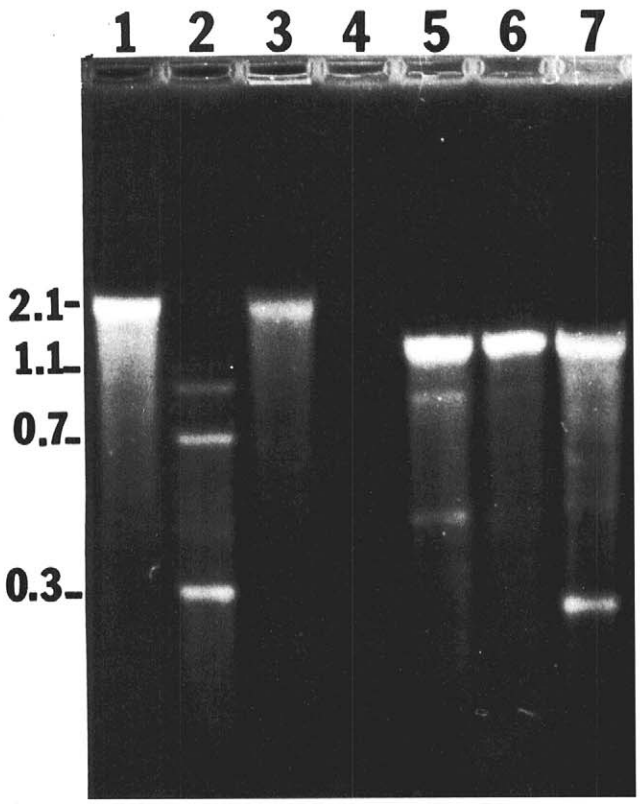

Fig. 5. A 1% agarose gel showing virion RNAs after denaturation with glyoxal and DMSO and subsequent electrophoresis. Lane 1, tobacco mosaic virus RNA; 2, brome mosaic virus RNAs; 3, turnip yellow mosaic virus RNA; 4, extract from healthy *Zea mays*; 5, potassium phosphate-purified California isolate of sorghum yellow banding virus RNA; 6, sodium acetate-purified California isolate of sorghum yellow banding virus RNA; and 7, panicum mosaic virus and satellite panicum mosaic virus RNAs.

These were not detected in similar RNA preparations from virions purified using sodium acetate (Lane 6, Fig. 5). It was not immediately clear whether these minor ss-RNAs represented encapsidated RNAs, degradation products, or contaminating host RNAs. Ds-RNAs from infected tissue and northern hybridization of virion and ds-RNAs were used to further address this question.

Ds-RNAs extracted from SYBV-CA-infected *Z. mays* differed in mobility from PMV, SPMV, and CP-SBMV ds-RNAs when compared by PAGE (Fig. 6). The larger ds-RNA (3.0×10^6 MW) was estimated to be twice the size of SYBV-CA ss-RNA and is presumed to be the replicative form of this RNA. The lower molecular weight ds-RNA, which migrated slightly slower than SPMV ds-RNA and slightly faster than the smaller CP-SBMV ds-RNA, was estimated to be approximately 0.60×10^6 MW. This pattern and the molecular weight estimates differed appreciably from that expected if the two less prominent ss-RNAs seen in some virion RNA preparations (Lane 5, Fig. 5) were in fact virion RNAs. No ds-RNAs were detected that corresponded in size estimate with these minor RNAs, and no ss-RNA was seen in virion RNA preparations that corresponded to the smaller (0.6×10^6 MW) ds-RNA.

The relationships of SYBV-CA ds-RNAs and virion ss-RNA were investigated using northern hybridization. SYBV-CA virion ss-RNA hybridized with the cDNA probe to produce one prominent band corresponding to the 1.5×10^6 virion ss-RNA (Fig. 7). There was no evidence for hybridization with the two minor ss-RNAs seen in Lane 5, Figure 5. Ds-RNAs purified from SYBV-CA-infected *Z. mays* hybridized with the cDNA probe to produce two bands that corresponded in size estimate to the two ds-RNA bands seen in Lane 2, Figure 6. Weak reactions were seen with healthy total RNA extracts (data not shown), but these were much less intense than the virion RNA reaction and were of lower molecular weight than the two less prominent ss-RNAs seen in Lane 5, Figure 5. These hybridization reactions, along with the ds-RNA profile, suggest that the two minor ss-RNA bands in question are most likely degradation products. In addition, the

