Etiology

Taxonomy of Potyviruses Infecting Maize, Sorghum, and Sugarcane in Australia and the United States as Determined by Reactivities of Polyclonal Antibodies Directed towards Virus-Specific N-Termini of Coat Proteins

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This work was carried out when D. D. Shukla and M. Tosic were on sabbatical leave at the University of Illinois, Urbana. We thank Drs. G. T. Benda, W. G. Dougherty, D. T. Gordon, and J. H. Hill (Table 1) for supplying some of the viruses and antisera used in this investigation; Dr. F. Forsberg, Deparment of Agronomy, University of Wisconsin, Madison 53706, for oat seeds; Dr. C. W. Ward for invaluable discussions; and Mr. C. Mercer and Ms. L. Monarch for the photographs. D. D. Shukla and M. Tosic are grateful to the University of Illinois for providing laboratory support, and D. D. Shukla is also grateful to the Australian-American Educational Foundation for awarding a Fulbright Senior Fellowship.

Accepted for publication 7 August 1988.

ABSTRACT

Shukla, D. D., Tosic, M., Jilka, J., Ford, R. E., Toler, R. W., and Langham, M. A. C. 1989. Taxonomy of potyviruses infecting maize, sorghum, and sugarcane in Australia and the United States as determined by reactivities of polyclonal antibodies directed towards virus-specific N-termini of coat proteins. Phytopathology 79:223-229.

A large number of potyvirus isolates currently classified as strains of sugarcane mosaic virus (SCMV) are reported to infect maize, sorghum, and sugarcane in various parts of the world, although isolates originating in maize have been frequently referred to as strains of maize dwarf mosaic virus (MDMV). The taxonomic status and the serological interrelationships of these strains have never been clearly defined. In this paper we have compared 17 SCMV/MDMV strains from Australia and the United States on the basis of their reactivities, in electro-blot immunoassay, with cross-absorbed polyclonal antibodies directed towards surface-located, virus-specific N-termini of coat proteins. Our results clearly demonstrate that the 17 SCMV/MDMV strains belong to four distinct potyviruses for which the names Johnsongrass mosaic virus (SCMV-JG and MDMV-O), maize dwarf mosaic virus (MDMV-A, MDMV-D, MDMV-E, and MDMV-F), sugarcane mosaic virus (MDMV-B, SCMV-A, SCMV-B, SCMV-D, SCMV-E, SCMV-SC, SCMV-BC, and SCMV-Sabi), and sorghum mosaic virus (SCMV-H, SCMV-I, and SCMV-M) have been proposed.

A large number of virus isolates currently classified as strains of sugarcane mosaic virus (SCMV), a definitive member of the potyvirus group (20), have been reported to infect maize, sorghum, sugarcane, and other poaceous plant species in various parts of the world (26). The strains generally induce a mosaic type of symptom in their hosts (26) and are known to be responsible for significant crop losses (9,17,42). Traditionally, strains originating in sugarcane were designated as strains of SCMV (1) and those originating in maize as strains of maize dwarf mosaic virus (MDMV) (18). However, strains of SCMV and MDMV were reported to be interrelated serologically (5,27,38), and therefore, the latter is now considered a strain of the former (26). SCMV and MDMV are still considered distinct viruses in the United States (16), and the two names continue to be used in the literature (3,8,17,21).

In the United States, 13 strains of SCMV—SCMV-A, SCMV-B, SCMV-C, SCMV-D, SCMV-E, SCMV-F, SCMV-G, SCMV-H, SCMV-I, SCMV-J, SCMV-K, SCMV-L, and SCMV-M (1,14,39, 41,46,47)—and eight strains of MDMV—MDMV-A, MDMV-B, MDMV-C, MDMV-D, MDMV-E, MDMV-F, MDMV-KS1, and MDMV-O (13,18,19,21,38)—have been described. The SCMV strains have been differentiated primarily on reactions in selected sugarcane cultivars, whereas strains of MDMV have been distinguished by infectivity on Johnsongrass, sorghum lines, maize inbreds, and oats (21,25).

In Australia, four strains of SCMV have been described on the basis of natural and experimental host ranges, symptoms in certain differential plant species, and antigenic properties. These strains were designated as Johnsongrass (SCMV-JG), sugarcane (SCMV-SC), Queensland blue couch grass (SCMV-BC), and Sabi grass (SCMV-Sabi) (40).

The interrelationships among MDMV and SCMV strains are complex and difficult to determine (7,8,16,26). Some investigations of antigenic properties have shown that MDMV/SCMV strains can be divided into different groups on the basis of their

close and distant serological relationships (5,13,28,34,38). However, such groupings have not been found consistent with the results of other workers (27,44). Moreover, some MDMV/SCMV strains have recently been shown to be even serologically unrelated when compared with the other strains of the virus (8,21,28,34). On the basis of these and other findings, Francki et al (6) noted that some of the SCMV strains differ so much from each other that they merit the status of distinct viruses. These observations highlight the present confusion concerning the status and the relationships of the virus isolates included as strains of SCMV (26). This situation is not unique to SCMV and its strains but is typical of the whole potyvirus group. The unsatisfactory state of potyvirus taxonomy is due to the large size of the group (at least 152 definitive and possible members), the vast variation among the viruses, and the lack of satisfactory taxonomic parameters which will distinguish viruses from strains (6).

