Identification of Sugarcane Mosaic Virus Strain H Isolate in Commercial Grain Sorghum

LAURA M. GIORDA, Graduate Student, and R. W. TOLER, Professor, Department of Plant Pathology and Microbiology, and F. R. MILLER, Professor, Department of Soil and Crop Sciences, Texas Agricultural Experiment Station, Texas A&M University, College Station 77843

ABSTRACT

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A virus isolated from grain sorghum resistant to maize dwarf mosaic virus (MDMV) was examined and identified as an isolate of sugarcane mosaic virus strain H (SCMV-H) on the basis of host range, particle morphology, and serological relationships. The host range was similar to that of SCMV-H except for differences in the severity of reactions of three sorghum accessions. The virus was serologically related to SCMV-I and SCMV-H. No serological relationship was detected among strains A, B, and D of SCMV or among strains A, B, D, E, and F of MDMV. The average length of particles of the sorghum isolate (SI) was 706 nm and did not differ from that of SCMV-H (708 nm). The SI was more closely serologically related to SCMV-H than to other SCMV and MDMV strains. PAG3387 and DR1125 (sorghum accessions) were differential hosts for the SI; they are resistant to MDMV-A and susceptible to the SI of SCMV-H and the type isolate SCMV-H. Four sorghum accessions were immune from the SI of SCMV-H. These were QL3-Texas, QL3-India, SC0097-14E, and QL11. SCMV-H was confirmed as a pathogen of commercial grain sorghum.

Sugarcane mosaic virus (SCMV) was described in sugarcane in 1919 by Brandes (4); sorghum was first shown to be a host in 1923 (5). The virus is transmitted in a nonpersistent manner by at least seven species of aphids (12). Toler and Fazli (17) identified SCMV in corn and sorghum in Texas in 1970 but did not classify it to strain. SCMV strain H (SCMV-H) was first identified from commercial cane fields in Louisiana in 1950 (1). In 1969, sugarcane was brought from Louisiana to Texas to inititate commercial sugarcane production in the Rio Grande Valley. SCMV-H was first reported on sugarcane in Texas in 1974 (18). Thirteen strains of SCMV including SCMV-Jg(MDMV) were reported by 1978 (16). SCMV and maize dwarf mosaic virus (MDMV) are differentiated mainly in that strains of SCMV do not go to johnsongrass (15) (*Sorghum halepense* L.), which is infected by all strains of MDMV except strain B.

The appearance of mosaic and red-leaf symptoms on an MDMV-resistant hybrid (DR1125) in 1980 at Rio Hondo, TX, prompted further investigation of the causal agent. Our objectives were to determine if resistance to MDMV had been overcome, to determine the identity of the virus causing infection of MDMV-resistant sorghum, and to identify resistance sources if a new virus or strain was responsible.

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MATERIALS AND METHODS

The sorghum isolate (SI) of the virus from a naturally infected MDMV-resistant hybrid (PAG3387) was mechanically transmitted and maintained in an insect-free greenhouse on a sudan hybrid (Trudan-5, Northrup King Corp., Edina, MN). The SCMV-H isolate was obtained from H. Koike, USDA Sugarcane Field Station, Huma, LA. Inoculum for mechanical transmission was prepared by macerating leaf tissue 1:5 (w/v) in 0.1 M potassium phosphate buffer, pH 7, with 0.5% activated charcoal and 1% 600-mesh Carborundum. Plants for host range studies were grown in pots or flats containing a steam-sterilized mixture of peat, sand, and perlite medium (2:1:1, v/v) at 25–31 C in the greenhouse. Host plants tested were johnsongrass, 32 grain sorghum accessions, and sugarcane cultivars CP 31-294 and CP 31-588. Reactions of 26 accessions to SCMV-H and SI infection were compared in a field test at Texas A&M University Research Plantation, College Station. A split-plot design with three replicates was used, with the sorghum cultivars as main plots and the two treatments (inoculated and uninoculated half-rows) as subplots. Plants remaining symptomless after inoculation were assayed serologically (Ouchterlony test) using antisera produced to the SI. Plants were rated for virus infections with the disease severity index (DSI), which considers the number of symptomless plants and those infected that fall in each category of a scale of 1–5, where 1 = symptomless plants; 2 = mild mosaic; 3 = mosaic, yellowing; 4 = any red-leaf symptoms, stunting with or without severe mosaic, and yellowing; and 5 = plants dead or dying with extensive leaf necrosis.

