Comparative Study of Two Maize Dwarf Mosaic Virus Strains Infecting Corn and Johnsongrass in Israel

YEHESKEL ANTIGNUS, Department of Virology, ARO, The Volcani Center, P.O.B. 6, Bet Dagan, Israel

ABSTRACT

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Two strains of maize dwarf mosaic virus (MDMV) were identified in Israel on corn and johnsongrass. One strain, designated M-C, induces mosaic in some common sorghum cultivars; the other, designated M-D, is responsible for the appearance of red lesions and systemic necrosis on the same cultivars. M-D-infected corn plants were severely dwarfed compared with M-C-infected plants when inoculated at the three-leaf stage. Both strains caused a reduction of about 30% in ear yield. Serological tests, including enzyme-linked immunosorbent assay, immunoelectron microscopy, and sodium dodecyl sulfate immunodiffusion tests, indicated weak serological cross-reactions between M-C and M-D. Strain M-C reacted strongly with antisera against M-A and sorghum red stripe virus (SRV) and very weakly with antiserum against strain H of sugarcane mosaic virus (SCMV-H), whereas strain M-D reacted weakly with M-A and not at all with SRV or SCMV-H antisera.

Maize dwarf mosaic virus (MDMV) includes several strains that are part of the sugarcane mosaic virus complex (16,20) whose members have properties of the potyvirus group of plant viruses. The virus has worldwide distribution and has been reported from the United States (4,21), Europe (17,18), Asia (1,6), and Australia (11). Strains have been differentiated serologically (15,16) and by host range (8,17). The two principal strains are A (M-A), which infects johnsongrass, and B (M-B), which does not (9). MDMV and sugarcane mosaic virus (SCMV) strains were classified into four serogroups: 1) MDMV-A and SCMV-J; 2) MDMV-KS1; 3) MDMV-B, and SCMV-A, SCMV-B, and SCMV-D; and 4) SCMV-H, SCMV-I, and SCMV-M (3). The first report of this disease from Israel was in 1973 (6). This paper presents some information on the purification and biological and serological properties of two strains of MDMV found in Israel on corn and johnsongrass.

MATERIALS AND METHODS

Host range assays and cultural conditions. Corn plants showing mosaic symptoms were collected from different parts of Israel. Leaf samples were homogenized in the presence of 0.01 M phosphate buffer, pH 7, and were inoculated to the following Carborundum-dusted test plants: *Bromus rubens* L., *Capsicum annuum* L. cv. Maor, *Chenopodium amaranticolor* Coste & Reyn., *Cucumis sativus* L. cv. Bet Alfa, *Gomphrena globosa* L., *Hordeum vulgare* L., cv. Omer, *Lycopersicon esculentum* Mill. cv. Marmande, *Nicotiana tabacum* L. cv. Samsun, *Paspalum dilatatum* Poir., *Saccharum officinarum* L., *Sorghum bicolor* L. cv. 610, *S. halepense* (L.) Pers., *Triticum aestivum* L. cv. Miriam, and *Zea mays* L. cv. Jubilee. All grasses were grown from seeds, mechanically inoculated at the three-leaf stage, and observed for 4 wk. All plants were back-assayed to sweet corn (*Z. mays* cv. Jubilee), which served also as virus propagation host. All plants were grown in an insectproof greenhouse fumigated weekly with nicotine sulfate.

Purification. Leaves from corn plants infected by two Israeli strains of MDMV, designated M-C or M-D (characterized under Results), were ground by a power-driven crusher (Merkel Bruschal, Federal Republic of Germany). The crushed tissue was placed in the appropriate buffer and homogenized in a cold Waring Blendor for 1 min, then different procedures were adopted for each virus strain. Strains M-C and M-D were purified according to methods described by Jones and Tolin (5) and von Baumgarten and Ford (20), respectively. Further purification steps included sucrose density-gradient (10–40%) centrifugations (2.5 hr at 24,000 rpm in a Sorvall OTD50 ultracentrifuge with an AH627 rotor). Fractionation and photometric scanning (254 nm) of processed gradients were done by an ISCO instrument (Instrumentation Specialities Co., Lincoln, NE).

Sucrose gradient fractions containing viral particles were concentrated by high-speed centrifugation for 3 hr at 27,000 rpm in a Beckman 30 rotor. An extinction coefficient $A^{0.1\%}_{260nm} = 2.4$ (13) was used to estimate virus concentration in the viral preparations.