During our investigations on coat proteins of potyviruses (10,29,30,32,33,35–37), we observed that their structural properties can be used to clearly differentiate between individual potyviruses and their strains. Our results demonstrated that 1) distinct members of the group possess a sequence homology of 38–71% with major differences in length and sequence of their N-termini, whereas strains of individual viruses exhibit a sequence homology of greater than 90% and have N-terminal sequences that are very similar (37); 2) the N- and C-termini of coat proteins are located on the particle surface. These termini can easily be removed from the intact particles by mild enzyme treatment, and their removal does not affect the infectivity and morphology of virus particles (35); 3) the N-terminus is the only large region in the entire coat protein of a potyvirus that is virus-specific (37), and it contains major virus-specific epitopes (35): 4) distinct members possess extensive sequence homology in the core region of their coat protein, and antibodies directed to this region of one potyvirus were found to recognize all 15 distinct potyviruses tested (31,35, *unpublished results*).

On the basis of these findings we developed an affinity chromatographic procedure (31) to isolate antibodies directed towards N-terminal peptide regions of coat proteins from polyclonal antisera to purified particles of potyviruses. Such antibodies were found to be virus-specific as they recognized only the homologous viruses, whereas the original unfractionated polyclonal antisera from which these antibodies were isolated contained antibodies directed to conserved internal epitopes and reacted with several biologically and serologically distinct potyviruses (31).

In this paper we have investigated the serological relationships of 13 American and four Australian strains of MDMV and SCMV using polyclonal antibodies directed towards N-termini of their coat proteins. Our results show that these 17 strains can be grouped into four classes, which we propose represent four distinct potyviruses.

MATERIALS AND METHODS

Virus strains. The MDMV/SCMV strains investigated are shown in Table 1 along with their sources. Except for the four Australian SCMV strains (SCMV-JG, SCMV-SC, SCMV-BC, and SCMV-Sabi) (40) all other strains originated in the United States. SCMV-JG (Australia) has recently been classified as a strain of Johnsongrass mosaic virus (JGMV) (29). However, for the sake of uniformity the SCMV-JG nomenclature will be used throughout this paper. The strains were maintained in corn, cultivar Gold Cup by mechanical inoculation of sap obtained by grinding infected tissue in 0.05 M sodium borate buffer, pH 8.0, using Carborundum as abrasive.

Virus purification. The strains, propagated in corn, were purified by clarification of sap with chloroform and carbon tetrachloride, one cycle of differential centrifugation, and one or two centrifugations on sucrose-cesium sulphate density gradients (31).

Antisera. The antisera to MDMV/SCMV strains used are shown in Table 1, along with their sources. All antisera were produced in rabbits to purified particles of the strains.

Isolation of virus-specific antibodies. Virus-specific antibodies from the polyclonal antisera to MDMV-A, MDMV-B, MDMV-D, MDMV-E, MDMV-F, MDMV-O, SCMV-H, SCMV-I, SCMV-JG, SCMV-SC, SCMV-BC, and SCMV-Sabi (Table 1) were isolated by the affinity chromatographic method essentially as described by Shukla et al (31). Briefly, the surface exposed N-terminal peptide region from 10 mg of purified virions of MDMV-A (R. E. Ford, Table 1) was removed by a 30-min incubation of the viral preparation with lysyl endopeptidase (Wako Chemicals, Dallas). The coat protein core was dissociated using formic acid and coupled to 1 g of CNBr-Sepharose (Pharmacia, Sweden). One hundred microliters of each antisera was washed through the column and the antibodies not bound to the column were collected and used in immunoasaay.

Electro-blot immunoassay. The immunoassay was performed after sodium dodecyl sulfate-polyacrylamide gel electrophoresis (15) of either purified viral preparations or infected sap using nitrocellulose strips and horseradish peroxidase-conjugated second antibodies according to the manufacturer's directions (Bio-Rad, Richmond, CA) as described previously (11,23). Purified viral preparations or infective sap were mixed with an equal volume of the Laemmli sample buffer (15), heated for 3 min in boiling water, and stored at −20 C in small aliquots until used in the immunoaasay. Electrophoresis (Model Protean II) and transfer apparatus used were those from Bio-Rad. About 5 μg of the purified viral preparations or 10 μl of 1:10 diluted sap preparation (23) was used per slot of the gel. The antisera were diluted 1:1000 in 5% nonfat milk powder containing 0.1% Antifoam A (Sigma, St. Louis, MO) before use (31). The Bio-Rad prestained standards for marker proteins used were: phosphorylase b (130,000), bovine serum albumin (75,000), ovalbumin (50,000), carbonic anhydrase (39,000), soybean trypsin inhibitor (27,000), and lysozyme (17,000). The prestained marker proteins for BRL standards were: myosin, H-chain (200,000), phosphorylase b (97,400), bovine serum albumin (68,000), ovalbumin (43,000), α chymotrypsinogen (25,700), β-lactoglobulin (18,400), and cytochrome C (12,300). Five, ten, or twenty microliters of the prestained standards was used per slot of the gel.

