Molecular Characterization of Sorghum Chlorotic Spot Virus, a Proposed Furovirus

By T. L. KENDALL,1† W. G. LANGENBERG2 AND S. A. LOMMEL1*

1Department of Plant Pathology, Throckmorton Hall, Kansas State University, Manhattan, Kansas 66506 and 2Agricultural Research Service, United States Department of Agriculture and Department of Plant Pathology, University of Nebraska, Lincoln, Nebraska 68583, U.S.A.

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SUMMARY

A virus morphologically and physicochemically similar to the type member of the furovirus group, wheat soil-borne mosaic virus (WSBMV), has been isolated from Sorghum bicolor and partially characterized. The virus, sorghum chlorotic spot virus (SCSV), causes symptoms which include distinct elongated chlorotic spots and ring spots as well as yellowing on systemically infected leaves of sorghum and inbred Zea mays lines. SCSV is mechanically transmissible to and produces symptoms on the inoculated leaves of Nicotiana clevelandii, Chenopodium amaranticolor and C. quinoa.

The virus is bipartite with two distinct rods, 20 nm in diameter and 260 and 140 nm in length. Virions are composed of a single 20.5K capsid protein and two non-homologous, non-polyadenylated, genomic RNAs of approx. 6.2 kb ($M_r$ $2.2 \times 10^6$) for RNA-1 and 3.5 kb ($M_r$ $1.2 \times 10^6$) for RNA-2. SCSV and WSBMV capsid proteins are serologically related as determined by Western blot and immunogold cytochemical analysis. Northern blot hybridizations indicated that there is no homology between SCSV RNA and WSBMV RNA under high stringency conditions. Unfractionated SCSV RNAs direct the synthesis of 180K, 170K, 110K, 50K, 42K, 25K and 20.5K polypeptides in vitro. The 110K, 25K and 20.5K products are immunoprecipitated by antiserum raised against SCSV capsid protein. Comparative in vitro translation analysis with WSBMV suggests that the SCSV capsid protein cistron resides on the 5' terminus of RNA-2. A 1.8 kb cDNA clone was synthesized using SCSV RNA-2. T7 transcripts from this clone directed the synthesis of several polypeptides, none of which was immunoprecipitated by antiserum to the capsid protein. SCSV is similar to, but distinct from, WSBMV and is proposed to be a new member of the furovirus group.

INTRODUCTION

The furovirus group is characterized by viruses with rigid, hollow, rod-shaped virions, plasmodiophoraceous fungal vectors, and a divided genome (Shirako & Brakke, 1984a). Wheat soil-borne mosaic virus (WSBMV) is the type member of the furovirus group (Shirako & Brakke, 1984a) and several other plant viruses have been suggested as members, including beet necrotic yellow vein virus (BNYVV) (Tamada, 1975; Putz, 1977), potato mop-top virus (PMTV) (Harrison, 1974; Roberts & Harrison, 1979), Hypochoeris mosaic virus (Brunt & Stace-Smith, 1978), Nicotiana velutina virus (Randles et al., 1976), and peanut clump virus (Thouvenel et al., 1976; Thouvenel & Fauquet, 1981).

Several of these viruses have been extensively characterized at the molecular level. The BNYVV genome has been cloned as cDNA and completely sequenced (Bouzoubaa et al., 1985, 1986, 1987) and in vitro translation studies have been used to determine its genomic organization

† Present address: Department of Plant Pathology, Box 7616, North Carolina State University, Raleigh, North Carolina 27695-7616, U.S.A.
(Richards et al., 1985; Ziegler et al., 1985). Likewise, an Indian isolate of peanut clump virus (IPCV) has been characterized (Reddy et al., 1983) by in vitro translation (Mayo & Reddy, 1985). WSBMV has been extensively studied (Tsuchizaki et al., 1975; Brakke, 1977; Shirako & Brakke, 1984a, b; Hsu & Brakke, 1985; Shirako & Ehara, 1986) and a considerable amount of information concerning the genomic organization of the virus has been deduced.

A comparison of physicochemical properties and the genomic organization of WSBMV, BNYVV and IPCV indicates that these viruses are indeed similar and share features consistent with characteristics of the furovirus group. WSBMV and IPCV, in particular, are morphologically and physicochemically very similar (Shirako & Brakke, 1984a; Brakke, 1977; Tsuchizaki et al., 1975; Reddy et al., 1983; Mayo & Reddy, 1985) and have a common vector, Polymyxa graminis Led. (Estes & Brakke, 1966; Rao & Brakke, 1969; Brakke et al., 1965; Reddy et al., 1983).