Serology. Antisera to M-C and M-D were prepared by injecting rabbits with purified viral preparations. Antigens (0.8–1 mg) were emulsified in Freund's complete adjuvant and injected twice intradermally followed by a single intramuscular and three intramuscular booster injections (0.5 mg each, with the same adjuvant). Injections were given at

Table 1. Comparative partial host range of M-C and M-D, Israeli strains of maize dwarf mosaic virus (MDMV)

Test plant	Symptoms	
	M-C	M-D
Bromus rubens	Mosaic + tillering	Symptomless
Hordeum vulgare cv. Omer	–[a]	–
Paspalum dilatatum	Mosaic	Purple spots + mosaic
Saccharum officinarum	–	–
Sorghum bicolor cv. 610	Mosaic	Purple spots + mosaic
S. halepense	Mosaic	Mosaic
Triticum aestivum cv. Miriam	–	–
Zea mays cv. Jubilee	Mosaic	Mottling
Various dicots[b]	–	–

[a] – = No symptoms and negative reinoculation to corn.

[b] *Capsicum annum* cv. Maor, *Chenopodium amaranticolor*, *Cucumis sativus* cv. Bet Alfa, *Gomphrena globosa*, *Lycopersicon esculentum* cv. Marmande, and *Nicotiana tabacum* cv. Samsun.

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intervals of 2 wk. The rabbits were bled 11–12 wk after the first injection. Partially purified immunoglobulins from M-C and M-D antisera were obtained by using DE_{23} diethylaminoethyl cellulose (Whatman) for column chromatography (2). Enzyme-linked immunosorbent assays (ELISA) were carried out in polystyrene plates (Dynatech M129A) essentially according to Clark and Adams (2), using an indirect procedure (7). Wells were coated with the viruses (20 μg/ml or as indicated) for 4 hr at 37 C. Diluted rabbit antiserum or purified globulins were incubated for 1.5 hr at 37 C and overnight at 4 C followed by conjugated goat antirabbit globulin purchased from Bio-Yeda Ltd., Israel (3.5 hr at 37 C). Substrate hydrolysis time was 1 hr. ELISA values for the controls (mock virus and buffer) were 0.1 A_{405nm} or as indicated. Immunoelectron microscopy (IEM) was done according to Milne and Louisoni (10), using crude antisera and purified preparations of M-C and M-D. Immunodiffusion tests with sodium dodecyl sulfate (SDS) were conducted according to Purcifull and Batchelor (14), using crude antisera and purified preparations of M-C and M-D. Antisera against sugarcane mosaic virus strain H (SCMV-H) PVAS 51 and MDMV-A PVAS 558 were purchased from the American Type Culture Collection, Rockville, MD. Antiserum against sorghum red stripe virus (SRV) was kindly provided by M. Conti, Laboratorio di Fitovirologia Applicata, Torino, Italy.

Electron microscopy (EM). EM was used for detecting virions in sucrose gradient fractions and for IEM tests. All preparations were mounted on carbon-coated Formvar membranes, and preparations were negatively stained in 2% aqueous uranyl acetate. Microscopic examinations were done with a Jeol JEM-100 C × II electron microscope operating at 80 kV.

Field experiments. To compare corn plant responses to infection with M-C and M-D, plants were grown in an insectproof screenhouse (4 × 18 × 2.5 m) to avoid natural infections in the controls. Seeds of sweet corn (*Z. mays* cv. Jubilee) were planted in three 15-m-long rows spaced 1 m apart. Seeds were planted to obtain seven plants per row meter.

A randomized complete block design with three replicates was used; each block consisted of three treatments: M-C- and M-D-inoculated plants and an uninoculated control. Inoculum was prepared as described and inoculated mechanically to plants at the three-leaf stage. Measurements of plant height and ear characteristics were subjected to analysis of variance by Duncan's multiple range test.

RESULTS

Host range. M-C is the common MDMV strain in Israel. It was identified in samples collected from different parts of the country; however, M-D was identified only in few sites along the Jordan Valley and at one site in the coastal region. The partial host range for the Israeli strains of MDMV is summarized in Table 1. Sweet corn reacts to M-C by mild mosaic and to M-D by bright mosaic and mottling (Fig. 1). Both

Fig. 1. Symptoms induced by Israeli strains M-C and M-D of maize dwarf mosaic virus (MDMV) on corn (*Zea mays* cv. Jubilee). **(Left)** Bright mosaic and mottling induced by M-D, **(center)** leaf from a healthy plant, and **(right)** mild mosaic induced by M-C.