TABLE 1. Sources of viruses and antisera used

Virus[a]	Source of virus[b]	Source of antiserum[b]
MDMV-A	D. T. Gordon[1] (21), R. E. Ford (22)	R. E. Ford (22), J. H. Hill[4] (12) M. A. C. Langham (16)
MDMV-B	D. T. Gordon[1] (21), R. E. Ford (22)	R. E. Ford (22), J. H. Hill[4] (12) M. A. C. Langham (16)
MDMV-D	R. W. Toler (8)	M. A. C. Langham (16)
MDMV-E	R. W. Toler (8)	M. A. C. Langham (16)
MDMV-F	R. W. Toler (8)	M. A. C. Langham (16)
MDMV-O	D. T. Gordon[1] (21)	D. T. Gordon[1] (21), M. A. C. Langham (16)
SCMV-A	G. T. Benda[2]	-
SCMV-B	G. T. Benda[2]	-
SCMV-D	G. T. Benda[2]	-
SCMV-E	D. T. Gordon[1]	-
SCMV-H	R. W. Toler (8)	R. W. Toler (8)
SCMV-I	-[c]	R. W. Toler (8)
SCMV-M	R. W. Toler (8)	-
SCMV-JG	D. D. Shukla (29)	D. D. Shukla (28)
SCMV-SC	D. D. Shukla (29)	D. D. Shukla (28)
SCMV-BC	D. D. Shukla (29)	D. D. Shukla (28)
SCMV-Sabi	D. D. Shukla (29)	D. D. Shukla (28)
TEV	W. G. Dougherty[3] (2)	

[a] MDMV = Maize dwarf mosaic virus, SCMV = sugarcane mosaic virus, TEV = tobacco etch virus.

[b] [1] Department of Plant Pathology, Ohio State University, Ohio Agricultural and Development Center, Wooster, OH 44691;
[2] USDA, ARS, Houma Sugarcane Research Center, U.S. Sugarcane Field Laboratory, Houma, LA 70361;
[3] Department of Microbiology, Oregon State University, Corvallis 97331;
[4] Department of Plant Pathology, Iowa State University, Ames 50011.

[c] This strain or this antiserum was not used.

RESULTS

When unfractionated antisera to MDMV-A (Fig. 1A), MDMV-B (Fig. 1C), and MDMV-O (Fig. 1E) were analyzed in electro-blot immunoassay they were found to react to varying degrees with the eight strains of MDMV/SCMV tested as well as with tobacco etch virus (TEV), an unrelated, distinct member of the potyvirus group (20). In contrast, the antibodies obtained by cross-absorption over a column of coat protein cores showed restricted specificities and allowed the 17 MDMV/SCMV strains examined to be placed into four distinct groups.

The eight MDMV/SCMV strains examined in Figure 1 were found to fall into three groups. The first consisted of MDMV-A only (Fig. 1B): the second comprised MDMV-B, SCMV-SC, SCMV-BC, SCMV-Sabi, and SCMV-E (Fig. 1D): and the third group contained SCMV-JG and MDMV-O (Fig. 1F). Electro-blot immunoassay of additional strains revealed that MDMV-A was serologically related to MDMV-D, MDMV-E, and MDMV-F (Fig. 2A) and that the group containing MDMV-B, SCMV-E, and the three of the Australian SCMV strains (Fig. 1D) also included the U. S. strains SCMV-A, SCMV-B, and SCMV-D (Fig. 2B). Further analysis of MDMV/SCMV strains confirmed these findings (Fig. 3A, C, and D) and revealed the existence of an additional group containing SCMV-H and SCMV-M (Fig. 3B). The complete set of reactivities of the 12 cross-absorbed virus-specific antibodies with 16 MDMV/SCMV strains are