Two procedures were used for virus purification. The first was a modification (10) of that described by Berger and Zeyen (2). The second procedure (8) was modified by adding 0.5 M deionized urea to the supernatant containing 5% Triton X-100. The procedure was further

Table 1. Host range analysis of the sorghum isolate (SI) of sugarcane mosaic virus strain H demonstrating the expressed symptoms and percentage of infected plants

Host	Symptoms[a]	Infected plants (%)
Sorghum		
QL3-Texas[b]	–	0
QL3-India[b]	–	0
QL11[a]	–	0
Q7539	M	10
Haygrazer	M,RL[c]	57
Trudan-5	M	31
Trudan-7	M,RL	34
Dekalb E59+	M	46
Cargill 20	M,RL	41
Bug-off	M	65
OKY8	M	18
Atlas	M,RL	27
Rio	M	27
Btx398	M	36
SA8735	M	32
N. Mex 31	M,RL	51
SC0097-14E	–	0
SC00175-14E	M	36
Pioneer 8199	M	23
BTx623	M,RL[c]	39
RTx430	M	10
RTAM428	M	23
BTx378	M,RL	61
RTx09	M	36
BTx3197	M,RL	56
SA394	M,RL	8
PI 35038	M	20
DR1125	M,RL	50
PAG3387	M,RL	55
Sugarcane		
CP 31-294	–	0
CP 31-588	M	100
Johnsongrass	–	0

[a] M = mosaic, RL = red-leaf, and – = no symptoms. Readings were taken 2 and 4 wk after inoculation.
[b] Symptomless plants were tested serologically against SI antiserum, all with negative results.
[c] Only one plant with RL.

modified by adding 0.5 M deionized urea + 0.5% sodium metaphosphate to the supernatant containing 5% Triton X-100. Deionized urea (0.5 M) instead of Triton X-100 was used after clarification in the first low-speed centrifigation. Sucrose density gradients (10–40%) were prepared in 0.05 M sodium borate, pH 8.0, with 0.01 M EDTA.

Antiserum for the SI, MDMV strains A, B, D, E, and F and SCMV strains A, B, D, H, I, and M was prepared by intramuscular and subcutaneous injections of rabbits weekly for 5 wk with 0.5 mg of virus suspended in 1 ml of buffer and emulsified with 1 ml of Fruend's complete adjuvant. Rabbits were bled weekly after the fifth injection.

Immunodiffusion tests were used to study the relation of the SI to MDMV strains A, B, D, E, and F (15,11) as well as SCMV strains A, B, D, H, I, and M (1,9). Tris-HCL buffer, 0.05 M, pH 7.2, was used as antigen diluent and a solution of tris-HCl buffer, 0.5 M, pH 7.2, containing 0.85% NaCl, 5% bovine serum albumin, and 0.02% NaN was used as antiserum diluent.

Sodium dodecyl sulfate (SDS)-agar double-immunodiffusion tests were performed in plastic petri dishes 50 × 6 mm (14). The medium was 0.8% Agarose Type I (Sigma), 0.5% SDS, and 1.0% NaN in 0.025 M, pH 7.0, tris-HCl buffer (14). Wells 5 mm in diameter were spaced 4 mm apart.

Leaf-dip preparations of infected sorghum tissue were examined with a Hitachi HS 7S electron microscope. For ultrastructural examinations, diseased tissue was embedded in Poly/bed 812 (Polyscience, Inc., Warrington, PA) after double fixation in glutaraldehyde and osmium tetroxide, both in 0.01 M Pipes buffer, pH 7.4. Reynolds' uranyl acetate-lead citrate was used to stain sections.

Serologically specific electron microscopy (SSEM), as described by Derrick and Brlansky (6,7), was used to determine serological relationships of SI with MDMV and SCMV strains, using normal sera as a control. Serological relatedness between any two viruses was studied using serial dilutions of 1:10, 1:100, 1:500, and 1:1,000 for each antigen. Electron micrographs taken for SSEM and ultrastructure studies using a Hitachi HU-11E electron microscope were used to determine particle length of the SI and SCMV-H.