Some discrepancies are apparent between the proposed members of the furovirus group, particularly between WSBMV and BNYVV. Although both viruses have plasmidiophoraceous fungus vectors, the vector of BNYVV is P. betae (Tamada, 1975; Fujisawa & Sugimoto, 1976) rather than P. graminis. BNYVV is composed of four discrete RNAs separately encapsidated in four rod-shaped virions (Putz, 1977; Putz et al., 1983; Richards et al., 1985) and BNYVV RNA is polyadenylated (Putz et al., 1983) whereas WSBMV contains no polyadenylated sequence (Bouzoubaa et al., 1986). BNYVV and IPCV are mechanically transmissible and infect dicotyledonous plants whereas WSBMV is essentially not mechanically transmissible and infects monocotyledonous plants. Molecular characterization of the genome of WSBMV or other WSBMV-like furoviruses is needed to define in greater depth areas of observed discrepancies within the furovirus group.

A virus that is morphologically and physicochemically similar to WSBMV has been isolated from sorghum, Sorghum bicolor. Sorghum chlorotic spot virus (SCSV) is the name proposed for this virus as the dominant symptom in infected sorghum is an elongated chlorotic spot. SCSV is mechanically transmissible to several monocotyledonous and dicotyledonous hosts and is serologically related to WSBMV. Results from the characterization of SCSV indicate that it is more similar to WSBMV than to other members of the furovirus group. The possible use of SCSV to define further the genomic organization of WSBMV and, more specifically, to identify the genes responsible for mechanical transmissibility, host specificity and vector transmission, is discussed.

**METHODS**

*Virus host range, maintenance and purification.* SCSV was mechanically inoculated to Chenopodium amaranticolor, C. quinoa, S. bicolor, Triticum aestivum, Zea mays, Arachis hypogaea, and N. clevelandii. Leaves from field-collected sorghum that were showing symptoms were ground in 10 mM-potassium phosphate buffer pH 7.0 with 1% Celite (w/v) and inoculated to maintenance and host range plants. Inoculated plants were maintained in the glasshouse at a constant temperature of 25 °C.

Virus for purification was grown in Z. mays (N28Ht, an inbred maize line). Virus was purified from systemically infected leaves using the method described for WSBMV (Shirako & Brakke, 1984a), except that tissue was ground in liquid nitrogen and then allowed to thaw in extraction buffer rather than being homogenized in a blender.

*Capsid protein analysis and serum production.* Virus was denatured and electrophoresed in a 12% SDS-polyacrylamide gel using the discontinuous buffer system of Laemmli (1970). Protein bands were stained with Coomassie Brilliant Blue R after fixing in 50% methanol and 10% acetic acid (v/v).

Denatured capsid protein for antiserum production was isolated by SDS-PAGE. Capsid protein was visualized by incubating gels in cold 250 mM-KCl, followed by excision and elution of the protein from the matrix by grinding. Immunization with purified protein (1 mg/ml) was performed as described by Lommel et al. (1982). Antiserum collected 6 weeks after the initial injection was used in Western blot and in vitro translation studies.

Protein was transferred from SDS-PAGE gels to nitrocellulose in Towbin's buffer (Towbin, 1979) by electroblotting for 4.5 h at a constant current of 120 mA. Following transfer, nitrocellulose was fixed, probed with WSBMV or SCSV antiserum and stained with Protein A-alkaline phosphatase (Leach et al., 1987).

*Electron microscopy.* Immunostaining was carried out as described by Langenberg (1985). Colloidal gold enhancement of IgG-decorated virus particles by goat anti-rabbit IgG-colloidal gold treatment was performed according to the two-step method of Lin & Langenberg (1983). Preparations were examined using a Philips EM-201 transmission electron microscope.
**Characterization of SCSV**

**RNA extraction and analysis.** Virion RNA was extracted according to the procedure of Morris et al. (1979) and electrophoresed under both non-denaturing and denaturing conditions. Non-denaturing agarose gels were prepared in 90 mM-Tris pH 8.0, 90 mM-boric acid and 2 mM-EDTA. For Northern blot analysis RNA was separated by formaldehyde-denaturing agarose gel electrophoresis (Rave et al., 1979).

