Purification and Serology of Maize Dwarf Mosaic and Sugarcane Mosaic Viruses

T. E. Snazelle, J. B. Bancroft, and A. J. Ullstrup

Plant Pathologist, ARS, USDA, and Professors of Plant Pathology, Purdue University, respectively, Lafayette, Indiana 47907. Present address of senior author: Life Science Department, Rock Valley College, Rockford, Illinois 61101.

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ABSTRACT

Maize dwarf mosaic virus (MDMV) and sugarcane mosaic virus (SCMV) were partially purified by extraction of the viruses in a medium containing 0.14 M 2-mercaptoethanol, 0.17 M ascorbic acid, and 0.01 M Na diethyldithiocarbamate followed by chloroform clarification and differential ultracentrifugation. Rate-zonal, sucrose, density-gradient centrifugation was the final step in purification. On the basis of serological testing and host range studies, the various SCMV and MDMV isolates can be relegated to groups. The Johnson grass-infecting isolates of MDMV would comprise one group distinct from the isolates which do not infect Johnson grass. Sugarcane mosaic virus-A, B, D, E, and Australian SCMV (SCMV-AUS) would form another group. Sugarcane mosaic virus-H would be distinct from all other groups. Phytopathology 61:1059-1063.

Additional key words: sorghum susceptibility, microprecipitin test.

Since the appearance of maize dwarf mosaic in 1962 in Scioto County, Ohio (8), there has been confusion as to the relationship of maize dwarf mosaic virus (MDMV) to sugarcane mosaic virus (SCMV) which causes sugarcane mosaic. The purpose of this investigation was to attempt to clarify the relationship between the various isolates of SCMV and MDMV.

MATERIALS AND METHODS.—*Virus isolates.*—The Ohio isolate of maize dwarf mosaic virus (MDMV-Ohio I or MDMV-A) and the 13B isolate of MDMV (MDMV-13B or MDMV-B) were furnished by L. E. Williams, Ohio Agricultural Experiment Station, Wooster. The A, B, D, and H isolates of SCMV were obtained from A. G. Gillaspie, US Sugarcane Field Station, Houma, La. The St. Augustine grass isolate of SCMV or SCMV-E was provided by J. L. Dean, US Sugarcane Field Station, Canal Point, Fla.

Virus culture.—The single-cross corn (*Zea mays* L.) hybrid C103 Rf × Hy Rf was used for routine culture of all viruses. Johnson grass, *Sorghum halepense* (L.) Pers., was grown from seed provided by R. Hull, University of Rhode Island, Kingston. The sorghum cultivars, *Sorghum bicolor* (L.) Moench, were obtained from D. T. Rosenow, Texas A&M University Agricultural Research and Extension Center, Lubbock, Tex. Sugarcane (*Saccharum officinarum* L.) was obtained from A. G. Gillaspie. The seed pieces were grown in 10-gal cans, and nodal sections were removed for propagation as needed.

The corn, Johnson grass, and sorghum cultivars were mechanically inoculated by finger-rubbing aluminum oxide-dusted plants with virus inoculum. Sugarcane was mechanically inoculated by rubbing the leaves and by multiple hypodermic injections of sap into the leaf whorl.

Purification.—The following method was usually successful for all MDMV and SCMV isolates. Virus-infected corn leaves were harvested 3 to 4 weeks after inoculation, and were usually chilled for 15-18 hr at 4 C as whole leaves in sealed polyethylene bags. The leaves were cut into ca. 1-inch sections and were homogenized in 1.5 to 2.0 ml/g tissue of a cold, freshly prepared medium containing 0.14 M 2-mercaptoethanol, 0.17 M ascorbic acid, and 0.01 M Na diethyldithiocarbamate. The extract was expressed through two layers of cheesecloth, and emulsified with cold chloroform (1 ml/3 g tissue) by shaking for 1 min in an Erlenmeyer flask. The emulsion was broken by centrifugation at 8,000 rpm in a GSA rotor in a Sorvall centrifuge. The clear supernatant liquid was removed and centrifuged at 28,000 rpm in a type 30 rotor in a Spinco preparative ultracentrifuge. Pellets were suspended in 0.1 M pH 8.2 borate buffer (2-3 ml/100 g tissue). The suspensions were clarified by centrifuging at 8,000 rpm in a SS-1 rotor for 10 min in a Sorvall centrifuge, and the supernatant liquids were retained. A method similar to this one was used (A. G. Gillaspie, *personal communication*) to partially purify SCMV. Delgado-Sanchez & Grogan (5) used a method similar to this one to partially purify potato virus Y.