Table 2. Symptomatology of the sorghum isolate (SI) and sugarcane mosaic virus strain H (SCMV-H) compared on 26 sorghum accessions

	SI		SCMV-H	
Accessions	DSI[a]	RL[b] (%)	DSI	RL (%)
RTx7000	4.6	91	4.5	89
N. Mex. 31	4.5	84	4.3	91
Atlas	4.5	89	4.3	81
BTx3197	4.0	81	4.7	97
RTx3048	4.1	82	4.1	81
SA394	3.9	77	4.0	84
PI 35038	3.3	22	3.4	26
SC0175-14E	3.4	29	3.0	11
Rio	3.1	17	3.2	6
BTx378	3.1	51	3.1	52
RTx09	3.6	58	2.6	0
RTx412	2.5	0	2.5	0
RTx430	2.1	29	2.0	46
BTx623	2.5	1	2.4	2
SA7078	2.2	0	2.6	0
RTAM428	2.2	0	2.5	0
RTx414	2.4	9	2.3	0
OKY8	2.2	0	2.2	0
SA8735	2.5	0	1.9	0
Pioneer 8199	2.0	0	2.2	2
BTx398	2.0	0	2.1	0
Hegari	1.8	0	2.0	0
SC0097-14E	1.0	0	1.0	0
QL11	1.0	0	1.0	0
QL3-Texas	1.0	0	1.0	0
QL3-India	1.0	0	1.0	0

[a] Disease severity index rating: 1 = no symptoms, 2 = mild mosaic, 3 = severe mosaic and chlorosis, 4 = red-leaf, and 5 = necrosis.
[b] RL = red-leaf: percentage of plants showing lesions that develop red discoloration because of anthocyanin development.

RESULTS

Of 32 sorghum accessions tested for host susceptibility to SI, 28 were susceptible and four were immune. Of the

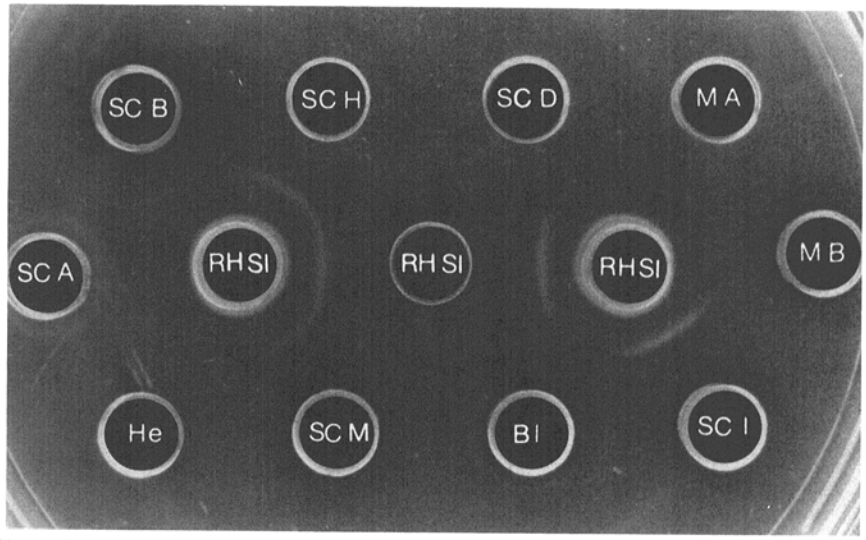

Fig. 1. Ouchterlony agar diffusion test. Antiserum to the sorghum isolate (RHSI) with strains of sugarcane mosaic virus (SCMV) and maize dwarf mosaic virus (MDMV) as antigens. SCA (SCMV-A), SCB (SCMV-B), SCD (SCMV-D), SCH (SCMV-H), SCI (SCMV-I), SCM (SCMV-M), MA (MDMV-A), MB (MDMV-B), RHSI (sorghum isolate), He (healthy), and Bl (blank well).