Viral RNA was fractionated on an oligo(dT)-cellulose column. Polyadenylated RNA was eluted from the column with 10 mM-Tris–HCl pH 7.5, 1 mM-EDTA and 0.05% SDS (w/v). Polyadenylated and non-polyadenylated RNA fractions were recovered and assayed by agarose gel electrophoresis. Cowpea mosaic virus (CPMV) was used as polyadenylated positive (El Manna & Bruening, 1973) and WSBMV as a negative control (Bouzoubaa et al., 1986).

**cDNA cloning of SCSV RNA.** Purified viral RNA (10 μg) was enzymically polyadenylated and primed with oligo(dT) for the first-strand cDNA synthesis using Moloney murine leukaemia virus reverse transcriptase as described by Carrington & Morris (1984). Second-strand synthesis and homopolymer tailing was performed as described by Gubler & Hoffman (1983). The dC-tailed double-stranded cDNA was then annealed to PstI-cleaved dG-tailed pBR322 as described by Maniatis et al. (1982). Competent Escherichia coli strain RR1 cells were prepared and transformed by the calcium chloride procedure (Maniatis et al., 1982). Transformants recovered from tetracycline plates were analysed for inserts by the alkaline lysis minipreparation method (Birnboim & Doly, 1979).

**Northern blot hybridization.** Northern blots were hybridized with SCSV and WSBMV cDNA probes and a nick-translated SCSV cDNA clone. SCSV and WSBMV randomly primed 32P-labelled cDNA and 32P-labelled nick-translated SCSV cDNA clone probes were synthesized according to Maniatis et al. (1982). SCSV and WSBMV RNAs were electrophoresed under denaturing conditions for 3 h at 120 mA and transferred to GeneScreen Plus (DuPont), prehybridized and hybridized according to the manufacturer’s directions.

**In vitro transcription and translation.** pSCS007, the largest SCSV-specific cDNA clone (1.8 kb), was subcloned into the PstI site of the transcription vector, pGEM-blue. T7 and SP6 transcripts were synthesized from (T7) HindIII- and (SP6) EcoRI-linearized plasmid. Viral RNA and the RNA transcripts generated in vitro were translated in rabbit reticulocyte lysates (Green Hectares, Madison, Wis., U.S.A.). The lysate was made dependent on exogenous RNA by micrococcal nuclease treatment (Pelham & Jackson, 1976; Dougherty & Hiebert, 1980). In vitro translation was carried out as described by Dougherty & Hiebert (1980). The 32S-labelled translation products were analysed by 12% SDS–PAGE followed by fixing and impregnation with fluor. The gel was then dried and fluorographed at −70 °C. Immunoprecipitation of in vitro translation products was carried out as described by Hiebert & Purcifull (1981) with Staphylococcus aureus strain Cowan I (10% suspension) and polyclonal antiserum raised against SCSV capsid protein. The immunoprecipitated proteins were analysed by SDS–PAGE and fluorography.

**RESULTS**

**Biological properties**

SCSV was mechanically transmitted to *Z. mays* (N28Ht), *C. amaranticolor*, *C. quinoa*, and *N. clevelandii* (Table 1). *Z. mays* was the only plant in which inoculation resulted in a systemic infection. Systemic symptoms included elongated chlorotic spots, yellowing, and a mild mosaic on uninoculated tissue. Spots sometimes contained green centres and resembled the ringspots that are typical of infection by several dicotyledonous plant viruses. No necrosis was observed in infected tissues. Purification of SCSV from infected *Z. mays* resulted in 40 to 100 μg virus/g tissue. Infected *N. clevelandii* exhibited irregular necrotic lesions and a general yellowing on inoculated leaves. Purification of SCSV from those inoculated leaves of *N. clevelandii* that were exhibiting symptoms yielded 200 to 500 μg virus/g tissue indicating that replication occurs in inoculated tissue but that movement out of the initially inoculated leaves appears to be limited (Table 1). Distinct chlorotic local lesions were produced on inoculated leaves of *C. amaranticolor* and concentric rings on *C. quinoa*. Inoculations on *S. bicolor*, *A. hypogaea*, and several monogenic WSBMV-susceptible and resistant varieties of *T. aestivum* did not result in symptoms or infection as determined by back inoculation of inoculated tissue to *C. amaranticolor* (Table 1).