Fig. 2. Symptom development in a *Sorghum bicolor* cv. 610 plant infected by M-D, an Israeli strain of maize dwarf mosaic virus. The appearance of purple lesions is followed by their coalescing, and finally, by withering of the whole leaf.

Table 2. Effects of infection with M-C and M-D, Israeli strains of maize dwarf mosaic virus (MDMV), on sweet corn (Jubilee) plant height and ear quality[v]

Treatment	Plant height[w] (mm)	Ear characteristics[x,y] Weight (g)	Diameter (cm)	Length (cm)	Missing kernels[z] (%)
M-C-infected	1,573 b	159 a	4.2 a	16.6 a	42 a
M-D-infected	1,360 a	151 a	4.2 a	16.5 a	40 a
Uninfected	1,835 c	232 b	4.6 b	19.5 a	27 b

[v] Plants were inoculated at the three-leaf stage, and measurements were taken at maturity.
[w] Means of 100 infected or uninfected plants.
[x] Within columns, means followed by the same letter do not differ significantly ($P = 0.05$).
[y] Data represent means of 100 ears collected from infected or uninfected plants.
[z] Percentage of ear length with unfilled kernels.

Fig. 3. Effects of M-D and M-C, Israeli strains of maize dwarf mosaic virus, on corn (*Zea mays* cv. Jubilee) plant height. Plants were inoculated at the three-leaf stage and measured at maturity. **(Left)** Uninfected control plant, **(center)** M-C-infected plant showing moderate dwarfing, and **(right)** M-D-infected plant showing severe dwarfing.

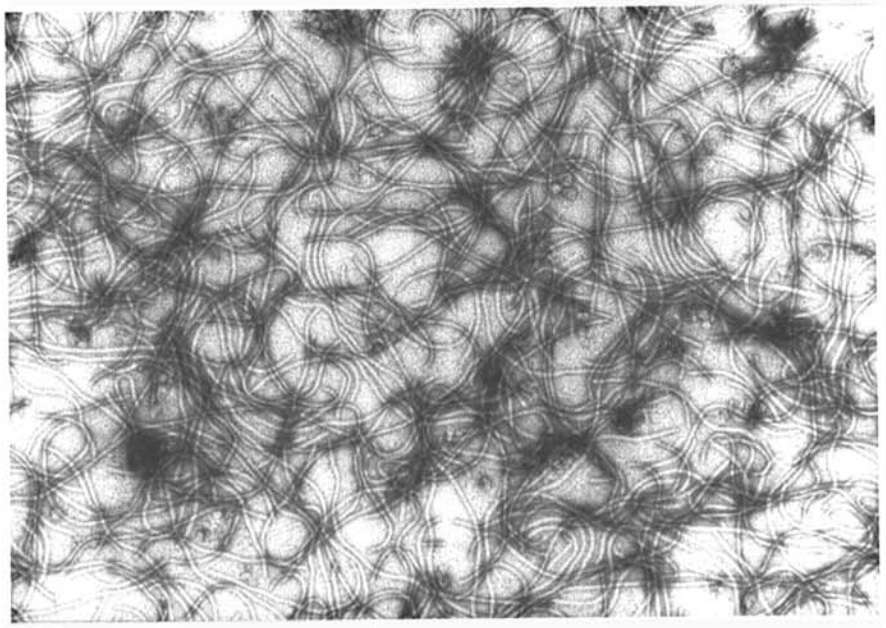

Fig. 4. Electron micrograph of purified maize dwarf mosaic virus strain M-D virions. Stain was 2% uranyl acetate (×20,000).

Fig. 5. Serological relationship between the Israeli strains M-C and M-D of maize dwarf mosaic virus obtained by sodium dodecyl sulfate (SDS) immunodiffusion tests. Purified preparations of both strains were treated with SDS before reaction. The wells were loaded as follows: C = M-C undiluted antiserum, 1 and 3 = purified preparation of M-C (0.5 mg/ml), 4 and 6 = purified preparation of M-D (0.5 mg/ml), 2 = buffer without virus, and 5 = SDS-treated purified tobacco mosaic virus (0.5 mg/ml).

strains induce mosaic in johnsongrass, which probably serves as a natural source for virus spread. In sorghum plants, M-C induced systemic mosaic, whereas M-D induced purple lesions that continued to enlarge and ultimately cause plant death (Fig. 2).