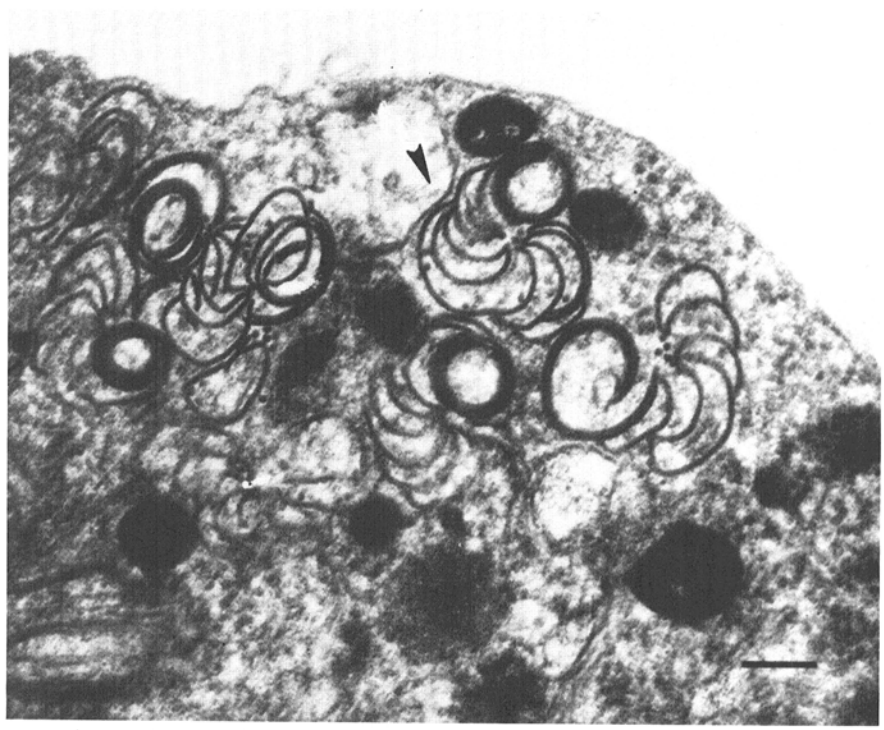

Fig. 2. Pinwheel inclusions in sorghum infected with the sorghum isolate of sugarcane mosaic virus strain H. Scale bar = 0.25 μm.