Temperature appeared to be an important parameter in disease development and symptom expression. The percentage of infected *Z. mays* was much higher when the plants were maintained at a relatively constant temperature of 25 °C. During the hot summer months when glasshouse temperatures frequently exceed 35 °C, percentage infection in N28Ht was considerably lower and often less than 10%. Symptom development in *Chenopodium* spp. and
Table 1. *Host range, symptomatology and infection of SCSV*

<table>
<thead>
<tr>
<th>Host</th>
<th>Inoculated</th>
<th>Systemic</th>
<th>Infection (%)†</th>
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<tr>
<td>Chenopodiaceae</td>
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</tr>
<tr>
<td>Chenopodium amaranticolor</td>
<td>L</td>
<td>–</td>
<td>100</td>
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<tr>
<td>C. quinoa</td>
<td>R</td>
<td>–</td>
<td>100</td>
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<tr>
<td>Gramineae</td>
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<tr>
<td>Sorghum bicolor</td>
<td>–</td>
<td></td>
<td>–</td>
</tr>
<tr>
<td>Triticum aestivum</td>
<td>–</td>
<td></td>
<td>–</td>
</tr>
<tr>
<td>Zea mays (N28Ht)</td>
<td>–, Y‡</td>
<td>S, Y, M</td>
<td>15–75§</td>
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<tr>
<td>Leguminosae</td>
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<tr>
<td>Arachis hypogaea</td>
<td>–</td>
<td></td>
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<tr>
<td>Solanaceae</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nicotiana clevelandii</td>
<td>Y, N</td>
<td>–</td>
<td>100</td>
</tr>
<tr>
<td>N. tabacum cv. Turkish</td>
<td>–</td>
<td>–</td>
<td>–</td>
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<tr>
<td>N. tabacum cv. Turkish (NN)</td>
<td>–</td>
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* Y, yellowing; N, irregular necrotic lesion; R, ringspot; L, chlorotic local lesion; S, elongated chlorotic spot; M, mild mosaic; –, no symptoms and infection at 25 °C, as assayed by back inoculation to *C. amaranticolor* of inoculated (for local) and uninoculated (for systemic infection) leaf extracts.
† Percentage of plants expressing either systemic or local symptoms after inoculation, and assay for infection by back assay to *C. amaranticolor*.
‡ Mild or non-existent symptoms but virus present in the inoculated tissue.
§ Temperature-dependent.

*N. clevelandii* did not appear to be temperature-dependent. Attempts to induce infection by planting host plants either at the location from which SCSV was originally isolated, or by planting into field-collected soil, were unsuccessful (data not shown).

**Physicochemical properties**

The $M_r$ of the single SCSV capsid protein was estimated to be 20.5K by SDS–PAGE; this is similar in size to the 19.7K WSBMV capsid protein (Shirako & Brakke, 1984a, b) (Fig. 1). The capsid protein was associated with two rigid rod-shaped components of discrete size. Each component measured 20 nm in diameter and either 260 or 140 nm in length and had a hollow centre (Fig. 2). The larger virion was always present in higher concentrations than the smaller virion, as observed by electron microscopy of purified virus (Fig. 2).

Two RNA species were observed after electrophoresis of phenol-extracted SCSV virions (Fig. 3). The $M_r$ of the largest RNA species, RNA-1, was approx. $2.2 \times 10^6$ (6200 nucleotides) and RNA-2 was approximately $1.2 \times 10^6$ (3500 nucleotides); these values are similar to the 2.28 × 10^6 (6500 nucleotides) and 1.23 × 10^6 (3500 nucleotides) reported for WSBMV RNA-1 and RNA-2, respectively (Gumpf, 1971; Shirako & Brakke, 1984a). As illustrated in Fig. 3, the relative concentrations of RNA-1 and RNA-2 from purified virions were the opposite of those reported for WSBMV (Shirako & Brakke, 1984a). The concentration of WSBMV RNA-2 was approximately 10 times higher than RNA-1, and at times, RNA-1 was not detected. SCSV yielded a much greater amount of RNA-1 than RNA-2. In addition to RNA-1 and RNA-2, electrophoretic analysis of RNA liberated from SCSV virions consistently resulted in a diffuse band of low $M_r$ nucleic acid (Fig. 3).