Effects on corn plants and ear characteristics. Measurements of growth and corn yield were done at harvesttime. The data presented in Table 2 indicate that M-C and M-D reduced the average heights of infected corn plants 86 and 74%, respectively, compared with uninfected controls. This effect is demonstrated in Figure 3. Under the experimental conditions, both strains similarly affected ear weight, ear diameter, and percent missing kernels but not ear length (Table 2). All comparisons of virus-infected vs. healthy, except ear length, were statistically significant ($P = 0.05$).

Purification. The purification procedure used for M-C (5) yielded 9 μg of virus per gram fresh weight of infected tissue; however, the method was ineffective for purifying strain M-D, which was successfully purified by a different method (20) and yielded 6 μg of virus per gram fresh weight. A satisfactory degree of purification of M-D was obtained by the above-mentioned procedure, as confirmed by EM examination (Fig. 4).

Serology. In SDS immunodiffusion tests, purified viral preparations were adjusted to 0.5 mg/ml, disrupted with SDS (1.5% final concentration), and placed into wells in agar. M-D antiserum reacted with its homologous viral antigens but failed to react with M-C. M-C antiserum, however, reacted with both viruses, forming a spur (Fig. 5), which indicated partial serological relatedness. No precipitin lines were obtained when the antisera were reacted against healthy crude sap.

Further evidence for serological cross-reactivity between the Israeli strains was obtained by indirect ELISA (Fig. 6). Using this procedure, we found homologous and heterologous titers of M-C antiserum were 1:16,384 and 1:4,096, respectively, whereas those of M-D antiserum were 1:16,384 and 1:1,024, respectively. Indirect ELISA was also used to study the serological relationships among M-C, M-D, and some foreign antisera against MDMV.

The results (Fig. 7) suggest a close serological relationship between M-C antigen and SRV, M-A, and M-D antisera and a distant relationship with SCMV-H antiserum. Reactivity between M-D antigen and M-D and M-C antisera was strong, whereas the reactivity of M-D antigen with SRV, M-A, and SCMV-H antisera was weak. Serological relationships were also determined by IEM decoration tests.

As shown in Table 3, M-C particles reacted with all tested antisera, but

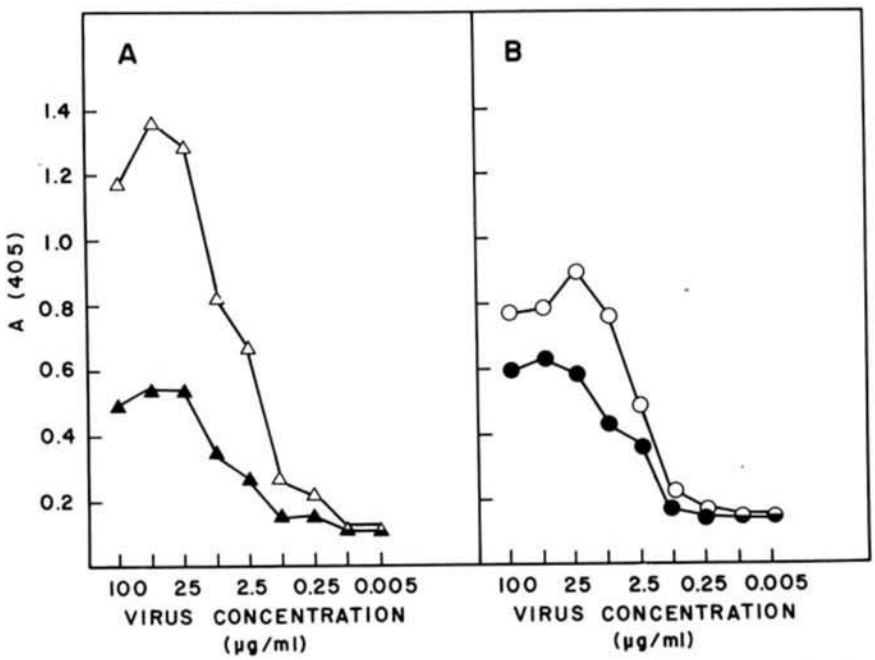

Fig. 6. Serological relationship between Israeli strains M-C and M-D of maize dwarf mosaic virus obtained by indirect ELISA. Immunoglobulins against both strains were brought to the same concentration (0.9 A_{280nm}) and used at a dilution of 1:128. **(A)** Activity of anti-M-C globulins with M-C (Δ) and M-D (▲) viral preparations. **(B)** Activity of anti-M-D globulins with M-D (o) and M-C (●) viral preparations. The mean ELISA value for both the buffer and the purified healthy controls was 0.08 A_{405nm}.