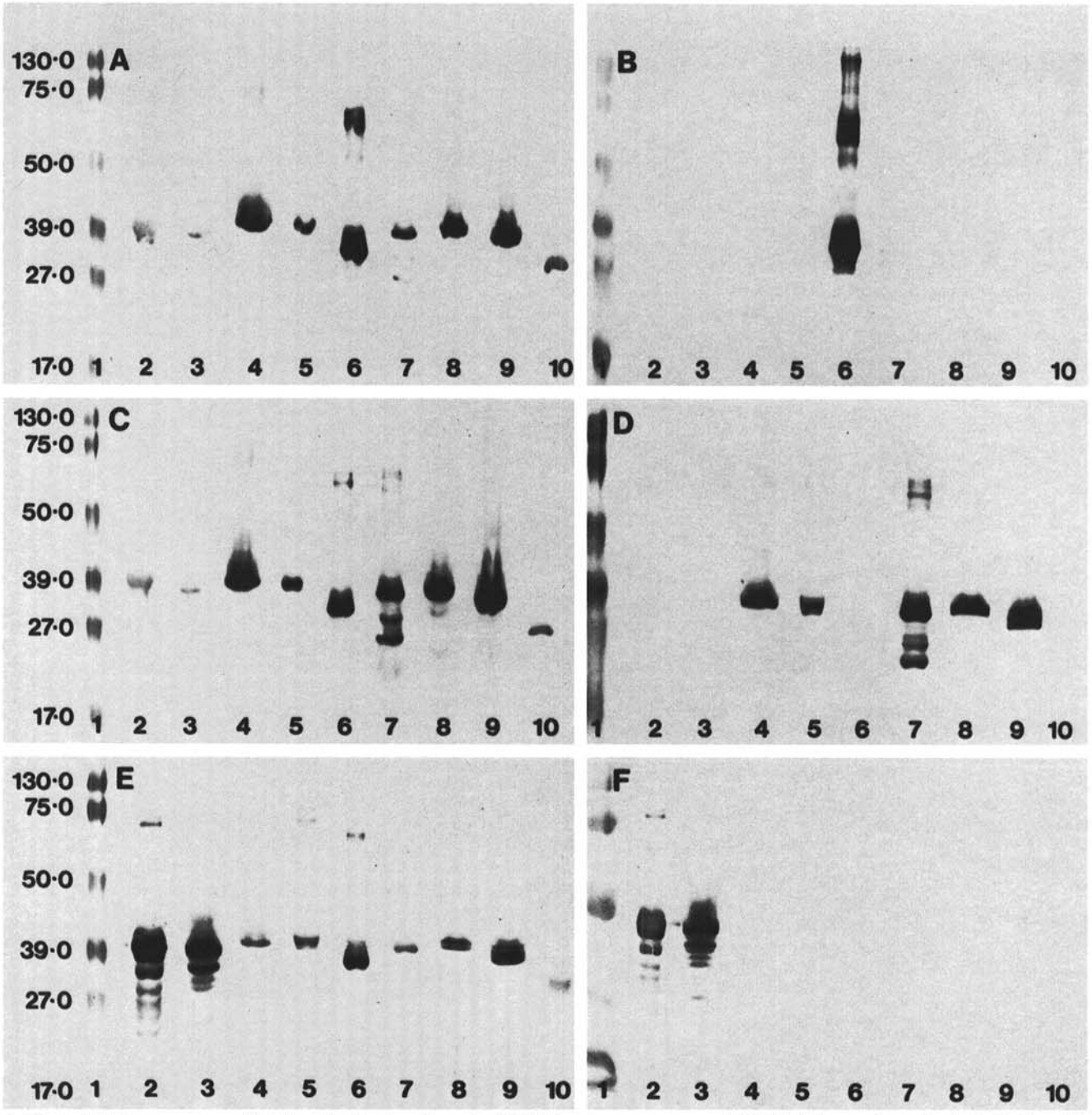

Fig. 1. Electro-blot immunoassays of strains of maize dwarf mosaic (MDMV) and sugarcane mosaic (SCMV) viruses. Lane 1 in A, B, C, D, and E is Bio-Rad- and F BRL- (molecular weight markers as in Fig. 2A) prestained standards. Lanes 2–10 represent SCMV-JG, MDMV-O (Gordon), SCMV-SC, SCMV-E, MDMV-A (Ford), MDMV-B (Ford), SCMV-BC, SCMV-Sabi, and tobacco etch virus, respectively. A, C, and E were probed with unfractionated antisera to MDMV-A (Ford), MDMV-B, and MDMV-O (Gordon), respectively. B, D, and F were probed with cross-absorbed, virus-specific antisera to MDMV-A, MDMV-B, and MDMV-O, respectively. Lanes 2, 3, and 6 are purified preparations; other lanes are infected sap. Numbers to the left are molecular weight ($\times 10^3$) of marker proteins in lane 1.

summarized in Table 2. From this table it can be seen that the 17 investigated strains fall into four distinct groups, 1) MDMV-A, MDMV-D, MDMV-E, and MDMV-F; 2) MDMV-B, SCMV-A, SCMV-B, SCMV-D, SCMV-E, SCMV-SC, SCMV-BC, and SCMV-Sabi: 3) SCMV-H, SCMV-I, and SCMV-M; and 4) SCMV-JG and MDMV-O.

The lack of specificity shown for unfractionated antisera to MDMV-A, MDMV-B, and MDMV-O (Fig. 1A, C, and E) was also observed with all other antisera to MDMV and SCMV strains used (results not shown) except for the SCMV-JG antiserum (Fig. 3A). Besides reacting with the MDMV/SCMV strains these antisera also reacted with TEV in all oases. To determine the time of appearance of the cross-reacting antibodies, antisera to MDMV-A (16) from different bleedings of a rabbit were analyzed with MDMV/SCMV strains and TEV. The results show that antiserum from the first bleeding obtained 4 wk after immuniza-