Unfractionated SCSV virion RNA did not bind to oligo(dT)–cellulose (Fig. 3), indicating the lack of a significant polyadenylated sequence located either internally or terminally. Non-polyadenylated WSBMV RNA (Bouzoubaa *et al.*, 1986) did not bind to the column, whereas polyadenylated CPMV RNA (El Manna & Bruening, 1973) was bound (Fig. 3).
Characterization of SCSV

Fig. 1. Analysis of proteins separated by SDS-PAGE detected by Coomassie Brilliant Blue R and alkaline phosphatase staining of Western blots. Lane S contains M, standards: phosphorylase B 97.4 K, bovine serum albumin 68 K, ovalbumin 43 K, α-chymotrypsinogen 25.7 K, β-lactoglobulin 18.4 K, and lysozyme 14.3 K. Lanes 1 and 2 contain purified SCSV and WSBMV capsid proteins respectively. Panel (a) is a silver-stained SDS–PAGE gel and panels (b) and (c) are Western blots of SDS–PAGE gels probed with anti-SCSV and anti-WSBMV antibodies respectively.

Fig. 2. Electron micrographs of SCSV particles (a) purified using the sodium borate purification procedure published for WSBMV (Shirako & Brakke, 1984); (b) immunostained with WSBMV capsid protein antiserum; and (c) immunostained with TMV capsid protein antiserum. The antiserum bound antigen was detected by gold-labelled goat anti-rabbit IgG.

Serological relationships with other furoviruses

Immunospecific electron microscopy was performed to ascertain the serological relatedness of SCSV to WSBMV. Leaf dip preparations of SCSV-infected *Z. mays* were made in WSBMV, tobacco mosaic virus (TMV), and barley yellow mosaic virus (BaYMV N & M strain antisera; W. Huth, Institut für Viruskrankenheiten der Pflanzen, Braunschweig, F.R.G.) capsid protein
Fig. 3. Ethidium bromide-stained agarose gel following electrophoresis of viral RNA. Lanes 1 and 2 contain SCSV and WSBMV virion RNA respectively. SCSV, WSBMV and CPMV virion RNAs were passed through an oligo(dT)-cellulose column and separated into unbound (lanes 3, 5 and 7 respectively) and bound (lanes 4, 6 and 8 respectively) fractions.

Fig. 4. Northern blot hybridizations of SCSV and WSBMV virion RNAs electrophoresed under denaturing conditions. Lanes 1 and 2 contain SCSV and WSBMV virion RNA respectively. Blot (a) was hybridized with $^{32}$P-labelled SCSV cDNA and blot (b) with $^{32}$P-labelled WSBMV cDNA. Blot (c) was hybridized with $^{32}$P-labelled nick-translated pSCS007.

As with the immunoelectron microscopy, Western blot analysis indicated a serological relationship between the capsid proteins of WSBMV and SCSV (Fig. 1). WSBMV antisera positively recognized SCSV capsid protein and SCSV antisera recognized WSBMV capsid protein; however, the reactions between capsid protein and homologous antisera were much stronger than those between capsid protein with non-homologous antisera, indicating that, although the capsid proteins are serologically related and undoubtedly share common epitopes, the serological relationship was not complete. A Western dot blot assay performed with BNYVV and PMTV antisera indicated no serological relationship with SCSV (data not shown).

Hybridization studies

cDNA cloning of SCSV RNA resulted in several small (less than 500 bp) virus-specific clones as well as a single 1800 bp clone, pSCS007. The smaller clones were not characterized. Clone pSCS007 was labelled with $[^{32}\text{P}]\text{dATP}$ by nick translation and used to probe Northern blots of unfractionated WSBMV and SCSV RNAs. Clone pSCS007 hybridized exclusively with SCSV RNA-2 but not with SCSV RNA-1 or WSBMV RNA-1, RNA-2, or the WSBMV RNA-2 deletion mutant (Fig. 4). In addition, pSCS007 did not hybridize to the diffuse band of nucleic acid in the SCSV RNA preparation (Fig. 4).
Characterization of SCSV

Randomly primed [32P]dATP-labelled cDNA probes were prepared using unfractionated WSBMV and SCSV RNA. The cDNA probes were used to determine the extent of nucleic acid homology between the two viruses under conditions of high stringency. For each virus, each probe was hybridized to the virion RNA isolated from denaturing gels and transferred to GeneScreen Plus. The SCSV cDNA probe hybridized strongly with SCSV RNA-1 and RNA-2 and not to WSBMV RNA-2 or the WSBMV RNA-2 deletion mutant (Fig. 4). The SCSV cDNA probe hybridized to the diffuse band of nucleic acid found in all SCSV RNA preparations. The WSBMV cDNA probe hybridized strongly with WSBMV RNA-1 and RNA-2, as well as the RNA-2 deletion mutant (Fig. 4).