The partially purified preparations were further purified by rate-zonal, density-gradient centrifugation (4) for 2.5 hr at 23,000 rpm in a SW 25.1 rotor. The density-gradient columns were prepared by layering 4, 7, 7, 7, ml of 100, 200, 300, and 400 mg sucrose/ml 0.01 M Na citrate in 2.5 × 7.6 cm cellulose nitrate tubes prior to overnight storage at 4 C. The contents of the tubes were monitored and quantified by peak areas in an ISCO Model D density-gradient fractionator, and the virus-containing zones collected. These zones, the contents of which were infective, were easily seen and separated from contaminating plant constituents which

remained near the top of the tubes. Virus collected from the gradient tubes was sometimes diluted in 0.01 M Na citrate and concentrated by centrifugation for 1 hr at 38,000 rpm in a type 40 rotor. The pellets were suspended in 0.01 M Na citrate and centrifuged at 10,000 rpm in a type 40 rotor for 10 min, and the supernatant liquid was collected. Such purified preparations were used as antigens.

Serology.—New Zealand rabbits were bled for normal serum, then injected intravenously with virus purified as described. The rabbits received eight to nine injections of 1- to 2 mg virus/injection over a 5- to 15-week period. Antisera were stored in an equal volume of glycerol at −8 C.

The microprecipitin test (2) was used to establish serological relationships. All antigens were purified prior to use in these tests. Controls consisted of normal serum and healthy plant constituents obtained three different ways as noted in Table 1. No reactions occurred between viral antisera and the controls. We are satisfied that our antisera are specific.

The SCMV-AUS antiserum was provided by Taylor & Pares (13). Shepherd (12) kindly sent us his MDMV-C and St. Augustine isolate of SCMV (SCMV-SA) antisera.

RESULTS.—*Time of harvest and storage of tissue.*—The physical yield of MDMV-A, as measured in rate-zonal, density-gradient columns, increased 5-fold between 1 and 3 weeks after inoculation. Between 3 and 5 weeks after inoculation, there was an additional increase of less than 10% in the virus yield. Consequently, virus-infected tissue was usually harvested 3-4 weeks after inoculation.

The feasibility of storing tissue was examined. Corn leaves were harvested, cut, and randomized. The virus from one sample was purified and quantified immediately. Other samples were frozen at −6 C for 15 and 33 days. These yielded only 30% as much virus as was obtained from the freshly harvested material. Tissue kept for 15 and 33 days at 4 C yielded, respectively, about 50% and 7% as much virus as did the fresh material. Therefore, all purifications were made from fresh tissue.

Comparison of extraction, concentration, and suspension methods.—The purification method already outlined was used because it gave high MDMV and SCMV yields. In extraction, if the reducing agents and diethyldithiocarbamate were omitted, yields were decreased by about 60%. Use of 0.5 M Na citrate in the extraction medium (12) reduced MDMV-A by about 30%. This extraction method was only successful with MDMV-A; neither MDMV-B nor the SCMV isolates yielded amounts of virus detectable in the density-gradient tubes after 0.5 M Na citrate extraction. Maize dwarf mosaic virus-A yields from 0.5 M Na citrate extractions were double those from 0.03 M Na citrate extractions. No virus was recovered after 2 M Na citrate extraction. Extraction of SCMV-H in 0.02 M Na_2SO_3 containing the reducing agents and diethyldithiocarbamate, followed by adjustment to pH 4.7, as in Pirone & Anzalone's method (11), yielded only 15% as much virus as from the routine schedule. Incorporation of 0.5 M urea in the usual extraction medium reduced the yield of SCMV-A by 50%.