dilution end points differed among the various antisera. Here, the homologous, M-A, and SRV antisera were reactive in the range of 1:128 to 1:512. M-C antigen and M-D and SCMV-H antiserum reacted out to a dilution end point of 1:4. In contrast, the M-D antigen reacted with its homologous antiserum out to a dilution of 1:512, and the remaining antisera were either reactive at dilutions of 1:16 (M-C) or 1:8 (M-A) or were nonreactive (SRV and SCMV-H). The serological differences among the tested antisera were also visible in the intensity and arrangement patterns of antibodies adhering to the viral particles in IEM tests. In Figure 8, M-C particles were heavily coated with the closely related M-A antiserum, whereas SRV and SCMV-H antisera produced a thin coating of antibodies arranged in clusters or spirals along the particle axis. Virus particles were not decorated using antiserum prepared to tobacco mosaic virus. A similar effect is demonstrated when a mixture of viral particles was treated with only homologous antisera (Fig. 9).

DISCUSSION

The Israeli strains of MDMV infected corn and johnsongrass and thus should be designated MDMV-A (9). The mosaic symptoms induced by strain M-C on sorghum cultivars resemble those described by Tosic and Ford (19) for MDMV-A on sorghum cultivar Atlas. The serological data (Fig. 7A, Table 3) also indicate that M-C is closely related to MDMV-A and SRV but has a distant relationship with SCMV-H. These results agree with previous reports on the cross-reactivity between MDMV-A and SCMV-H (3,16) and also agree with the findings of Jarjees and Uyemoto (3), who grouped MDMV-A and SCMV-H in different serogroups but found a weak serological cross-reaction between these two viruses by direct ELISA. The similarity of M-C and MDMV-A is also supported by the ability to extract M-C with Na citrate, which was reported to be efficient only for the extraction of MDMV-A and not for MDMV-B or SCMV-H isolates (16).

Strain M-D is distinguishable from strain M-C in terms of its response to purification procedures, serological properties, and host reaction. M-D