Translation studies

Unfractionated SCSV RNA directed the synthesis of 180K, 170K, 110K, 50K, 42K, 25K and 20.5K polypeptides in a rabbit reticulocyte in vitro translation system (Fig. 5 and 6). Translation profiles from SCSV and WSBMV RNA were similar yet distinct. Differences were

Fig. 5. Fluorogram of [35S]methionine-labelled SCSV- and WSBMV-directed products of a rabbit reticulocyte translation system. Lane 1 contains 14C-labelled protein Mr standards: myosin (heavy chain) 200K, phosphorylase B 97.4K, bovine serum albumin 68K, ovalbumin 43K, α-chymotrypsigen 25.7K, β-lactoglobulin 18.4K. Translation products directed by SCSV RNA are in lane 2 and by WSBMV RNA in lane 3. SCSV RNA-directed translation products immunoprecipitated by anti-SCSV capsid protein antiserum are in lane 4 and by anti-WSBMV capsid protein antiserum in lane 5. WSBMV RNA-directed translation products immunoprecipitated by the SCSV serum are in lane 6 and by anti-WSBMV serum in lane 7. Translation products from rabbit reticulocyte lysates not containing exogenous RNA are in lane 8.
observed primarily in the higher $M_r$ polypeptides. SCSV capsid protein antiserum precipitated three proteins from the total SCSV RNA translation products, the 20.5 K capsid protein and polypeptides of 25 K and 110 K. Similarly, WSBMV antiserum precipitated three proteins from WSBMV translation products, the 19.7 K capsid protein and polypeptides of 25 K and 90 K (Fig. 5). Immunoprecipitations of SCSV translation products with WSBMV antiserum and vice versa resulted in the precipitation of minor amounts of the three polypeptides immunoprecipitated by the homologous antiserum (Fig. 5).

The T7 RNA transcript (presumed positive-polarity) generated from RNA-2-specific pSCS007 directed the synthesis of two major polypeptides of $M_r$ 50 K and 45 K, as well as several minor polypeptides (Fig. 6). The translation products of the RNA transcript were not immunoprecipitated with capsid protein antisera (data not shown). An SP6 negative-polarity RNA transcript did not yield significant or definite products in vitro.

**DISCUSSION**

SCSV was originally isolated from an inbred sorghum line from a breeding plot in Kansas. Interestingly, attempts to transmit the virus mechanically to commercial sorghum hybrids
proven negative (Table 1). Thus, either mechanically mediated infection is dependent on the
correct sorghum genotype (inbred rather than hybrid sorghum), or more likely, sorghum can
only become infected by another inoculation method, possibly by a fungal vector. Polymyxa
graminis, the WSBMV vector, colonizes sorghum and maize roots (W. G. Langenberg,
unpublished data). Attempts to detect soil transmission by seeding plants in soil from the
location where the virus was first isolated were unsuccessful; however, work in this area is being
continued. The infection pattern of SCSV in the original field is consistent with there being a
soil-borne vector, specifically virus-infected plants were along irrigated rows and in the lower
part of the field. Given that physicochemically SCSV is a furovirus, we predict that it can be
transmitted in nature by a plasmodiophoraceous fungus.

SCSV is readily transmitted to several inbred maize varieties by mechanical inoculation,
resulting in systemic symptoms similar to those on the naturally infected sorghum. Systemic
infection and symptom expression are optimal at 25 °C. Infection decreases significantly above
35 °C or below 20 °C. This is in contrast to WSBMV, which causes systemic infection between
15 and 17 °C (Brakke & Rao, 1967). In addition to systemic infection of maize, SCSV infects N.
clevelandii and limited movement of the virus through inoculated leaves is observed. Mosaics,
yellowing and high virus titres result in inoculated leaves, but symptoms and virus are not
detected on uninoculated leaves, suggesting a limitation in long distance movement through the
vascular tissue. SCSV is unusual in that it systemically infects both monocotyledonous and
dicotyledonous (limited systemic infection) hosts.