The efficacy of polyethylene glycol (PEG), as opposed to that of centrifugation as a method for concentrating MDMV-A, was examined. Tissue was blended in 0.5 M citrate and emulsified in chloroform, and the emulsion broken by low-speed centrifugation. The PEG, at 4, 8, 12, and 16% (w/v) was added directly to the chloroform-saturated 0.5 M Na citrate supernatant liquid. Polyethylene glycol 600 (average mol wt 600) did not precipitate the virus up to 16% polymer. Polyethylene glycol 1540 (average mol wt 1,540) precipitated virus at 16%, but not at 12%. Virus was precipitated by PEG 4,000 (average mol wt 4,000) at 8%, but not at 4%, and by PEG 6,000 (average mol wt 6,000) at 4%. Yields after PEG precipitation were never as great as those of the centrifuged controls, nor did PEG improve the purification product qualitatively. Consequently, centrifugation was used in the routine purification schedule.

High-speed pellets were routinely resuspended in 0.1 M pH 8.2 borate buffer. Addition of 0.5 M urea to the borate buffer increased MDMV-A yields by less than 10%. Addition of 0.5 M urea to sucrose used in density-gradient columns had no effect on MDMV-A, and 0.05 to 0.1% Igepon T-73 destroyed the virus. The use of CsCl for equilibrium-zonal centrifugation gave no advantage over the rate-zonal method.

Serology.—The serological relationships among the MDMV and SCMV isolates are summarized in Table 1. Maize dwarf mosaic virus-A reacted homologously with its own antiserum and against Shepherd's MDMV-C antiserum prepared against an MDMV isolate from Fresno County, Calif., and his SCMV-SA antiserum prepared against a Florida St. Augustine grass isolate of SCMV (12). We have previously shown that MDMV isolates from across the United States react with our MDMV-A antiserum (3). Furthermore, the reaction of maize mosaic virus from Yugoslavia against our MDMV-A antiserum shows that Balkan virus is related to the American ones (M. Tosic, *personal communication*).

The SCMV-A, B, D, E, and MDMV-B isolates reacted heterologously against MDMV-A antiserum. Thus, these viruses are related to MDMV-A, although they are not serologically identical to that virus. The same isolates, but not MDMV-B, reacted against antisera made to SCMV-A and SCMV-AUS. Interestingly, the reciprocal tests involving MDMV-A against SCMV-A or SCMV-AUS antisera showed no reactions. The epitope, common to MDMV-A and SCMV-A at least, is probably less reactive or more strongly antigenic in MDMV-A than in SCMV-A. This nonparallelism between antigenicity and reactivity of determinant groups has been noted by van Regenmortel (14) in reactions between tobacco mosaic virus and cucumber virus-4 and their respective antisera. Sugarcane mosaic virus-H reacted only homologously, and is not related to either MDMV-A, MDMV-B, or the other SCMV strains. On the basis of our data, MDMV-A, MDMV-

TABLE 1. Serological relationships among strains of maize dwarf mosaic virus (MDMV) and sugarcane mosaic virus (SCMV)

	Antisera						
Antigens[a]	SCMV-A	SCMV-AUS[b]	SCMV-H	SCMV-SA[c]	MDMV-A	MDMV-C[c]	Normal serum
SCMV-A	+(128)[d]	+(16)	[e]		+(64)		
SCMV-B	+(32)	+(64)			+(32)		
SCMV-D	+(64)	+(32)			+(32)		
SCMV-E	+(64)	+(32)			+(16)		
SCMV-H			+(64)				
MDMV-A				+(64)	+(128)	+(64)	
MDMV-B				+(64)	+(16)		
MMV	0[f]	0	0	0	+(?)	0	0
HCP[g]		+(8)					
HCPFI[h]							
HCPVD[i]							

[a] All viral antigens were partially purified, then subjected to density-gradient centrifugation. Virus-containing zones were removed from the density-gradient tubes, and the virus was used at an initial concentration of about 0.15 mg/ml in all serological tests except the MDMV-A antiserum versus maize mosaic virus (MMV) test. The test with MMV was done by M. Tosic in Yugoslavia with clarified sap against our antiserum. No titer was provided.