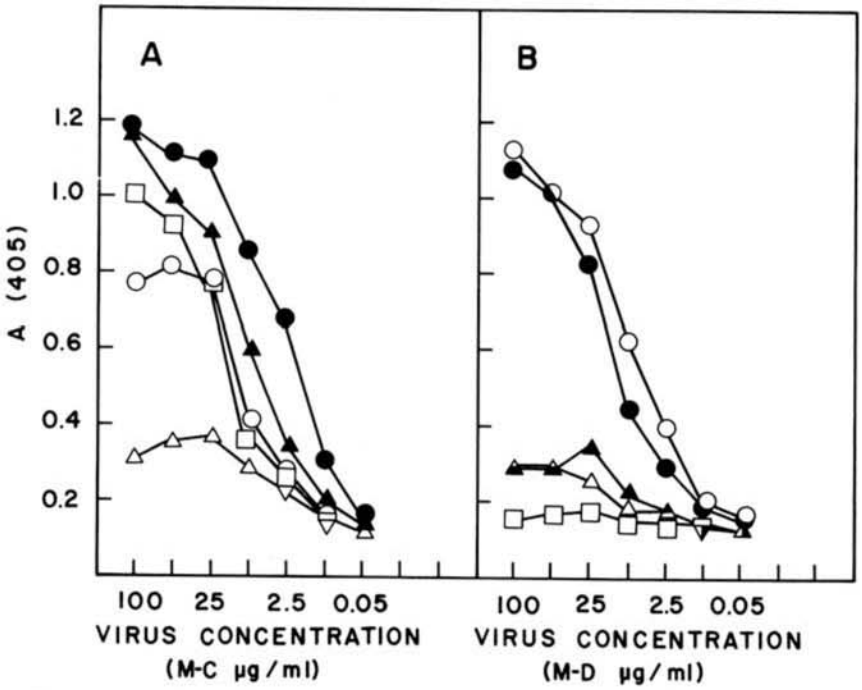

Fig. 7. Serological relationships (indirect ELISA) among antigens and antisera of M-C and M-D, Israeli strains of maize dwarf mosaic virus (MDMV), and three foreign sources of MDMV antisera. All antisera used in this test were in the crude form and were diluted 1:128 before use. **(A)** Reaction of M-C antigen with the following: homologous antiserum (●), M-D antiserum (o), M-A antiserum (▲), SCMV-H antiserum (Δ), and SRV antiserum (□). **(B)** Reactions of M-D antigen with the following: homologous antiserum (o), M-C antiserum (●), M-A antiserum (▲), SCMV-H antiserum (Δ), and SRV antiserum (□). The mean ELISA value for both the buffer and the purified healthy controls was 0.08 A_{405nm}.

Table 3. Dilution end point values obtained from immunoelectron microscopy reactions of M-C and M-D, Israeli strains of maize dwarf mosaic virus (MDMV), with their homologous and heterologous antisera

	Antiserum type[a]				
MDMV strain	**M-C**	**M-D**	**SRV**	**M-A**	**ScMV-H**
M-C	+[b] (512)	+ (4)	+ (128)	+ (256)	+ (4)
M-D	+ (16)	+ (512)	–[c]	+ (8)	–

[a] All antisera were used undiluted.
[b] Positive reaction (reciprocal dilution end point in parentheses).
[c] Negative reaction.

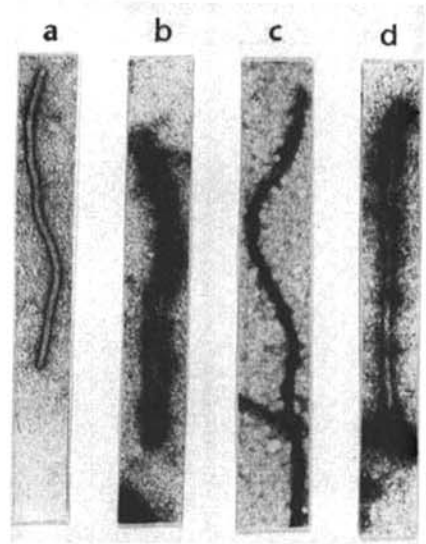

Fig. 8. Purified particles of M-C, an Israeli strain of maize dwarf mosaic virus, showing different decoration patterns after treatment with various antisera: a = TMV antiserum (1:10 dilution), b = M-A antiserum (1:100 dilution), c = SRV antiserum (1:64 dilution), and d = SCMV-H antiserum (undiluted).

Fig. 9. Mixed particles of M-C and M-D, the Israeli strains of maize dwarf mosaic virus, decorated with M-A crude antiserum at a titer of 1:5. M-C particles are heavily coated with the closely related M-A antiserum, whereas those of M-D show a thinner antibody coating.

induced severe mosaic symptoms in corn plants, and its effects on plant height were twice as great in number and severity as the comparable effects in M-C-infected plants. This may explain Szirami's observation (17) on the appearance of two symptom types in Hungary, a milder type that is common and a severe type that is more rare and the occurrence of which varies from year to year.

The symptoms induced by strain M-D in sorghum cultivars resemble those reported for SRV (12); however, M-D failed to react with SRV antiserum either by ELISA or by IEM decoration tests, indicating that there is no serological relationship between these two viruses.

The serological differences between M-C and M-D were more distinct in SDS immunodiffusion and IEM tests than by indirect ELISA. This discrepancy can be explained by the broad specifity and the higher sensitivity of indirect ELISA (7), which was used in this work.

The distinct differences in the biological, serological, and physical properties of Israeli M-C and M-D strains support the statement by Snazelle et al (16) that the flexuous rod viruses attacking corn and sugarcane constitute a homogeneous group in terms of gross physical shape but are heterogeneous in terms of their responses to purification schedules, antigenicity, and host range.

The IEM decoration technique was reported to be a reliable tool for determining strain relationships of elongated viruses (8). The present results with MDMV agree with this generalization and indicate a potential use of this IEM procedure for determining serological relationships within the SCMV complex as well as for diagnostic purposes where strain differentiation is needed.

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