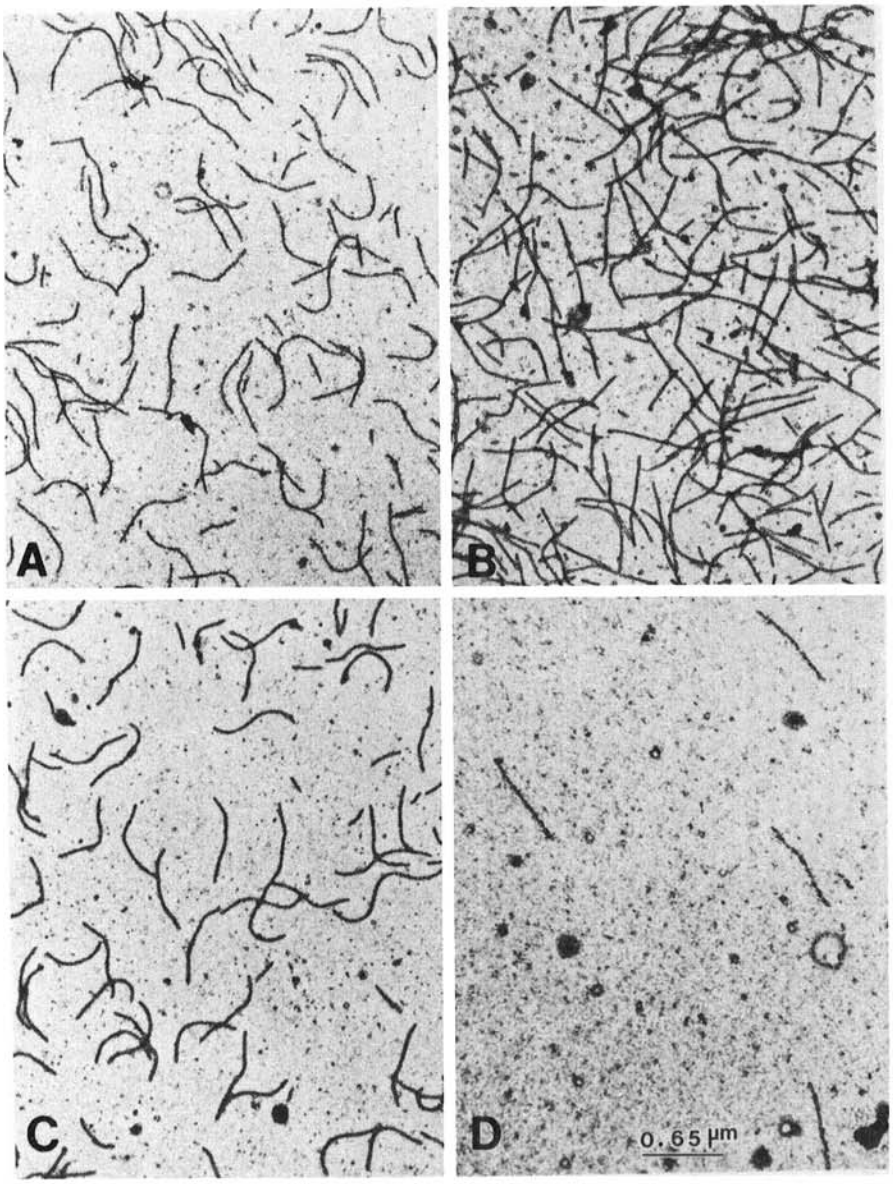

Fig. 3. Serologically specific electron microscopy (SSEM) of sorghum isolate (SI), maize dwarf mosaic virus strain F (MDMV-F), and sugarcane mosaic virus strain H (SCMV-H). Scale bar = 0.65 µm. **(A)** Homologous reaction, SI antiserum vs. SI antigen; **(B)** homologous reaction, MDMV-F antiserum vs. MDMV-F antigen; **(C)** heterologous reaction, SI antiserum vs. SCMV-H antigen; and **(D)** heterologous reaction, SI antiserum vs. MDMV-F antigen.

susceptible sorghum accessions, 14 showed red-leaf symptoms and 13 showed only mosaic symptoms. The virus was not recovered from symptomless sorghums QL3-Texas, QL3-India, QL11, and SC0097-14E. Plants that were not infected included johnsongrass and sugarcane (CP 31-294) (Table 1).

Symptomatology of the SI and SCMV-H was compared on 26 sorghum accessions (Table 2). Symptom reactions differed between SI and SCMV-H on three accessions. A major difference was observed on RTx09, where the SI isolate had a 3.6 DSI and 58% red-leaf compared with a 2.6 DSI and 0% red-leaf for SCMV-H. The SI and SCMV-H symptom difference for Tx414 was 2.4 DSI and 9% red-leaf compared with 2.3 DSI and 0% red-leaf for SCMV-H. Pioneer 8199 showed 2% red-leaf when inoculated with SCMV-H and a 2.2 DSI compared with 0% red-leaf and a 2.0 DSI when inoculated with the SI.

Results of immunodiffusion studies comparing SI and SCMV and MDMV strains are shown in Table 3. Strong precipitin reactions were formed when SI antiserum was tested against SCMV-H and SCMV-I antigens. Weaker reactions were noted with SCMV-M antigen. No reaction was observed with other strains of SCMV or MDMV (Table 3, Fig. 1). Examination of leaf-dip preparations revealed filamentous rods. Pinwheel inclusions typical of the potyvirus group were observed in fixed sections of SI-infected cells (Fig. 2).

Pretreatment of electron microscope grids for SSEM with antiserum (1:1,000) resulted in an increased number of virus particles trapped when coated with the homologous rather than the heterologous serum. Ratios of the number of heterologous and homologous serum-strain combinations are shown in Table 4. Viruses with the highest ratios are closely related and could be placed in the same serological group. SCMV-H and SI were closely related. The SI was not related to the heterologous virus MDMV-F (Fig. 3, Table 4). Ratios were sufficiently close to

Table 3. Serological relationships among maize dwarf mosaic virus (MDMV) and sugarcane mosaic virus (SCMV) strains based on double immunodiffusion tests