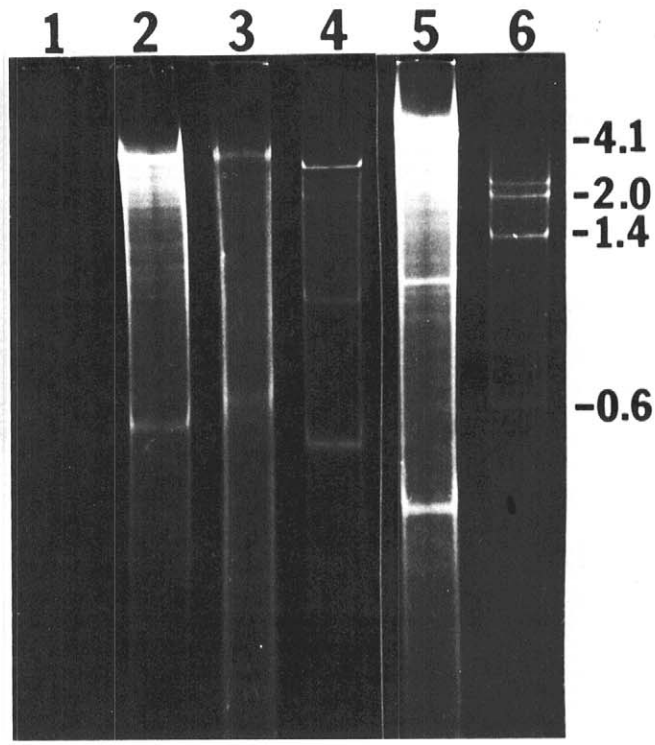

Fig. 6. A 6% polyacrylamide slab gel showing double-stranded (ds) RNAs. Lanes represent ds-RNAs extracted from: 1, healthy *Zea mays*; 2, *Z. mays* infected with California isolate of sorghum yellow banding virus; 3, *Vigna unguiculata* infected with cowpea strain of southern bean mosaic virus; 4, *Setaria italica* infected with panicum mosaic virus and satellite panicum mosaic virus; 5, *Nicotiana tabacum* infected with tobacco mosaic virus; and 6, *Hordeum vulgare* infected with brome mosaic virus.

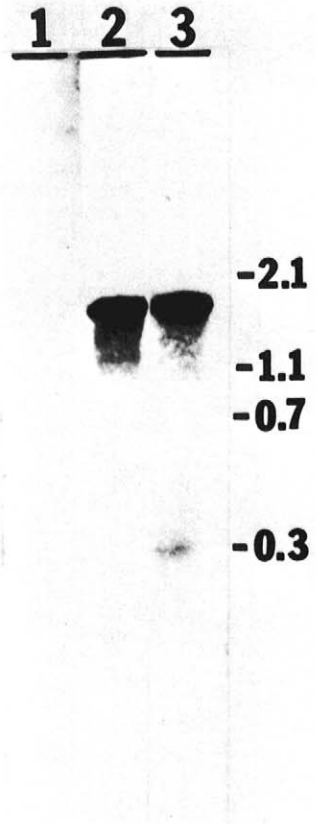

Fig. 7. Northern blot analysis of California isolate of sorghum yellow banding virus (SYBV-CA) single-stranded and double-stranded (ds) RNAs denatured with glyoxal and DMSO, electrophoresed through a 1% agarose gel, transferred to nitrocellulose, and hybridized with a ^{32}P-labeled cDNA probe made to the SYBV-CA. The three lanes contain RNAs from: 1, panicum mosaic virus and satellite panicum mosaic virus; 2, SYBV-CA; and 3, ds-RNA extracted from *Zea mays* infected with SYBV-CA.

presence of a single nonencapsidated subgenomic RNA is indicated by the smaller ds-RNA (0.6×10^6 MW).

PMV virion RNA did not hybridize with the cDNA probe, indicating that there is little or no sequence homology between the SYBV-CA and PMV virion RNAs.

DISCUSSION

AO ss-RNA molecular weight of approximately 1.5×10^6 for SYBV-CA RNA is a taxonomic character that places SYBV-CA with several other small isometric viruses of the gramineae. The SYBV-CA capsid protein molecular weight of 29,000 is closer in size to that of PMV, which has a molecular weight of 30,000 (3), than to the size of the coat protein of the other isometric viruses of gramineae. However, there was no serological cross-reactivity with any of the tested viruses except the Texas isolate of SYBV which also was estimated to have a capsid protein of 29,000 MW (7). SYBV-CA and PMV are further distinguished because SYBV-CA-infected plants contained a prominent small ds-RNA that differed in mobility from the subgenomic ds-RNA of PMV (13). Although the smaller ds-RNA for SYBV-CA has a similar electrophoretic mobility to ds-RNA from SPMV, it hybridizes with cDNA to the SYBV-CA virion RNA and therefore is suggestive of a subgenomic mRNA which is produced in SYBV-CA-infected plants but is not encapsidated. The lack of a satellite virus is also suggested by the observation that SYBV-CA virion preparations contain a single component sedimenting at approximately 109 S whereas PMV and SPMV show two components sedimenting at 109 S and 42 S (3). Furthermore, there was no indication of a second capsid protein similar to that of SPMV. The ds-RNA pattern for SYBV-CA resembled that of CP-SBMV with ds-RNAs of 0.76×10^6 and 3.0×10^6.

The taxonomy of small isometric viruses of the gramineae is complex. Francki et al (6) treated even fairly well-characterized viruses such as PMV as ungrouped. Based on present knowledge, SYBV-CA also fits this category.

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