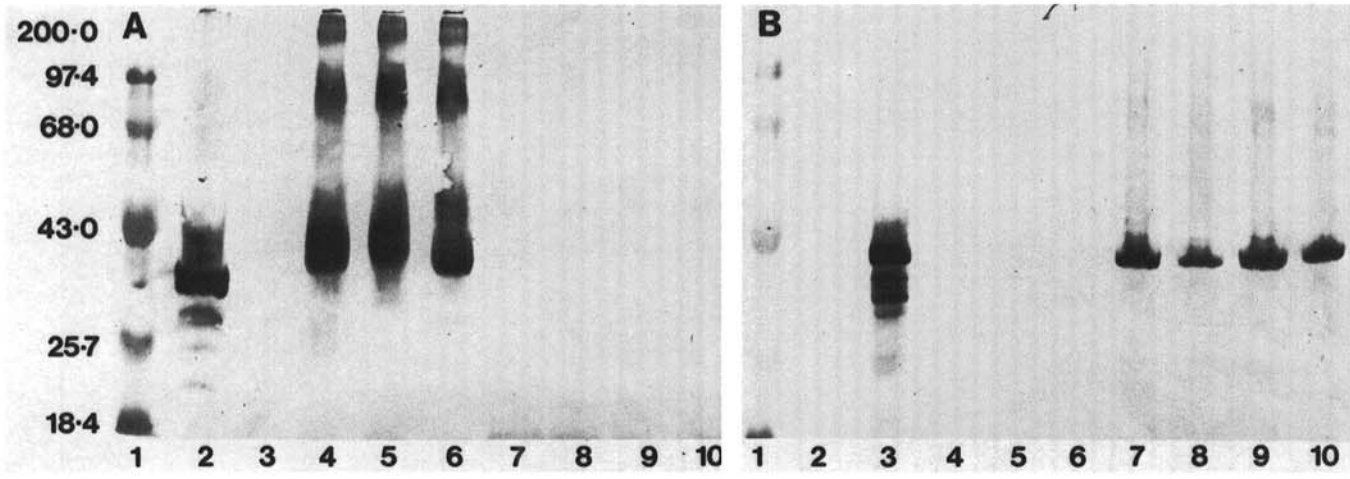

Fig. 2. Electro-blot immunoassays of strains of maize dwarf mosaic (MDMV) and sugarcane masaic (SCMV) viruses. Lane 1 is prestained BRL standards. Lanes 2–10 represent MDMV-A (Ford), MDMV-B (Ford), MDMV-D, MDMV-E, MDMV-F, SCMV-A, SCMV-B, SCMV-D, and SCMV-E, respectively. A and B were probed with cross-absorbed, virus-specific antisera to MDMV-A (Ford) and MDMV-B (Ford), respectively. Lanes 2 and 3 are purified preparations; other lanes are infected sap. Numbers to the left are molecular weight ($\times 10^3$) of marker proteins in lane 1.