SCSV is composed of two rod-shaped particles of approximately 260 nm and 140 nm in length
and 20 nm in diameter. Each particle is composed of a single capsid polypeptide of 20-5 K
separately encapsidating two RNA species having little or no homology. RNA-1 is approx. 6200
nucleotides and RNA-2 is approximately 3500 nucleotides. The particle morphology and
genome size of SCSV is essentially identical to WSBMV, although the observed ratio of virion components and, consequently, RNA components in SCSV is the inverse of that reported for
WSBMV. Electron microscopy of purified SCSV virions (Fig. 2) reveals a greater number of
260 nm particles and this is reflected in the relative concentration of RNA-1 compared to
RNA-2 (Fig. 3).

The nature of the diffuse nucleic acid band associated with RNA from purified virions has not
been determined, although there are several possible explanations of its occurrence in SCSV RNA preparations. The band may mask a distinct subgenomic or satellite RNA, or a third
RNA species, although the most likely explanation is that it represents RNA degradation products. As the RNA-2-specific clone, pSCS007, does not hybridize to the band and the SCSV
cDNA does (Fig. 4), it would suggest that the diffuse nucleic acid may be homologous to RNA-1,
possibly being either a subgenomic form of RNA-1 or the result of RNA-1-specific degradation.

WSBMV RNA-2 encodes capsid and inclusion body proteins and RNA-1 regulates virus
concentration and infectivity (Tsuchizaki et al., 1975). A proposed map of the WSBMV genome
(Hsu & Brakke, 1985) locates the capsid protein gene on the 5' end of RNA-2 with the 28 K and
90 K polypeptides resulting from readthrough of amber stop codons located 3' to the capsid
protein gene. In vitro translation (Ziegler et al., 1985) and sequence analysis (Bouzoubaa et al., 1986) of BYNVV RNA-2 identifies the 5'-terminal open reading frame as the 21 K capsid
protein gene. The 85 K polypeptide is produced by readthrough of an amber stop codon located
at the 3' end of the capsid protein gene. In vitro translation of IPCV RNA-2 demonstrates that
the capsid protein is encoded by this RNA but the location of the gene, specifically a 5' open
reading frame, has not been deduced. The placement of the coat protein cistron at the 5' end of
RNA-2 appears to be a feature common to the furoviruses.

The characterization of WSBMV and BNYVV allows for speculation on the genomic
organization of SCSV and particularly the organization of RNA-2. If the assumption is made
that the genomic organization of SCSV is similar to that of WSBMV, BNYVV and IPCV, and
specifically that the capsid protein gene is located on RNA-2, then the relatively low amounts of
capsid protein and the presumed readthrough products of 28 K and 110 K synthesized in vitro can
be explained. RNA-2 is present in much lower concentrations compared to RNA-1 and this
would be reflected in the lower yield of in vitro translation products from RNA-2. Furthermore,
it is probable that the capsid protein gene is located on the 5' end of RNA-2, as the in vitro translation products synthesized from the in vitro transcript of the RNA-2 clone, a clone most likely representing the 3' end of RNA-2, are not immunoprecipitated by antisera to the capsid protein. The RNA-2-specific clone, pSCS007, does not contain the complete capsid protein gene.

Results from the characterization of SCSV indicate that it should be classified as a furovirus. Particle morphology, physicochemical properties, and genomic organization of SCSV are similar to members of the furovirus group and essentially identical to WSBMV. SCSV and WSBMV were both initially isolated from monocotyledonous hosts and are morphologically and physicochemically indistinguishable. They are serologically related and their nucleic acids lack polyadenylation sequences. In vitro translation clearly demonstrates that the SCSV genome is organized much like the WSBMV genome. SCSV is obviously much more closely related to WSBMV than to the other furoviruses, and is in fact quite dissimilar to BNYVV.

SCCSV represents an ideal system for the study of the furoviruses in general and WSBMV in particular. Pseudorecombination studies involving SCSV and WSBMV may define areas of the furovirus genome responsible for host specificity. In addition, Shirako & Brakke (1984a, b) have suggested that a fungal transmission component may be associated with WSBMV RNA-2. The RNA-3 and RNA-4 of BNYVV appear to encode a determinant of fungus-mediated transmission (Lemaire et al., 1988). It is possible that furoviruses may encode a gene or genes that potentiate fungal transmission. Further cloning, sequencing and recombination experiments with SCSV are in progress to investigate this concept.

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Characterization of SCSV  2345


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