[b] Antiserum provided by R. H. Taylor, Victorian Plant Research Institute, Burnley, Victoria, Australia. We have no homologous titers. We also tested Australian MDMV and Johnson grass mosaic virus antisera, but did not obtain positive reactions with any of our antigens.

[c] Antisera provided by R. J. Shepherd, University of California, Davis.

[d] + = Positive reaction in the microprecipitin test after 2-4 hr at 25 C. The number in parentheses denotes the reciprocal of the titer.

[e] Blank space = No reaction at an antiserum dilution of 0.5.

[f] 0 = No test.

[g] HCP = Partially purified corn extract not subjected to density-gradient centrifugation.

[h] HCPFI = Presumably, fraction 1 protein from healthy corn removed from near the tops of density-gradient tubes.

[i] HCPVD = Samples removed at the depth of virus from density gradient tubes on which HCP had been placed.

B, and Shepherd's SCMV-SA appear to be related. Heterologous testing of SCMV-SA with our MDMV-A, SCMV-A, and SCMV-H antisera was not possible, as an infective isolate of this virus was no longer available.

On the basis of the serological tests presented here and previously (3), the various virus isolates can be relegated to groups. The Johnson grass-infecting isolates of MDMV would comprise one group distinct from the isolates such as MDMV-B which do not infect Johnson grass. Sugarcane mosaic virus-A, B, D, E, and probably SCMV-AUS would form another group. Sugarcane mosaic virus-H would be distinct from all other groups.

Host range.—Only MDMV-A infected Johnson grass, on which it caused a mosaic. The other viruses were each inoculated to 20 Johnson grass plants, but none developed symptoms, and no virus was recovered by back inoculation to corn. Both of the sugarcane cultivars C.P. 31-294 and C.P. 31-588, used by Abbott & Tippett (1) as symptom differentials, were susceptible to all SCMV isolates but not to MDMV-A or MDMV-B, neither of which could be recovered from the symptomless plants. The symptoms produced by SCMV-A, B, D, and H on C.P. 31-294 and C.P. 31-588 were typical of those described by Abbott & Tippett. Thus, MDMV-B could be differentiated from MDMV-A or the SCMV isolates by its inability to infect either Johnson grass or sugarcane. Reactions of SCMV and MDMV isolates on the sorghum cultivars are shown in Table 2. Symptoms were observable in all cases from 2 to 4 weeks after inoculation. Even though MDMV-A and SCMV-H infected all the sorghum differentials, they could be easily distinguished, as SCMV-H was lethal to five of them, whereas MDMV-A was not.

DISCUSSION.—The flexuous viruses attacking corn or sugarcane comprise a homogeneous group in regard to gross physical characteristics such as size and shape, but are really heterogeneous in terms of their response to purification schedules, and in regard to serology and host range. Thus, MDMV-A can be differentiated from other viruses by its response to 0.5 M Na citrate extraction. Serologically, the viruses present a general pattern which can be used, as we have shown, to group them. Differential host ranges present yet another means of classification. MacKenzie (10) designated isolates of MDMV that might infect both corn and Johnson grass as MDMV-A and isolates which only infect corn as MDMV-B. Louie & Knoke (9) were able to differentiate four MDMV isolates from southern Ohio on a series of corn lines. On the basis of symptomatology, Zummo (16) was able to differentiate six mosaic-inducing virus isolates from California, Georgia, Kentucky, Mississippi, and Virginia which were found infecting corn, Johnson grass, and sorghum on the sorghum cultivar Rio. Fazli et al. (6) reported that the sorghum cultivar Martin offered some degree of resistance to SCMV-H and MDMV-A. In our study, this cultivar was very susceptible to MDMV-A and SCMV-H. Yet, with all these methods available, confusion still remains as to how these viruses are related. We do not believe the difficulty is primarily due to methodology, but rather results from the multiplicity