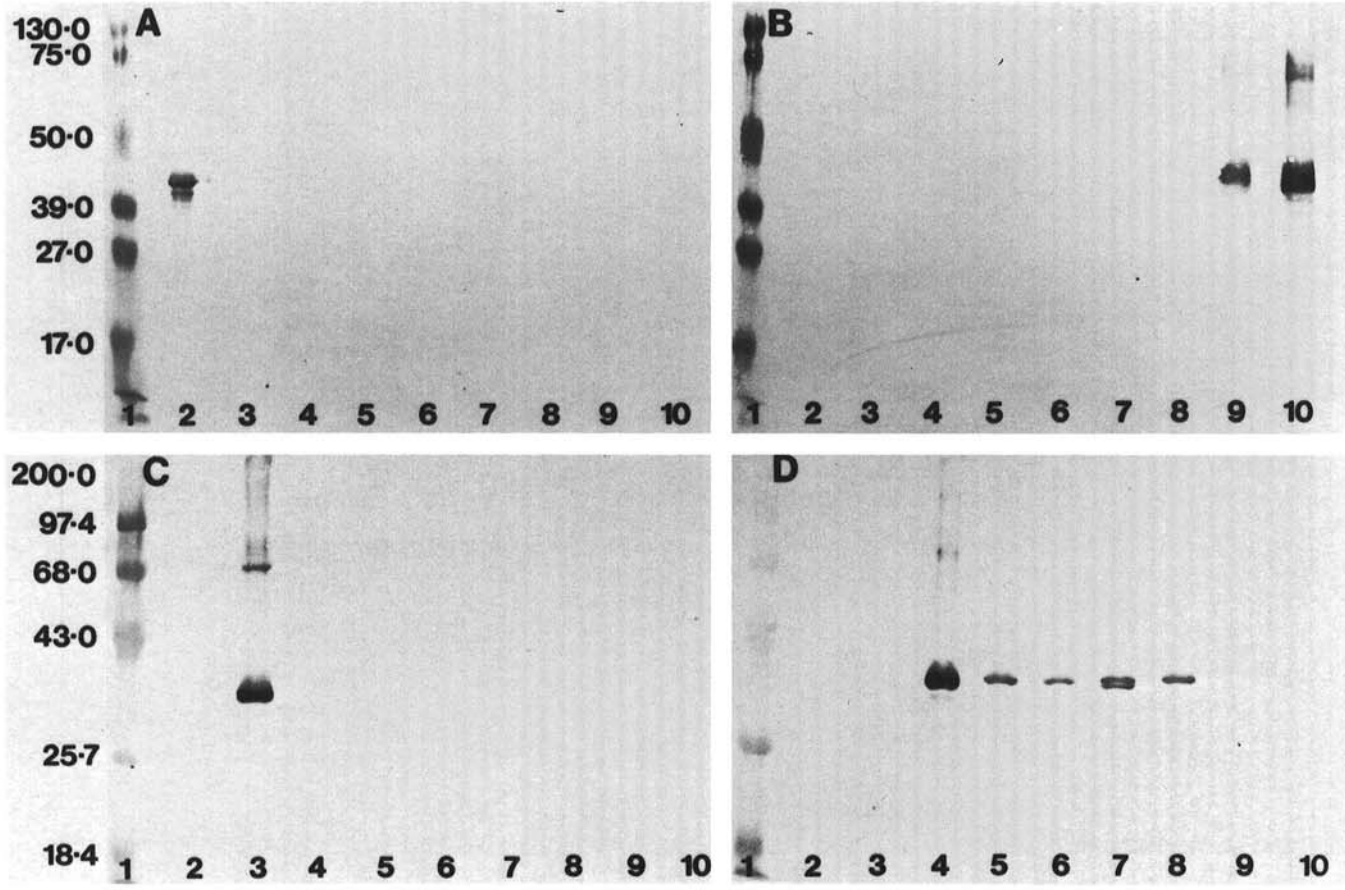

Fig. 3. Electro-blot immunoassays of strains of maize dwarf mosaic (MDMV) and sugarcane mosaic (SCMV) viruses. Lane 1 in A and B is Bio-Rad-and C and D BRL-prestained standards. Lane 2–10 represent SCMV-JG, MDMV-A (Ford), MDMV-B (Ford), SCMV-A, SCMV-B, SCMV-D, SCMV-E, SCMV-H and SCMV-M, respectively. A is probed with unfractionated antiserum to SCMV-JG and B, C, and D with cross-absorbed, virus-specific antisera to SCMV-H, MDMV-A (Ford), and MDMV-B (Ford), respectively. Lanes 2 and 3 are purified preparations; other lanes are infected sap. Numbers to the left are molecular weight ($\times 10^3$) of marker proteins in lane 1.

tion contained populations of antibodies that reacted strongly with MDMV-A, very weakly with SCMV-SC, SCMV-E, MDMV-B, SCMV-BC, and SCMV-Sabi, and not at all with SCMV-JG, MDMV-O, and TEV (Fig. 4A). However, antisera obtained 8 (Fig. 4B), 11 (Fig. 4C), and 13 wk (Fig. 4D) after immunization contained an increasing proportion of antibodies that reacted with other potyviruses.

As indicated in Table 1, three isolates of MDMV-A, three isolates of MDMV-B, and two isolates of MDMV-O were used in these studies, and the results were not affected by the isolates or antisera employed. The lower molecular weight bands seen in some lanes of Figures 1–4 are degradation products of the coat proteins, and the higher molecular weight bands are coat protein dimers which display the same serological properties as the corresponding coat protein monomers (35). The weak bands of marker proteins in lane 1 of some figures reflect lower loading of the prestained standards.

DISCUSSION

In this paper we have compared the reactivities of cross-absorbed antibodies directed towards virus-specific N-termini of

TABLE 2. Reactivities, in electro-blot immunoassays, of virus-specific polyclonal antibodies to maize dwarf mosaic and sugarcane mosaic virus strains

Strain[a]	Virus-specific antibodies to:											
	MDMV-A	MDMV-B	MDMV-D	MDMV-E	MDMV-F	MDMV-O	SCMV-H	SCMV-I	SCMV-JG	SCMV-SC	SCMV-BC	SCMV-Sabi
MDMV-A	+[b]	−	+	+	+	−	−	−	−	−	−	−
MDMV-B	−[c]	+	−	−	−	−	−	−	−	+	+	+
MDMV-D	+	−	+	+	+	−	−	−	−	−	−	−
MDMV-E	+	−	+	+	+	−	−	−	−	−	−	−
MDMV-F	+	−	+	+	+	−	−	−	−	−	−	−
MDMV-O	−	−	−	−	−	+	−	−	+	−	−	−
SCMV-A	−	+	−	−	−	−	−	−	−	+	+	+
SCMV-B	−	+	−	−	−	−	−	−	−	+	+	+
SCMV-D	−	+	−	−	−	−	−	−	−	+	+	+
SCMV-E	−	+	−	−	−	−	−	−	−	+	+	+
SCMV-H	−	−	−	−	−	−	+	+	−	−	−	−
SCMV-M	−	−	−	−	−	−	+	+	−	−	−	−
SCMV-JG	−	−	−	−	−	+	−	−	+	−	−	−
SCMV-SC	−	+	−	−	−	−	−	−	−	+	+	+
SCMV-BC	−	+	−	−	−	−	−	−	−	+	+	+
SCMV-Sabi	−	+	−	−	−	−	−	−	−	+	+	+

[a] MDMV = maize dwarf mosaic virus, SCMV = sugarcane mosaic virus.
[b] + = positive serological reaction.
[c] − = negative serological reaction.