	Antisera								
Antigens	**SCMV-I**	**SCMV-H**	**SI[a]**	**SCMV-D**	**MDMV-A**	**MDMV-B**	**MDMV-D**	**MDMV-E**	**MDMV-F**
SCMV-A	−	−	−	++[b]	−	+	−	−	−
SCMV-B	−	−	−	++	−	+	−	−	−
SCMV-D	−	−	−	++	−	+	−	−	−
SCMV-H	++	++	++	−	−	−	−	−	−
SI	++	++	++	−	−	−	−	−	−
SCMV-I	++	++	++	−	−	−	−	−	−
SCMV-M	−	±	±	−	−	−	−	−	−
MDMV-A	−	−	−	−	++	−	+	+	+
MDMV-B	−	−	−	+	−	++	−	−	−
MDMV-D	−	−	−	−	±	−	+	±	±
MDMV-E	−	−	−	−	±	−	±	+	±
MDMV-F	−	−	−	−	±	−	±	±	+

[a]Sorghum isolate.
[b]++ = Strong reaction, + = reaction, ± = weak reaction, and − = no visible reaction.

consider SCMV-H and SI as the same virus, and SCMV-H, SI, and SCMV-I form a group more closely related to each other than to the other strains tested (Table 5). No relationship could be demonstrated between SI and any of the MDMV strains. Ratios higher than 1 could be explained by different concentrations of virus in sap.

Particle lengths of SCMV-H and SI were measured from electron micrographs of SSEM grids (Fig. 4); 406 particles of SI and 346 of SCMV-H were measured (Fig. 5). The modal lengths were 702 and 714 nm for SI and SCMV-H, respectively. The most frequent particle lengths of SI were in the range of 670–760 nm and those of SCMV-H were in the range of 650–750 nm. Particle lengths were not significantly different between the two viruses by the t test ($P > 0.40$).

In three trials with the purification procedure of Berger and Zeyen (12), yields of the SI were 0.32, 0.62, and 1.10 mg/100 g of tissue. The A_{260}/A_{280nm} ratios were between 1.22 and 1.23. With the modified purification procedure of Gough and Shukla (8), where 0.5 M deionized urea was used, a yield of 0.78 mg/100 g of tissue was obtained and the A_{260}/A_{280nm} ratio was 1.14. Purified SI preparations gave a UV spectrum typical of a nucleoprotein. The virus particles produced by these two methods of purification, when negatively stained with 1% potassium phosphotungstate, revealed little evidence of aggregation.

DISCUSSION

On the basis of host range, symptomology, particle morphology, and serological relationships determined by immunodiffusion and SSEM methods, the virus isolate from MDMV-resistant commercial grain sorghum in Rio Hondo, TX, was SCMV-H.

Interrelationships among members of the SCMV group are complex and difficult to determine (13). The SDS-agar double-diffusion test was useful in identifying the virus as a member of the SCMV complex but only to place it in a group with SCMV-H and SCMV-I, which is relatedness group 2 according to Derrick's classification of SCMV strains (6).

In the SSEM study, relatedness between any two strains is expressed as the ratio of the number of virus particle heterologous and homologous serum-strain combinations (6). With the SI antigen and SI antiserum, the ratio was 1 and the most closely related virus tested was SCMV-H with a ratio of 2.20. With SCMV-H antiserum, the most closely related virus was SI with a ratio of 0.90. SSEM placed the relatedness of the strains much more specifically than the agar double-diffusion tests.

Variability of the particle lengths observed, 708 nm compared with the previous reports of 750 nm (3), is in part

Table 4. Relationships between sugarcane mosaic virus strain H (SCMV-H) and sorghum isolate (SI) expressed as the ratio of the number of particles from heterlogous and homologous serum-strain combinations, using dilution series[a]

Antigens	Antisera		
	SI[b]	SCMV-H	MDMV-F
Dilution 1:10			
SI	1.00[c]	1.50	0.03
SCMV-H	1.11	1.00	0.03
MDMV-F	0.06	0.09	1.00
Dilution 1:100			
SI	1.00	1.70	0.03
SCMV-H	0.60	1.00	0.03
MDMV-F	0.03	0.03	1.00
Dilution 1:500			
SI	1.00	1.30	0.02
SCMV-H	0.89	1.00	0.02
MDMV-F	0.05	0.09	1.00
Dilution 1:1,000			
SI	1.00	1.30	0.02
SCMV-H	0.70	1.00	0.00
MDMV-F	0.00	0.00	1.00

[a] Maize dwarf mosaic virus strain F (MDMV-F) is included an an unrelated control. The test used was SSEM (serologically specific electron microscopy).
[b] Read results by column.
[c] Ratio of the number of virus particles from heterologous to homologous serum-strain combinations.