TABLE 2. Reactions of sorghum differentials with the maize dwarf mosaic virus (MDMV) and sugarcane mosaic virus (SCMV) isolates

Sorghum	Viruses						
	SCMV-A	SCMV-B	SCMV-D	SCMV-E	SCMV-H	MDMV-B	MDMV-A
B Tx 378	[a]	+[b]			+[c]		+
(Redlan)	0/14[d]	2/12	0/11	0/15	14/14	0/14	12/12
Tx 7000	+	+	+	+	+[c]		+
(Caprock)	9/14	9/10	8/11	15/16	9/9	0/11	7/10
Tx 414				+	+		+
(7078 derivative)	0/13	0/14	0/14	7/15	16/16	0/15	14/14
Tx 7078				+	+		+
(7078)	0/15	0/15	0/15	3/15	14/14	0/15	14/14
B Tx 398				+	+		+
(Martin)	0/16	0/15	0/15	1/13	17/17	0/18	14/14
Tx 09	+	+	+	+	+	+	+
(Combine White Feterita)	11/13	13/15	4/14	9/14	15/15	8/11	11/14
New Mexico-31		+	+	+	+[c]		+
(New Mexico-31)	0/15	14/14	3/15	9/15	14/15	0/16	9/12
Tx 412	+	+	+	+	+	+	+
(Tx 09 × SA 403)	15/15	14/14	14/14	10/13	16/16	15/15	14/14
B Tx 3197		+	+	+	+[c]		+
(Combine Kafir-60)	0/12	5/15	3/15	3/12	13/13	0/14	16/16
SA 394		+	+		+	+	+
(Combine Shallu)	0/15	8/14	1/17	0/15	12/12	10/13	13/14
P.I. 35038		+	+	+	+		+
(Sumac)	0/14	14/14	16/16	15/15	15/15	0/13	14/14
B Tx 3048		+	+	+	+[c]		+
(Redbine selection)	0/15	10/14	9/14	4/15	14/14	0/12	10/13

[a] Blank spaces = plants not infected; no mosaic symptom.
[b] + = Plants infected; mosaic symptom.
[c] SCMV-H is lethal to these sorghum cultivars.
[d] Fraction of plants infected.

of isolates and stocks kept throughout the country. A consideration of various serological results underlines this possibility.

Some of our serological data do not agree with published results. On the basis of symptom expression on the sorghum cultivar Atlas and serology, Gordon & Williams (7) concluded that the 13B virus isolate of MDMV is closely related to SCMV-H. This result is in contradiction with our findings in which this isolate did not react with our SCMV-H antiserum. Wagner & Dale (15) found that their SCMV-H cross-reacted strongly with an antiserum made to an Arkansas isolate of MDMV which reacted with our MDMV-A antiserum (3). They purified SCMV-H, as did Shepherd (12), by the 0.5 M Na citrate method. We have been unable to purify our SCMV-H isolate, which originated in Abbott & Tippett's collection (1), by the 0.5 M Na citrate method. Shepherd (12) found that his SCMV-H cross-reacted moderately with his MDMV-C. MDMV was referred to as California corn mosaic virus by Shepherd. We could not show a relationship between our SCMV-H and MDMV-A, although we know the latter virus is related to Shepherd's MDMV-C. Furthermore, we could not show a relationship between our SCMV-E and Shepherd's SCMV-SA (supposedly the same virus as our SCMV-E). Shepherd also found that his SCMV-H reacted strongly with his SCMV-SA antiserum. Also, we were not able to purify SCMV-E by the 0.5 M Na citrate method used by Shepherd to purify SCMV-SA. The simplest explanation for the above differences is that different strains have been circulated under the same name. We have attempted to circumvent this difficulty by obtaining isolates at their primary source, and by testing them on differential hosts to preclude the possibility of cross-contamination. Along with careful maintenance of stocks, such precautions probably should be taken routinely in future investigations of this large group of viruses.

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