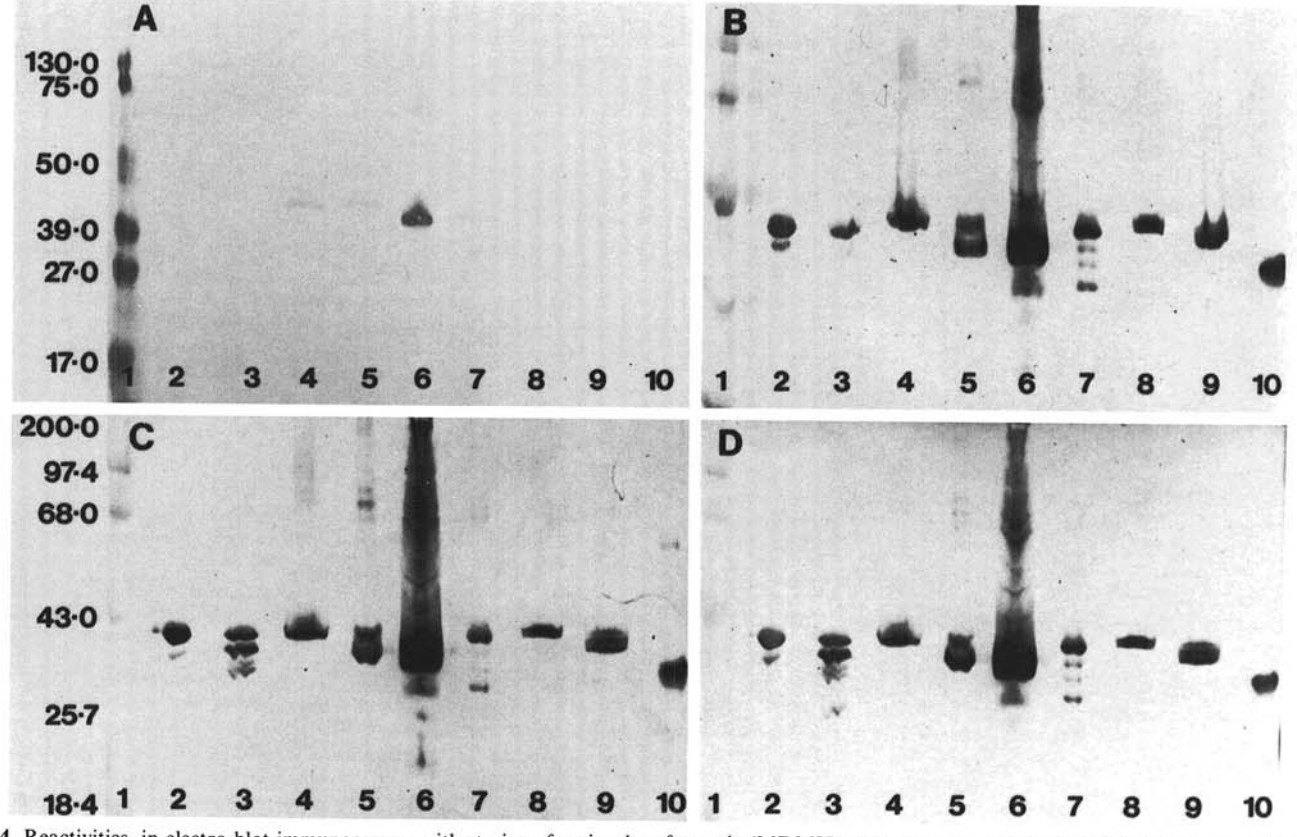

Fig. 4. Reactivities, in electro-blot immunoassays, with strains of maize dwarf mosaic (MDMV) and sugarcane mosaic (SCMV) viruses of antisera to MDMV-A (Langham) obtained after, **A,** 4 wk; **B,** 8 wk; **C,** 11 wk; and **D,** 13 wk of immunization. Lane 1 in A is Bio-Rad- and B, C, and D BRL-prestained standards. Lanes 2–10 represent SCMV-JG, MDMV-O (Gordon), SCMV-SC, SCMV-E, MDMV-A (Ford), MDMV-B (Ford), SCMV-BC, SCMV-Sabi, and tobacco etch virus. Lanes 2, 3, 6, and 7 are purified prepartions; other lanes are infected sap. Numbers to the left are molecular weight ($\times 10^3$) of marker proteins in lane 1.

coat proteins of 12 strains with purified or infective sap preparations of 16 strains of the SCMV group using electro-blot immunoaasay. The results clearly demonstrate that the 17 strains fall into four distinct groups (Table 3). Our recent work (31) has shown that the cross-absorbed antibodies are directed towards the N-termini of coat proteins of potyviruses and are virus-specific as they recognize only the homologous viruses. These findings suggest that the four groups distinguished may represent four distinct potyviruses (Table 3). This conclusion is supported by data on coat protein sequence homology and cross-protection experiments.

A detailed analysis of the coat protein sequences from 20 strains of nine distinct potyviruses (37) showed that coat protein sequence data could be used to establish whether potyviruses are related strains or independent members. The high-performance liquid chromatographic peptide profiling (32) and amino acid sequences of coat proteins (29) showed that the SCMV-SC, SCMV-BC, and SCMV-Sabi are related strains of SCMV, while SCMV-JG is a distinct member of the potyvirus group, as found here by comparative serology.

Furthermore, previously published reports on the lack of cross-protection by some MDMV/SCMV strains add further support to our assignment of these strains into four distinct viruses. It is generally accepted that related strains of the same virus will cross-protect each other, whereas distinct viruses will not. The reports that MDMV-A does not prevent multiplication of MDMV-B and SCMV-I (24,43), and that SCMV-H does not cross-protect against MDMV-A (27), are totally consistent with our allocation of those viruses (Table 3).

We have proposed the names Johnsongrass mosaic virus, maize dwarf mosaic virus, sugarcane mosaic virus, and sorghum mosaic virus for the four potyviruses distinguished among the 17 MDMV/SCMV strains (Table 3). On the basis of comparative coat protein structural and antigenic properties SCMV-JG has recently been classified as a strain of JGMV (29). Our present serological results (Table 2) suggest that MDMV-O (21) is also a strain of JGMV. A distinguishing feature of MDMV-O is its ability to infect oat cultivars, whereas U. S. strains of SCMV and MDMV have not been reported to infect oats (21). Three Australian strains, SCMV-JG (JGMV), SCMV-BC, and SCMV-Sabi, but not U. S. strains of MDMV/SCMV, were found to infect oat cultivars Marathan Wright and Garland, and the symptoms produced were similar to those caused by MDMV-O (21). Thus, the ability to infect oat does not appear to be restricted to JGMV strains.