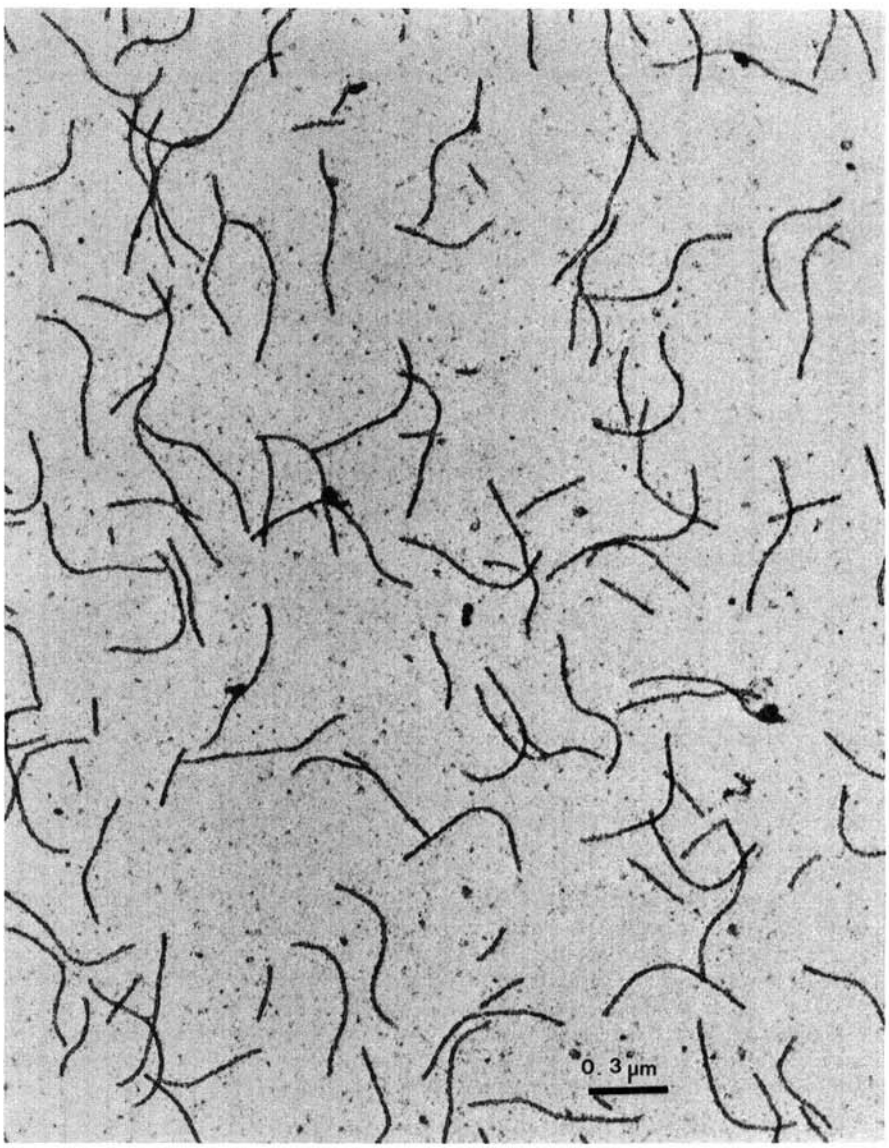

Fig. 4. Serologically specific electron microscropy of sorghum isolate (SI). Homologous reaction, SI antiserum vs. SI antigen. Scale bar = 0.3 µm.

Table 5. Relationship between any two viruses, expressed as the ratio of the number of particles from heterologous and homologous serum-strain combinations, from some sugarcane mosaic virus (SCMV) and maize dwarf mosaic virus (MDMV) strains[a]

	Antisera					
Antigens	**SCMV-I[b]**	**SCMV-H**	**SI[c]**	**SCMV-D**	**MDMV-A**	**MDMV-B**
SCMV-I	1.00	0.30	0.30	0.13	0.01	0.09
SCMV-H	6.70	1.00	2.20	0.83	0.20	0.08
SI	5.30	0.90	1.00	0.34	0.12	0.06
SCMV-D	1.00	0.20	0.03	1.00	0.03	0.20
MDMV-A	0.33	0.04	0.02	0.25	1.00	0.04
MDMV-B	0.66	0.00	0.05	1.20	0.03	1.00

[a]The test used was SSEM (serologically specific electron microscopy). Viruses with the highest ratios are closely related to each other.
[b]Read results by column only.
[c]Sorghum isolate.