MDMV-A was the first virus to be recognized to infect corn (45): MDMV-B and other MDMV-strains were described later (18,19). In the present work MDMV-D, MDMV-E, and MDMV-F were found to be closely related serologically only to MDMV-A (Table 2). Thus, the name MDMV proposed for this group of strains appears justified (Table 3).

TABLE 3. Grouping of maize dwarf mosaic virus and sugarcane mosaic virus strains from Australia and the United States on the basis of reactivities of cross-absorbed virus-specific antibodies

Sugarcane mosaic virus subgroup consists of four distinct potyviruses			
JGMV[a]	MDMV[b]	SCMV[c]	SrMV[d]
SCMV-JG (Aust.)[e]	MDMV-A (U.S.)	MDMV-B (U.S.)	SCMV-H (U.S.)
MDMV-O (U.S.)[f]	MDMV-D (U.S.)	SCMV-A (U.S.)	SCMV-I (U.S.)
	MDMV-E (U.S.)	SCMV-B (U.S.)	SCMV-M (U.S.)
	MDMV-F (U.S.)	SCMV-D (U.S.)	
		SCMV-E (U.S.)	
		SCMV-SC (Aust.)	
		SCMV-BC (Aust.)	
		SCMV-Sabi(Aust.)	

[a] JGMV = Johnsongrass mosaic virus.
[b] MDMV = Maize dwarf mosaic virus.
[c] SCMV = Sugarcane mosaic virus.
[d] SrMV = Sorghum mosaic virus.
[e] Aust. = Australia.
[f] U.S. = United States.

MDMV-B, SCMV-A, SCMV-B, SCMV-D, SCMV-E, and the three Australian SCMV strains (SCMV-SC, SCMV-BC, and SCMV-Sabi) were found to belong to the class identified as SCMV (Table 3). Because the name SCMV has been used in the past for all strains originating in sugarcane (1,14,39,46,47) and except for the SCMV-H, SCMV-I, and SCMV-M, all other strains examined are closely related serologically, it seems appropriate to use the name SCMV for these strains (Table 3).

Cross-absorbed antisera to SCMV-H and SCMV-I reacted only with SCMV-H and SCMV-M and not with any other MDMV/SCMV strains (Table 2), suggesting that these three strains represent a separate group of their own for which we have proposed the name sorghum mosaic virus (SrMV) (Table 3). Three reasons for recommending the name SrMV are: 1) the symptoms produced by these three strains in sugarcane cultivars cannot be clearly distinguished from those induced by other SCMV strains (1,14,41,46,47), and, therefore, a new name based on other symptoms in sugarcane is not logical; 2) SCMV-H has recently been reported to cause a severe disease of sorghum (8); 3) there is no recognized virus in the literature known as SrMV.

The groupings of the SCMV group of strains suggested here on the basis of their serological properties using virus-specific antibodies is in close agreement with those proposed by Derrick (5), Jarjees and Uyemoto (13), and Snazelle et al (38). However, these workers used only a small number of SCMV/MDMV strains, and they did not point out the fact that these groups represent distinct potyviruses.

Although we were able to classify 17 strains of the SCMV group occurring in Australia and the United States, the status of other strains described from the United States (MDMV-KS1, MDMV-C, SCMV-F, SCMV-G, SCMV-K, and SCMV-L) and other countries (3,25) is not known and needs investigation. It is possible that some of these strains may prove to belong to other than the four viruses distinguished here (Table 3). For example, MDMV-KS 1 of Jarjees and Uyemoto (13) was found to be distinct serologically from the strains in three other groups and was placed in a separate group of its own. Similarly, the biological and serological properties of a Venezuelan MDMV strain (25) and MDMV-D strain from Israel (3), respectively, were found to be substantially different from those of the other strains compared.

The cross-reactivity of unfractionated antisera observed here (Fig. 1A C, and E) is not unique to MDMV/SCMV strains. Similar results were obtained when 11 polyclonal antisera to potyviruses produced in different laboratories were analyzed with 12 distinct potyviruses (31). A majority of these antisera reacted with all or most of these viruses (31). This phenomenon is due to the presence, in these antisera, of antibodies directed towards the homologous core protein region (devoid of N- and C-termini) of the coat protein of potyviruses (31,35,37). As found here with MDMV-A antisera from different bleedings of a rabbit (Fig. 4), the proportion of such cross-reacting antibodies in antisera increases with an increase in the number of immunizations and an increase in time after immunization when sera are collected. These observations suggest that the past confusion concerning the taxonomic status of MDMV/SCMV strains may have been due to the presence of variable proportions of cross-reacting antibodies in the antisera used resulting in the observed serological relationships among viruses of the SCMV subgroup (26,27).

The results presented here clearly demonstrate that the use of virus-specific antibodies directed towards the N-termini of coat proteins (isolated from polyclonal antisera to intact particles of potyviruses by cross-absorption) is a very useful approach that has the potential to solve the problems of identification and classification for members of the potyvirus group. Similar taxonomic problems, found here with the SCMV subgroup, exist with other potyvirus subgroups particularly among the potyviruses infecting legumes (4,37). Use of virus-specific antibodies as described here or peptide profiling of the coat proteins (32) may resolve such problems. Our results also suggest that the cross-absorbed virus-specific antibodies should recognize most, if not all, strains of a potyvirus since the strains of individual potyviruses have highly homologous N-termini (37). Such antibodies may be

more useful than monoclonal antibodies whose specificity could be affected by minor sequence changes.

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