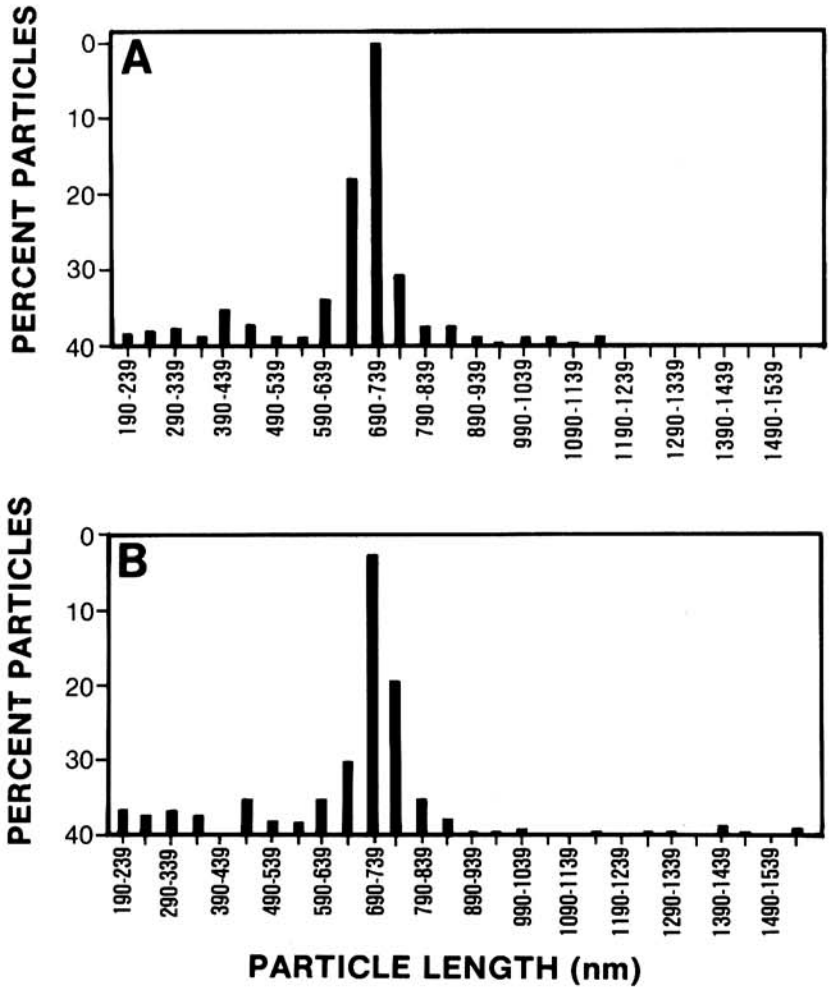

Fig. 5. Particle length distribution. **(A)** Sorghum isolate and **(B)** sugarcance mosaic virus strain H.

due to the purification procedures, although there was no difference between the SI and SCMV-H.

Two sorghum host differentials were identified that separated MDMV and the SI of SCMV-H; these were PAG3387 and DR1125. Both hybrids were resistant to MDMV-A and produced only mild mottling to this virus. These hybrids were susceptible to SCMV-H (SI) and produced severe red-leaf symptoms. Of the 26 accessions tested, four were immune to the SI and to the SCMV-H. These were QL11, QL3-India, QL3-Texas, and SC0097-14E. The host range was similar to that of SCMV-H except for some differences in the severity of reactions of three sorghum accessions. The differences between SI and SCMV-H isolates were based on differences in the aggressiveness on these three sorghum accessions.

The SI of SCMV-H causes a severe disease of sorghum, infecting even MDMV-A-resistant hybrids. Sugarcane is a reservoir and poses a threat to commercial grain sorghum when the two are grown in proximity. Johnsongrass is not a reservoir of SCMV and does not enter into the epidemiology of SCMV.

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